Rate of Disappearance of Cholesterol-C^{14} from the Bloodstream of Dogs.*† (27989)

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Rittenberg and Schoenheimer(1) fed deuterated water to mice and followed the specific activity of the H^2-labeled plasma cholesterol; they reported its half-time rate of disappearance to be 15 to 25 days. Pihl and associates(2) calculated the half time as 31 to 32 days after administration of C^{14}-acetate to rats. In a euthyroid patient, Kurland and Lucas(3) found the disappearance of C^{14}-cholesterol in the plasma to have a half time of 65 days; after induction of myxedema, the half time lengthened to 160 days; in another patient the half time increased from 68 to 100 days. Labeled cholesterol, which resulted from oral administration of DL-mevalonic acid-2-C^{14}, disappeared at 3 rates with half times of 1.5, 6.5 and 120 days, respectively, according to Gidez and Shreeve (4).

We have studied the disappearance of cholesterol from the plasma of dogs after direct intravenous injection of cholesterol-C^{14}. The disappearance curves were analyzed as a 4-compartment mammillary system.

Methods and materials. 4-C^{14}-cholesterol was emulsified with about 6 drops of Tween 80, 4 ml ethanol, and 10 ml of 0.9% saline. The solution was given intravenously to mongrel dogs.

A simplified method for measuring cholesterol-C^{14} in the plasma was designed: to 2 ml citrated plasma was added, under pressure, 28 ml of an acetone-alcohol mixture (1:1). After thorough mixing, the suspension was centrifuged and 15 ml of the supernatant solution (the extract from 1 ml of plasma) was transferred to a scintillation counting vial and evaporated to dryness. Radioactivity was measured after addition of the scintillation solution. The validity of the method depends on the assumption that the sterol ring is not metabolized(5-7); comparison of this "plasma extract method" with the conventional digitonide precipitation seemed to confirm the assumption.

Seven-dram glass vials with molded plastic stoppers were used for scintillation counting. Twelve milliliters of the following solution was used for each sample: 10 g of PBD (2-phenyl-5-[4-biphenyl]-1,3,4 oxadiazole) and 0.5 g of POPOP (1,4-bis-2-[5 phenyloxazolyl]-benzene) dissolved in 1 liter of analytical grade xylene.

Plasma sterol was determined by an adaptation of the Zak method(8).

Compartmental analysis. Semi-logarithmic analysis of the disappearance curves of C^{14} from the plasma of one dog (dog 1) by successive subtractions indicated that the curve could be subdivided into at least 3 components: $X_1 = C_1e^{-b_1t} + C_2e^{-b_2t} + C_3e^{-b_3t}$ (Fig. 1). The observed values seemed to fit a 4-compartment mammillary sytem such as that analyzed by Matthews(9) (Fig. 2). One may assume that the central compartment (V_1) represents the cholesterol in plasma and blood cells and in any other very rapidly exchanging compartments, such as the free cholesterol of the liver. Compartments V_3 and V_4 represent other extravascular pools with slower turnover. Compartment V_2 could represent excretion or a compartment (perhaps the central nervous system) where the movement of cholesterol is so slow as to appear to be unidirectional. In effect, the rate constant, k_{12}, represents rate of movement of cholesterol from the blood to a nonreturnable compartment (excretory rate).

Matthews(9) has calculated the intercompartmental rate constants for such a 4-compartment system; of these the excretory-rate

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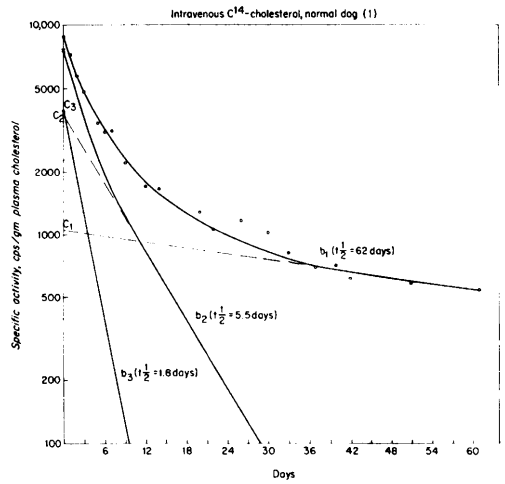

FIG. 1. Disappearance of C^{14} from plasma of dog 1, expressed as specific activity; the curve appears to be resolved into 3 components with slopes b_1, b_2, and b_3 and intercepts C_1, C_2, and C_3, respectively.

constant (k_{12}) is $\dfrac{1}{\dfrac{C_1}{b_1}+\dfrac{C_2}{b_2}+\dfrac{C_3}{b_3}}$. The calculated compartment sizes (V_3, V_4), based on the slopes of the 3 disappearance rates (b_1, b_2, b_3), and the 3 normalized Y-axis intercepts (C_1, C_2, C_3) are listed in Table I for dog 1. These calculations indicate that the excretory rate (k_{12}) was 6.4% of V_1 per day, or 1.5% of $V_1 + V_3 + V_4$ (the retained fraction of the dose) per day. Coincidentally, perhaps, this latter rate (1.5% per day) is in fair agreement with the slope of the slowest component (b_1) of the plasma specific activity-time curve (1.1% per day).

Analysis of the specific activity of the plasma of a second dog (dog 2) led to similar values (Table I). The terminal component of the plasma curve (b_1) had a slope of 1.25% per day; the rate for k_{12} was calculated as 1.6% of $V_1 + V_3 + V_4$ per day, again in reasonable agreement.

Thus, in 2 normal dogs on normal diets, a fair approximation was found between the calculated "excretory rate" of intravenously administered C^{14}-cholesterol and the terminal exponential component of the disappearance curve of plasma C^{14}-cholesterol. Although a broad generalization from these limited normal results is hazardous, certain approximations may be made by careful measurement of the last plasmatic curve (b_1) alone. For example, if the administered dose (D) is divided by C_1, which is the extrapolated value of the b_1 curve to time of injection of the labeled cholesterol, the quotient (D/C_1) reflects the extent of dilution of the dose into the volume represented by C_1. This quotient (D/C_1) may then be considered as the "exchangeable cholesterol pool" (ECP).

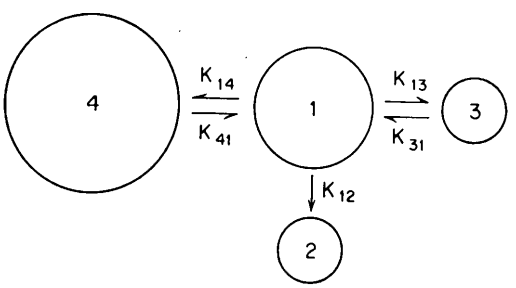

FIG. 2. Four-compartment mammillary system assumed to represent the distribution of cholesterol injected into bloodstream (compartment 1). Compartments (circles) labeled 1, 2, 3, 4 are described in the text as V_1, V_2, V_3, V_4.

TABLE I. Observed Rates and Y-Axis Intercepts in 2 Normal Dogs.*

	Dog 1	Dog 2
C_1	.122	.165
C_2	.422	.624
C_3	.456	.211
b_1 per day	.011	.0125
b_2 " "	.124	.154
b_3 " "	.381	.822
k_{12} " "	.064 V_1	.057 V_1
	.015 ($V_1+V_3+V_4$)	.016 ($V_1+V_3+V_4$)
k_{13} " "	.070	.118
k_{31} " "	.256	.676
k_{14} " "	.093	.097
k_{41} " "	.031	.041
V_3	.273 V_1	.175
V_4	3.0 V_1	2.35 V_1

* Observed rates (b_1, b_2, b_3) and Y-axis intercepts (C_1, C_2, C_3) in 2 normal dogs (Fig. 1). From these values and on the basis of a 4-compartment mammillary system (Fig. 2), rate constants (k_{12}, k_{13}, k_{31}, k_{14}, k_{41}) and volumes of the compartments relative to size of V_1 (where cholesterol-C^{14} was injected) were calculated.

Results. Since it was not until about 40 days after intravenous injection of radiocholesterol that the curve for specific activity of plasma appeared to become linear, the decline of plasma C^{14}-cholesterol was determined from about the 40th day in 17 dogs

TABLE II. Slope of Final Portion of Disappearance Curves of C^{14} from Plasma (b_1) Related to Diets and Plasma Cholesterol Levels in Dogs.*

Dog	Diet	b_1, $t\frac{1}{2}$ in days	Mean plasma cholesterol, mg/100 ml
	Restricted fat diet		
6†	1 + MER/29	13.6	76
3	2	20	116
4†	1 + MER/29	55	147
1	2	63	155
	High fat diet		
7a	1 + lard	9.2	185
7b	1 + corn oil	18	198
2	3	44	224
5a	3	32	221
5b	3‡	33	342

* The carbohydrate/protein/fat ratios of the diets were as follows: 1–24/38/38 %; 2–25/51/24; and 3–20/10/70.

† MER/29 was given orally in doses of about 250 mg every other day.

‡ Corn oil was substituted for lard.

injected with about 7 μc of 4-C^{14}-cholesterol. The dogs were studied while on high or low fat diets. Table II includes the data for diets ingested, level of plasma cholesterol (average during the study), and value of the slowest exponential decline (b_1) of the plasma C^{14}-cholesterol (Fig. 3).

Assuming that D/C_1 is a reflection of the exchangeable cholesterol pool (ECP), it was calculated that 2 dogs on a low fat diet (dogs 1 and 3, with average plasma cholesterol levels of 155 and 116 mg/100 ml) had exchangeable cholesterol pools of 4.3 and 2.2 g per kilogram of body weight. One of the dogs (dog 4) on a low fat diet plus added MER/29 (triparanol) was calculated to have an ECP of 4.1 g per kilogram (serum cholesterol, 147 mg/100 ml). On the other hand, 2 dogs on a high fat diet were found to have exchangeable cholesterol pools of 4.2 and 6.8 g per kilogram of weight (dogs 2 and 5, with serum cholesterol levels averaging 224 and 221 mg per 100 ml, respectively). Roughly, the higher the level of plasma cholesterol, the higher the ECP.

Since another method of elevating plasma cholesterol is to ablate the thyroid gland, this was done surgically in dog 1. The level of plasma cholesterol rose to an average of 583 mg per 100 ml from a preoperative average of 155 mg per 100 ml. The entire curve for specific activity of plasmatic C^{14} was depressed when the dog was myxedematous, in agreement with the concept of an expanding ECP (Fig. 4).

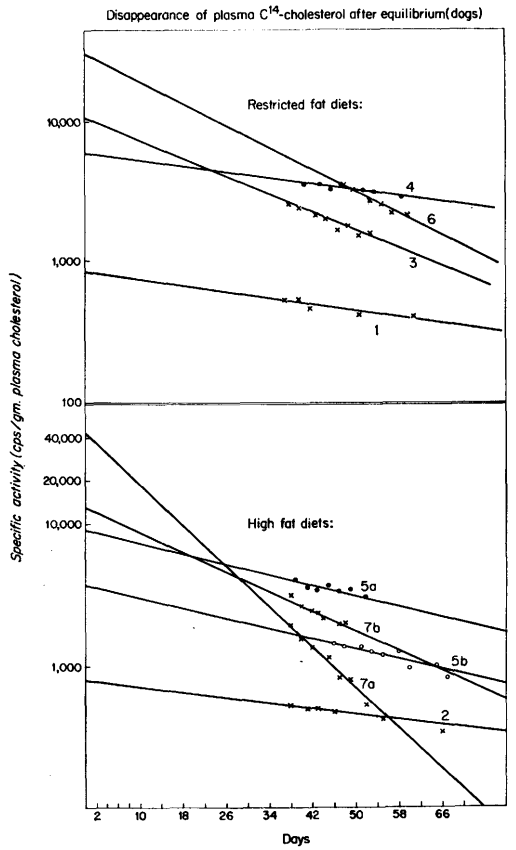

FIG. 3. Final slopes (b_1) of disappearance curves of C^{14} from plasma of dogs on restricted and on high fat diets (Table II).

Comment. These preliminary observations are suggestive of trends in the relationship of plasma cholesterol level, disappearance rate of C^{14}-cholesterol from the blood, exchangeable cholesterol pool, and excretory rate constant. The assumptions involved in the calculations are, at least in part, subject to experimental analysis: a more extensive comparison of disappearance rate (b_1) with rate constant (k_{12}), and an analysis of tissue C^{14} as a check on the assumed 4-compartment system. A direct measure of V_2 and of k_{12}, perhaps by assay of fecal radioactivity, if this is the excretory pathway, might lend validity to the mathematical analysis.

Summary. A simplified technic is offered

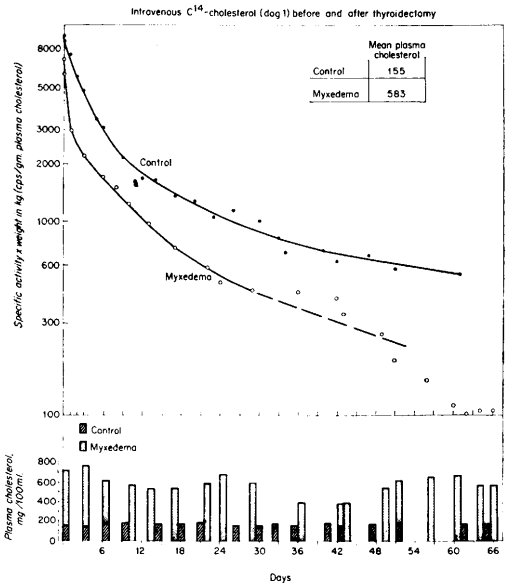

FIG. 4. Disappearance of C^{14} from plasma of dog 1 before and after thyroidectomy.

for measuring plasma cholesterol-C^{14} after intravenous injection of this sterol; it consists of liquid scintillation counting of an alcohol-acetone extract of the plasma. The distribution of cholesterol-4-C^{14} in 2 normal dogs was calculated from a 60- to 70-day study of plasmatic radioactivity, assuming a 4-compartment mammillary system. Excretory rate constants of 1.5 and 1.6% per day of the retained dose were derived. Preliminary estimates of the "exchangeable cholesterol pool" were made. The size of this pool appeared to increase as serum cholesterol rose, either as the result of a high fat diet or of thyroidectomy.

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Identification of Toxinogenic *C. diphtheriae* with Fluorescent Antitoxin: Demonstration of Its Nonspecificity.* (27990)

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Two recent reports have described the use of the immunofluorescence technic for identification of toxinogenic strains of *Corynebacterium diphtheriae*(1,2). These published procedures involve staining of diphtheria organisms with fluorescein-conjugated, commercially available diphtheria antitoxin. While working with this technic in our laboratory certain findings appeared which raised questions as to its specificity. These findings form the basis of this report.

Methods. Bacteria studied. Eleven culture isolates of *Corynebacterium diphtheriae* consisting of gravis, mitis and intermedius types, and one culture isolate of *Corynebacterium xerosis* were obtained from the Bacteriological Laboratories, Maryland State Department of Health, and from the American Type Culture Collection. Two culture isolates of diphtheroids and single culture iso-

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