samples stored for zero and one day. Uniform uptake values were found by extending the storage time of T_3-I^{131} up to 5½ weeks prior to use on uptake of T_3-I^{131} by RBC. The lot of T_3-I^{131} stored for 7½ weeks indicated a chemical breakdown of the triiodothyronine since a significant drop in its uptake was found. Within the levels studied, the changed concentration of exogenous tracer levels of T_3 was found to have no significant effect upon uptake of T_3-I^{131}.

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Metabolism and Excretion as Factors Influencing Serum Cholesterol Level in Two Lines of Chickens.* (28958)

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It has been demonstrated in various vertebrates including rats(1), humans(2), and chickens(3,4) that genotype influences blood level of cholesterol. The evidence is insufficient, however, to establish the precise mode of genetic transmission or to explain the physiological mechanisms involved. Hardy, Auger, and Wilcox(5) attempted to determine physiological contributors to blood serum cholesterol levels in 2 lines of White Leghorn chickens that differed in both combined and free cholesterol. Birds of both lines reacted similarly to fasting, injection of various hormones, and temperature stress. No line differences were observed with respect to: serum levels of fatty acids and phospholipids; cholesterol levels in testis, brain, adrenal, or liver; weights of various organs; or hematocrit values.

This study was undertaken further to investigate the physiological mechanisms that may be responsible for the different serum cholesterol levels in these 2 lines(4) of chickens. The immediate influence of the liver was checked by analyzing serum of post- and pre-hepatic blood of both lines. Comparative rates of sterol synthesis were studied *in vitro* by using acetate-1-C^{14} in whole blood and liver slices. The comparative catabolism, distribution, and excretion of injected cholesterol-4-C^{14} were determined in each line.

Materials and methods. Immediate liver effect. The chickens were progeny of high

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and low cholesterol lines(4) and were reared in comparable cage environments. They received the ration given by Wilcox *et al*(4) for birds raised in 1960-61 at Univ. of Maryland; this ration contained 1.5% animal protein.

Following laparotomy under sodium pentobarbital anesthesia, blood was taken from the right ventricle of the heart and the portal vein. The blood was incubated at 37°C for 1½ hours after which serum was decanted and frozen until analyzed(6).

Comparative rate of cholesterol synthesis. Non-fasted male birds 4 to 6 weeks of age were used. The incubation medium included: 2 μc sodium acetate-1-C^{14} (sp. act. 6.15 mc/mM) incorporated in 0.4 ml Ringer's solution, and an antibiotic solution (100 units penicillin, 0.5 mg dihydrostreptomycin) in 0.1 ml Ringer's solution. These materials were added to the side arm of a 50 ml center-well incubation flask. A filter paper strip moistened with 50% KOH was placed in the center well as a carbon dioxide absorber during incubation.

A sample of 5 ml of blood was taken from each bird by heart puncture, citrated, held in an ice water bath, then transferred to the outer well of an incubation flask and aerated with a gas mixture of 95% O_2 and 5% CO_2 at 5°C for 20 minutes. Following a 3-minute equilibration in a 39°C water bath the contents and rinsings of the side arm were mixed with the blood. Incubation proceeded 6 hours under continuous mild agitation. The blood was hydrolyzed(7), the digitonide was prepared(8), suspended in chloroform, transferred to a stainless steel planchet, and prepared for radioanalysis by evaporation of the solvent. All C^{14} measurements were made with a gas-flow proportional counter. Corrections were made for background radiation.

The liver was removed from recently decapitated birds and kept in a covered container with iced Ringer's solution until sliced inside a 5°C walk-in compartment. A portion of it was trimmed to a rectangular cube about 1 cm diameter, held firmly between two pieces of frosted glass and sliced with a razor blade (9). Liver slices totaling 500 $\pm$ 5 mg were taken from the liver of each bird and transferred to the outer well of an incubation flask containing 5 ml Krebs-Ringer phosphate buffer(9) slightly modified for isotonicity with chicken blood and with a pH of 6.9 to 7.1. Incubation media were aerated with oxygen at 5°C for 10 minutes and equilibrated at 37-38°C for 5 minutes. The contents of the side arm were mixed with the slices and buffered media and incubated for 3 hours under continuous mild agitation. The contents of the flask were separated into nonsaponifiable and saponifiable fractions (10). The digitonide was precipitated(8) from the nonsaponifiable fraction and submitted to radioanalysis along with aliquots of the saponifiable fraction.

The CO_2 absorbed during incubation was precipitated with barium hydroxide. The precipitate was washed 3 times with distilled water and one with methanol. It was then transferred in methanol to a filter paper disc of planchet size with the aid of a Hirsch funnel and mild suction, and submitted to radioanalysis.

Cholesterol catabolism, distribution, and excretion. Cholesterol-4-C^{14} (sp. act. 22.74 mc/mM), prepared for use with the aid of Tween 20(11) and containing 9.1×10^5 cpm, was injected into the wing vein of 18-day-old cockerels selected from families of both lines having low variability among sibs. After 48 hours a blood sample was taken for serum analysis of C^{14} and total cholesterol(6); the birds were sacrificed by decapitation and certain tissues were excised and frozen until analyzed. Feces, collected during the 48-hour period, were dried to constant weight at 70°C, pulverized, and stored in a desiccator until analyzed.

Liver, intestine, skin, adrenals, and carcass were placed separately in a media having a final concentration of 17% KOH and 50% ethanol and hydrolyzed 8 hours over a steam bath. The nonsaponifiable fraction was extracted from the hydrolysates(10), and duplicate aliquots were prepared in chloroform for C^{14} analysis. Bile acids were isolated from liver extracts(12) and C^{14} determined.

Duplicate samples were taken from the feces of each bird and extracted and fractionated into nonsaponifiable and saponifiable

TABLE I. Serum Cholesterol from Portal Vein and Right Ventricle of the Heart in 2 Lines of Chickens at 6 Weeks of Age.

"Low" line				"High" line			
Portal		Rt vent		Portal		Rt vent	
♂	♀	♂	♀	♂	♀	♂	♀
142*	129	141	131	191	185	188	185

* Values given are mean of 20 birds (mg/100 ml).

portions, and the bile acids were isolated(12). Aliquots were prepared in duplicate for C^{14} analysis of all fractions. As a control on efficiency of fractionation, digitonin solution was added to an aliquot of the saponifiable extract, however, no precipitate was observed.

Results. The line difference in serum cholesterol level was not changed whether blood was taken from the right ventricle of the heart or from the portal vein (Table I).

Labeled digitonin-precipitable materials (DPM) were isolated from the chicken blood incubated with acetate-C^{14}. Average values from blood of 7 chickens per line were: 50.9 and 50.6 cpm/ml of blood in the digitonide-C^{14} fraction and 6369 and 7790 cpm/ml in the $BaC^{14}O_3$ fraction from low and high lines, respectively. Average values for acetate-C^{14} incorporation into various fractions by liver slices from 10 chickens per line were 3062 and 2732 cpm/g of liver in digitonide-C^{14} fraction; 25492 and 28988 cpm/g in saponifiable fat fraction; 231,679 and 248,590 cpm/g in $BaC^{14}O_3$ fraction; from low and high lines, respectively. Statistical differences were not observed between the two lines in rate of acetate incorporation into these fractions as determined by incubation of blood or liver slices.

Following cholesterol-4-C^{14} administration into the wing vein, the relative differences between the 2 lines were approximately the same for serum cholesterol (190 and 264 mg per 100 ml) and for total C^{14} activity (3784 and 5230 cpm/ml). These line differences were highly significant.

The "low" line excreted significantly more nonsaponifiable C^{14}-labeled substances than did the "high" line. However, no differences were observed between the 2 lines in the saponifiable fraction of the feces; in the nonsaponifiable or saponifiable C^{14}-labeled sub-

TABLE II. Fecal and Tissue Analysis for C^{14}-Labeled Substances in Nonsaponifiable and Saponifiable Fractions Following Injection of Cholesterol-4-C^{14} into 18-day Old Male Chicks of High and Low Cholesterol Lines.*

Line	Serum cholesterol	Blood serum	Feces		Liver		Gut	Skin	Adrenal	Carcass
			NS	Sapon	NS	Sapon	NS	NS	NS	NS
	(mg/100 ml)	(cpm/ml)	(cpm/g)	(cpm/g)	(cpm/g)	(cpm/g)	(cpm/g)	(cpm/g)	(cpm/mg)	(cpm/g)
High	264	5230	1341	610	3582	1168	2366	1700	11.47	2161
Low	190	3784	1391	613	3533	1102	2398	1544	12.67	2041

* Values given are mean of 5 birds.

stances in the liver; or in the nonsaponifiable fraction of the gut, skin, adrenals, or carcass (Table II).

Discussion. Tissue incubation with labeled acetate followed by radioanalysis of the digitonin precipitate was considered adequate in determining relative rates and capabilities of tissue cholesterogenesis. However, some variability between birds in rate of conversion of labeled acetate to cholesterol would be expected due to the diverse pathways in acetate metabolism. The acetate-1-C^{14} incorporated into DPM of chicken blood was of similar magnitude as that obtained by Raut *et al* (7) with blood of the calf, pig or rat and higher than that obtained with blood of cow, piglet or rabbit.

Since cholesterol-4-C^{14} was administered intravenously, the only sources of fecal C^{14}-labeled substances would be through the intestinal wall, the bile system, or the urinary system. Quantitative differences in bile acids, the major metabolite of cholesterol(13), were not detected in either excreta or liver. However, the possibility exists that the 2 lines differed qualitatively or in balance of the various bile acids. This is pertinent since it has been shown that bile acids are important factors in other species in determining rate and route of cholesterol metabolism and rate of excretion of fecal sterols(14).

The fact that differences were not observed between the two lines with respect to distribution of the labeled cholesterol in the tissues of liver, intestine, skin, adrenal, or carcass would indicate that these tissues had little direct influence on maintaining genetic differences in serum cholesterol between the 2 lines.

Since rate of cholesterol-4-C^{14} excretion was significantly different between the 2 lines, it is suggested that mechanisms controlling mobilization and excretion of cholesterol are under genetic control. In the process of selecting these lines, differences in sensitivity to a critical site in the cholesterol homeostatic system may have been partitioned. This could have been expressed by increased excretion in the low line.

Summary. Two lines of White Leghorn chickens differing in serum cholesterol level were studied. The line difference in cholesterol was unchanged in serum from post-hepatic blood as compared to pre-hepatic blood. No line differences were observed in rate of *in vitro* cholesterogenesis from acetate-1-C^{14} incubated with blood or liver slices. Following administration of cholesterol-4-C^{14}, differences were not detectable between lines in labeled nonsaponifiable substances in liver, gut, skin, adrenal, or carcass, or in the labeled saponifiable fraction of liver or feces. Fecal excretion of labeled nonsaponifiable substances, however, was significantly higher in the low line, suggesting genetic regulation of cholesterol homeostasis through excretory pathways.

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