ney (NTS). Triparanol was given orally in doses of 25 mg/kg of body weight per day before, during, and for 2 weeks after administration of NTS. Total serum lipids, serum cholesterol, and serum and urinary proteins were determined before, during, and for several weeks after injection of NTS. Triparanol decreased serum cholesterol markedly and serum lipids slightly, but it had no influence on hypoproteinemia and proteinuria. The histologic changes in the kidneys were the same in the dogs receiving NTS plus triparanol as in the dogs receiving NTS alone, namely endothelial proliferation in glomerular tufts, adhesion of tuft to capsule at several points, focal fibrosis of glomeruli, hyalinization of tufts with periglomerular fibrosis, irregular thickening of basement membrane, and fusion of foot processes. The tubular epithelium showed hydropic degeneration. Triparanol aggravated the course of the disease.

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Incorporation of Plasma Cholesterol-4-C^{14} into Egg Yolk Cholesterol.* (29946)

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To date there is no direct evidence that cholesterol from the blood of the laying hen is transferred across the ovarian membranes into developing egg yolks. In previous work, laying hens have been given C^{14}-acetate or H^2-acetate. These labels have subsequently been found in all of the constituents of eggs, including the cholesterol of the yolk(1,2,3). However, because of the expected rapid conversion of the administered acetate to cholesterol in the liver and recycling of cholesterol into the blood, the finding of labeled cholesterol in the egg yolk could have been explained by at least two possible mechanisms. First, cholesterol of hepatic origin synthesized from the labeled acetate might have been transported *via* the blood into the yolk. Second, circulating labeled acetate might have been taken up by the ovary which then synthesized cholesterol and transferred it into the yolk. This latter hypothesis is

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supported by the *in vitro* work of Popjak and Tietz who found that ovarian membranes from laying hens synthesized cholesterol from acetate(4).

The present study was designed to measure directly the extent to which plasma cholesterol-4-C^{14} was transferred into the cholesterol of the egg yolk, and to ascertain the possibilities of producing egg yolks having a high content of cholesterol-4-C^{14}, with the radioactivity confined only to the cholesterol molecule of the yolk.

Materials and methods. White Leghorn hens, known to be steady egg producers, were given 50 to 100 μc of cholesterol-4-C^{14} intravenously. This isotope in 0.5 ml of ethanol with 1 mg of non-labeled cholesterol was mixed with 2.0 ml of chicken plasma and the entire 2.5 ml volume was injected over a 5-minute period into the wing vein of each hen. Eggs were collected subsequently and the yolks analyzed for radioactivity and total cholesterol content(5). Comparable assays of hen serum for radioactivity were made at selected intervals. The yolk lipids were extracted with 2:1 chloroform-methanol. The extracts were counted in a Packard Tri-Carb liquid scintillation counter.

Radioactivity was found only in the cholesterol component of the yolk and was not transferred to other lipids as indicated by the following procedures. The yolk extract was saponified and the cholesterol precipitated with digitonin. The cholesterol digitonide contained all of the radioactivity contained in the original lipid extract. The residues had no radioactivity. When the yolk lipids were separated by thin layer chromatography, radioactivity was found only in the cholesterol ester and free cholesterol fractions; no radioactivity was present in the triglyceride and phospholipid fractions. The stability of the C^{14} label at the 4 position of cholesterol after administration of this compound to animals has been demonstrated by others(6).

Results and discussion. Fig. 1 illustrates that a large amount of cholesterol-4-C^{14} was transferred from the plasma of the 4 laying hens into the yolks of eggs laid after administration of cholesterol-4-C^{14}. The isotope appeared first in eggs laid 2 days after injection. Specific activity in counts per minute per mg of cholesterol rose sharply to a peak for hens 2, 3, and 4 by the 5th to 7th days. Specific activity declined rapidly for the next few days and then exponentially. Hen 1 stopped laying from day 1 to day 11. The egg produced on day 11 had peak radioactivity and subsequent eggs of this hen had higher radioactivity than eggs from the other hens laid the same number of days following the initial injection of cholesterol-4-C^{14}. The reason for this difference was that the other hens had already eliminated as a constituent of eggs much of the injected cholesterol-4-C^{14}.

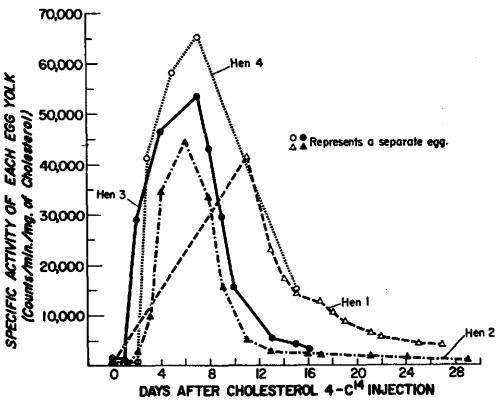

FIG. 1. Appearance of cholesterol-4-C^{14} in eggs laid successively after intravenous injection of this isotope in 4 hens. Each point on a curve represents radioactivity obtained from one egg expressed as specific activity in counts/min/mg of cholesterol. Hens 1, 3, and 4 received 100 μc in 1 mg of cholesterol; hen 2 was given 50 μc in 0.5 mg of cholesterol.

As listed for each hen in Table I, the recovery of cholesterol-4-C^{14} from eggs laid following its administration ranged from 31.1 to 45.8% of the total amount initially given. Thus, for a laying hen, a major excretory pathway of cholesterol-4-C^{14} is the production of eggs. With the technique described in this study, eggs containing from 1 to over 10 μc of cholesterol-4-C^{14} were readily produced. Furthermore, the cholesterol-4-C^{14} was incorporated into a natural food. Such a preparation might be useful in studies of the intestinal absorption and metabolism of cholesterol and offers advantages over the customary use of crystalline cholesterol-4-C^{14}

† Cholesterol-4-C^{14}, New England Nuclear, specific activity 50 μc/mg.

TABLE I. Cholesterol-4-C^{14} Activity in Eggs After Injection into Hens.

	Cholesterol-4-C^{14} given (μc)	Days after inj for egg with maximum radioactivity	Cholesterol-4-C^{14} found in eggs laid (μc)	% Recovered	No. of eggs with radioactivity above various levels: Above 1 μc	Above 5 μc	Above 10 μc
Hen 1*	100*	11*	31.1	31.1	8	1	1
2	50	6	22.9	45.8	5	2	0
3	100	7	40.4	40.4	6	5	0
4	100	5	36.5	36.5	4	3	2

* Given 50 μc on 2 successive days. No egg then laid for 11 days.

added artificially to foodstuffs. Administration of C^{14}-acetate to laying hens in order to obtain radioactive cholesterol in the yolk has the disadvantage that the C^{14} label is incorporated into other lipid and protein constituents of the yolk, as well as into the cholesterol. Such eggs would be of little value for the study of cholesterol metabolism *per se* after their ingestion in the diet because the C^{14} label would be in many compounds containing carbon and not confined to the cholesterol molecule.

Temporal relationships of serum and yolk radioactivities. The radioactivities of serum and egg yolk cholesterol had a well-defined temporal relationship (Fig. 2). Serum specific radioactivity was 54,670 counts/min/mg of cholesterol one day after intravenous injection of 50 μc of cholesterol-4-C^{14}. An egg laid at this time had no detectable radioactivity, since its yolk was completely formed before administration of the isotope. By day 2, however, cholesterol-4-C^{14} was found in the yolk, and in eggs laid subsequently, yolk specific activity rose to a peak between 5 and 7 days. Meanwhile, serum specific activity fell, so that when yolk radioactivity was highest, the serum radioactivity was much lower. In this actively laying hen, the crossover point of serum and egg radioactivities probably occurred at about day 4. After the 5- to 7-day peak, yolk specific activity tended to decline until a second intersection of serum and yolk specific activity curves took place. Specific activities of serum and egg yolks tended to equilibrate about day 16 and remained similar thereafter.

Because the ovarian yolk forms a new layer each day of the 7-9-day period of growth, the radioactivity of different layers of the yolk might vary widely if the yolk was being formed at a time when plasma radioactivity was changing rapidly. Accordingly, the radioactivities of 5 different layers of the yolk from an egg laid on day 11 after the injection of cholesterol-4-C^{14} were separately determined by appropriate sectioning of the yolk from an egg which had been hardboiled (Fig. 3). As can be noted, the center of the yolk, formed first, has a specific radioactivity of 8.8×10^3 cpm/mg of cholesterol and the specific activity was successively lower in more peripheral and more recently formed layers. In the outermost layer, the specific activity was lowest at 2.5×10^3 cpm/mg.

Our assumption from these data that the specific activities of the different layers of yolk reflected a changing plasma radioactivity is supported further by data comparing the specific activities of ova of different sizes with a mature yolk and with serum. As indicated in Table II, confirmatory information was obtained from 3 hens autopsied on the

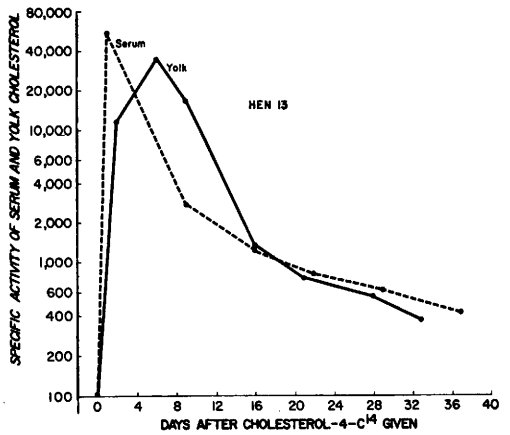

FIG. 2. Comparison of serum and egg yolk specific activities (counts/min/mg of cholesterol) in hen 13 after 50 μc of cholesterol-4-C^{14}.

TABLE II. Cholesterol Specific Radioactivities* of Serum, Mature Egg Yolks, and Ova of Different Sizes 7 Days After Administration of Cholesterol-4-C^{14}.

	Hen #5	#6	#7
Serum	6,038	4,989	8,453
Egg yolk	34,219	51,350	33,516
Ova, 3.0 cm†	25,627	25,721	22,752
2.0 "	8,844	6,628	14,576
.9 "	6,304	5,356	9,613

* Specific activity in counts/min/mg of cholesterol.

† Diameter of ova.

7th day after injection of cholesterol-4-C^{14}. For hen 5, for example, egg yolk specific activity was 34,219 compared with serum specific activity of 6,038. This egg yolk had developed over the previous 7-9 days when the radioactivity of the blood had been much higher. A large ovarian yolk, 3.0 cm in diameter, almost mature and somewhat more recently formed, had a specific activity of 25,627. Ova, smaller in size and younger in development, had specific activities resembling the level of the serum on day 7. Thus, the older ova and mature yolks represented a composite of previous serum radioactivities as well as the current level.

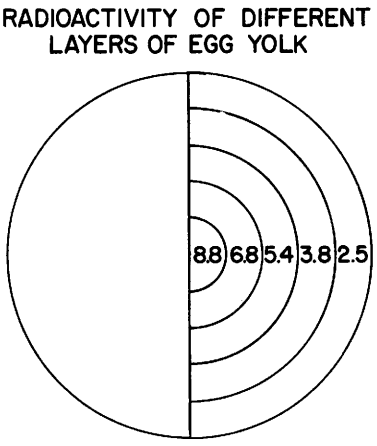

FIG. 3. Specific activity of cholesterol (counts/min/mg of cholesterol) contained in different layers of an egg (from hen 13) 11 days after cholesterol-4-C^{14} injection. Serum specific activity at this time was calculated at 2,340, approximately the same as the outermost layer of this egg. Specific activity of entire yolk was 4,426.

Summary. These data indicate that the laying hen transfers cholesterol-4-C^{14} from the blood across the ovarian membranes into developing yolks. Eggs laid after administration of cholesterol-4-C^{14} intravenously contained up to 45.8% of the administered isotope confined completely to the cholesterol fraction of the yolk. Many individual eggs contained from 5-10 μc of cholesterol-4-C^{14}. The specific radioactivity of yolk cholesterol always had a well defined temporal relationship to the specific radioactivity of cholesterol in the blood. While exact quantitative data are not yet available, the results of these experiments suggest that most, if not all, of the cholesterol in the egg yolk originates from the blood.

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