This problem is being studied in greater detail, with a particular attempt being made to correlate the laboratory data with the clinical findings.

Typical results obtained on a total of 20 hypercholesterolemic rabbits are shown in Table II. The experimental animals were fed Rockland rabbit pellets to which 5 g of cholesterol and 50 g of a hydrogenated vegetable oil (Mazola oil) dissolved in chloroform were added per kg, the whole thoroughly mixed and the chloroform allowed to evaporate. Blood was obtained by cardiac puncture at intervals and analyzed. It will be seen that the readily extractable fraction was very high in all cases, apparently comprising all of the cholesterol of the serum in some cases. All of the experimental animals at the time of autopsy showed some atherosclerotic lesions. Animals fed a similar diet but without added fat showed the same general picture except for a considerably greater fluctuation in the different cholesterol fractions amongst the individual animals.

Discussion. Although the significance of the variations in the concentration of the readily extractable cholesterol of the serum remains to be determined, it is of considerable interest that the two conditions in which it was found to be exceptionally high, namely clinical nephrosis and experimental hypercholesterolemia of rabbits, both tend toward the rather rapid development of atherosclerotic lesions. More extensive studies, however, are necessary before any conclusions regarding its significance can be drawn.

Summary. Chloroform extraction of normal serum, dried *in vacuo* from a frozen state, removed a relatively small but constant fraction of the total cholesterol. A very high percentage of the total cholesterol of the serum of nephrotic patients was extracted under the same conditions. The same was true of rabbits rendered hypercholesterolemic by the administration of cholesterol with their food. The readily extractable fraction was slightly elevated in all the hypothyroid cases studied and in some diabetic patients.

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Disappearance of Cholesterol Following its Intravenous Injection in Physiologically Emulsified Form.*

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Despite the implication of cholesterol in many vital processes, such as in the formation of hormones and in the genesis of atherosclerosis, very little is known concerning its metabolism. As technics in metabolic studies oral tolerance tests have consistently failed because of the slow and irregular absorption of cholesterol suspensions from the gastrointestinal tract, and the resultant insignificant rise in blood levels.[1-4] Artificial emulsions prepared with high pressure emulsifiers and various emulsifying agents, such as the lecithins and phospholipids have been unsatisfactory, either because the particle size obtained was too large for safe intravenous administration,

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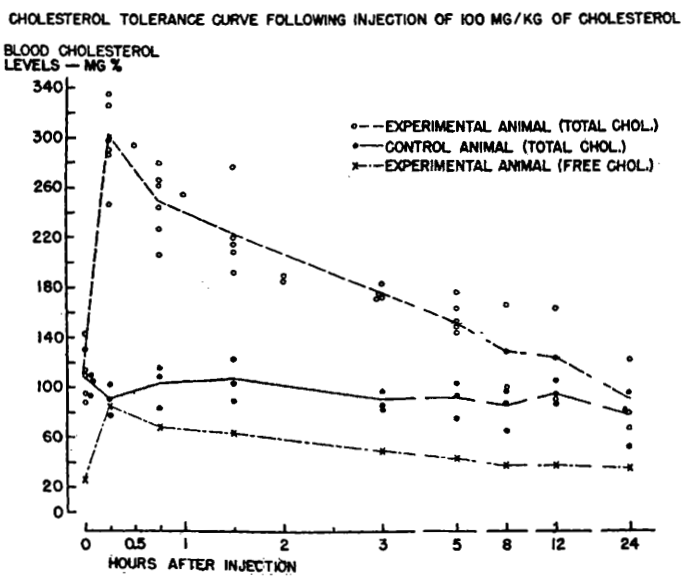

FIG. 1.

or because the concentration of cholesterol in the emulsion was too low.[5]

We have observed that chickens placed on a high cholesterol diet develop a hyperlipemia and hypercholesterolemia, with blood cholesterol levels frequently exceeding 2000 mg %.[6] It occurred to us that plasma from such blood could be employed in the study of cholesterol metabolism in the chicken. Relatively small volumes of the plasma would provide relatively large doses of cholesterol.

White leghorn chickens were fed a high cholesterol diet (4%) for at least 3 weeks, and then bled of 20 cc each. The heparinized blood was centrifuged, the milky plasma drawn off and passed through a Seitz filter in order to assure sterility. It was stored in the refrigerator until required. Plasma cholesterol determinations by the Schoenheimer-Sperry method[7] showed no change before or after filtration and storage for periods up to 10 days.

Tests of cholesterol disappearance were performed on normal adult white leghorn cockerels given the stored plasma intravenously in doses providing 100, 150 and 200 mg of cholesterol/kilo of body weight (approximately 6 cc of injected plasma). The blood levels were followed at intervals. A series of control animals which did not receive the plasma injection were subjected to the same tests. The injection of the lipemic plasma produced no injuries or deaths, and repeated injections caused no anaphylactic reactions.

Fig. 1 summarizes the curves obtained on 6 normal adult birds injected with lipemic plasma, providing 100 mg of cholesterol per kilo. Fifteen minutes after injection the average blood cholesterol level had risen from the initial value of 114 mg % to 297 mg %, then it declined gradually to a level somewhat below normal in 24 hours. The free cholesterol paralleled the total cholesterol levels throughout. The slope of the individual curves resembled the slope of the average curve.

The cholesterol levels on 3 normal adult control chickens remained relatively unchanged during the 24-hour period. In 2 additional adult chickens injected with a dose of 200 mg of cholesterol per kilo, and 1 with 150 mg per kilo, the peak levels were higher but the slope of the curve paralleled closely the average curve obtained with 100 mg per

[5] Cole, W. H., Clark, H., and Womack, N. A., *J. Lab. Clin. Invest.*, 1941, **26**, 1679.

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TABLE I.
Total Circulating Blood Cholesterol Above Control Value.*

Time after injection	100 mg/kg dose	200 mg/kg dose
15 min	220 mg	498 mg
45 "	160 "	428 "
90 "	132 "	350 "
3 hr	65 "	188 "
5 "	43 "	221 "
8 "	20 "	83 "
12 "	16 "	58 "
24 "	—27 "	—31.2 "

* The circulating blood volume was assumed to be 120 cc. The values given are averages, those connected by the lines in Fig. 1 are means.

kilo. At 24 hours, the levels had returned to normal.

The data in Table I show the total amount of cholesterol in the circulating blood in excess of the control value at the various time periods. The values are based on the total blood volume, estimated in our laboratory by the Evans blue method to be 120 cc for the adult chicken. The rate of disappearance of the injected cholesterol varies with its concentration above the control value. Thus, when the difference is 220 mg a fall of 60 mg occurs in 30 minutes, but when the difference is only 20 mg the fall is 4 mg in 4 hours. When the excess of circulating blood cholesterol is plotted against time on a logarithmic scale, a straight line relationship obtains up to 8 hours following injection; between 8 and 12 hours there is a decline in the slope; and between 12 and 24 hours the original relationship again obtains. The same relationship holds also for free cholesterol.

Discussion. Following the injection of physiologically emulsified cholesterol into the chicken consistent cholesterol disappearance curves were obtained. The highest blood levels were observed 15 minutes after injection, after which the cholesterol level fell gradually. The return of the cholesterol from the peak to the control level in 12 to 24 hours is in marked contrast to the return of glucose to the normal control level in 2 to 3 hours. We have shown that the removal of cholesterol from the blood is a function of its concentration in the blood, and that at high blood concentrations, the organism can remove relatively large amounts of cholesterol from the blood in short periods of time. The method has also been applied successfully in the rabbit.

Our technic involves the introduction of all the elements of the lipemic plasma, neutral fats, fatty acids, phospholipids, cholesterol, plasma proteins, etc., which are probably finely adjusted physiologically to maintain the lipemic plasma cholesterol in its native emulsified state. We can therefore essentially duplicate the conditions imposed by high dietary loads of cholesterol and thus obtain a picture of how the organism deals with exogenous cholesterol. The method is thus superior on theoretical grounds to those using artificially prepared emulsions, which the organism presumably must convert into physiologically acceptable form prior to disposal.

Summary. 1. A technic for obtaining an intravenous cholesterol tolerance test in the chicken is described.

2. Cholesterol is removed from the blood stream rapidly when the blood cholesterol levels are high and slowly when the blood cholesterol levels are low.

3. Total cholesterol and free cholesterol levels parallel each other throughout such tests.