# Regulation of Cholesterol Metabolism in the Dog

## I. EFFECTS OF COMPLETE BILE DIVERSION AND OF CHOLESTEROL FEEDING ON ABSORPTION, SYNTHESIS, ACCUMULATION, AND EXCRETION RATES MEASURED DURING LIFE

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ABSTRACT Six adult pedigreed dogs were studied as long as 3 yr in order to determine the effects of cholesterol feeding and of bile diversion on absorption, synthesis, and storage of cholesterol. These measurements were based on cholesterol balance and isotope kinetic studies.

In the six dogs fed a "cholesterol-free" diet with their enterohepatic circulations undisturbed, the rate of cholesterol synthesis ranged from 225 to 508 mg/day (mean 325 mg/day or 12.4 mg/kg/day). In two dogs studied subsequently on cholesterol-containing diets, absorption of cholesterol averaged 81% (range 71–94%) on a dietary intake of 0.5 g/day; on high cholesterol intakes (2.7–3.0 g/day) the rate of absorption dropped to 43–51% of daily intake, but the absolute amounts absorbed were increased. Feeding of cholesterol resulted in acceleration of bile acid formation and excretion, as well as nearly total inhibition of cholesterol synthesis. These two compensatory mechanisms were sufficient to maintain zero balance of cholesterol in the face of a high cholesterol intake. Plasma cholesterol concentrations in the two dogs increased by 37 and 44%.

In two other dogs bile was completely diverted into the urinary system for nearly 2 yr. When these dogs were studied on a cholesterol-free diet, the sum of acidic steroids excreted daily in urine plus neutral steroids excreted in feces was seven times as high as before the operation. Since these massive excretory losses could not have been sustained by mobilization from tissue cholesterol pools, and since the rate of disappearance of [4-$^{14}$C]cholesterol from the plasma after single intravenous injection was greatly increased after complete biliary diversion, total daily synthesis of cholesterol must have been greatly accelerated, for synthesis was totally unsuppressed. The increased output of fecal neutral steroids could be the result of transfer of plasma cholesterol across the gut wall or due to increased synthesis in the gut. Plasma cholesterol levels were reduced in these two dogs by 20 and 27%, and triglycerides decreased by 36 and 43%.

Accumulation of cholesterol in body pools in the cholesterol-fed dogs appeared to have been prevented, according to antemortem measurements: increased absorption of dietary cholesterol was exactly balanced by suppression of cholesterol synthesis and enhanced bile acid excretion. In the bile-shunted animals, depletion of tissue stores of cholesterol could not be predicted by antemortem measurements.

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## INTRODUCTION

Surgical exclusion of the ileum (ileal bypass) or feeding of cholestyramine results in partial interruption of the enterohepatic circulation; both procedures primarily affect the ileo-portal return of bile acids and to a lesser extent the entero-lymphatic absorption of cholesterol (1, 2). Complete diversion of bile, on the other hand, totally abolishes the enterohepatic circulation of bile acids and cholesterol.

The present experiments were designed to measure the degree to which cholesterol synthesis could be maxi-

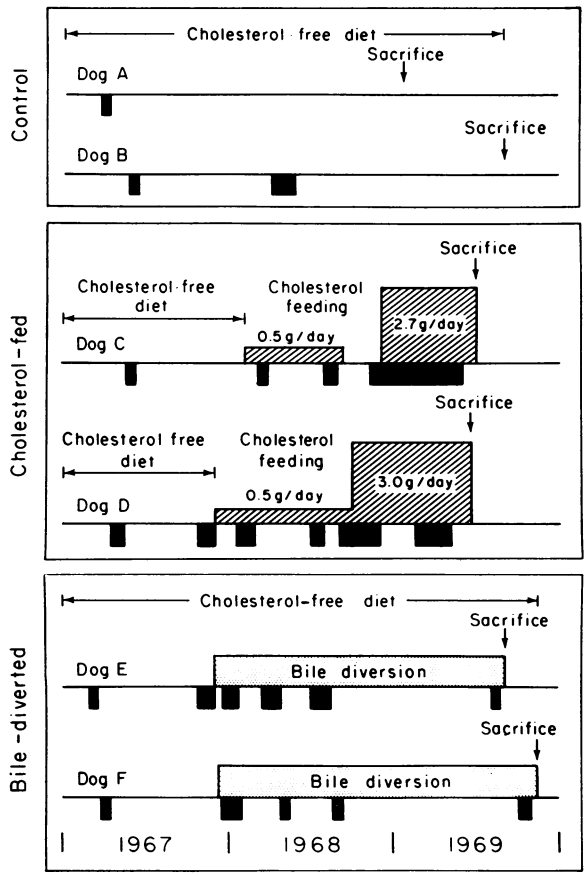


FIGURE 1 Diagrammatic outline of the experimental plan. The three groups of dogs (control, cholesterol-fed, and bile-diverted) were studied for 3 yr while on cholesterol-free or cholesterol-containing diets. The filled boxes below the line indicate the number and length of sterol balance studies for each animal.

mized in dogs by total interruption of the enterohepatic cycle. We also tested whether total exclusion of biliary bile acids and cholesterol from the enterohepatic circulation in dogs might result in depletion of sterols from tissue pools, while in a parallel study we attempted to swell these cholesterol pools by cholesterol feeding. Studies by Taylor and Gould on cholesterol-fed dogs (3) suggested an almost complete suppression of synthesis. Nevertheless, we questioned whether tissue cholesterol levels might increase despite such suppression because of the efficient absorptive capacity for cholesterol known to exist in dogs (4). Control dogs were maintained under similar conditions but without any intervention during the 3-yr study.

Our results showed that cholesterol feeding caused virtually complete cessation of cholesterol synthesis in the dog and that complete biliary diversion caused a sevenfold increase in cholesterol synthesis. Although there was a significant increase in plasma cholesterol concentrations in the cholesterol-fed dogs, it could be calculated that there were no significant differences between these dogs and the controls in total exchangeable cholesterol content; these calculations were confirmed at autopsy (see accompanying report). In the bile-diverted dogs plasma cholesterol concentrations decreased significantly. The magnitude of depletion of tissue cholesterol stores could not be calculated in vivo; however, autopsy data showed that tissue pools of cholesterol did not differ from those in the control and cholesterol-fed animals.

## METHODS

*Animals.* Six adult female dogs, two sets of three littermates from a cross-breed between Rhodesian Ridgebacks and German Shepherds, were studied over a period of 3 yr. One set was 20 and the other 15 mo old at the beginning of the study. One dog from each litter was designated control, cholesterol-fed, or bile-diverted; their respective weights were 31 and 27 kg, 27 and 22 kg, 28 and 23 kg. All animals were vacccinated for distemper, hepatitis, and leptospirosis upon arrival at The Rockefeller University; they were free of intestinal parasites. Individual indoor pens (3.25 $m^2$ surface) with outdoor runs (4.2 $m^2$ surface) were used for housing the dogs. Periodic balance studies lasting from 3 wk to 3 mo each were carried out in individual metabolism cages that permitted separate collection of urine and feces. These cages were designed to provide sufficient space (1 $m^3$ capacity) for the dogs' comfort; the metal cage floors were coated with Teflon to protect the paws of the animals from injury.

*Experimental plan (Fig. 1).* The control dogs A and B received a "cholesterol-free" diet throughout the experiment, and each animal was subjected to two metabolic balance studies before sacrifice. Dog A differed from dog B in that, after the first control study, an attempt was made in dog A to create a bilio-renal shunt; since this was technically unsatisfactory, the intended shunt was not created, but the gallbladder was anastomosed to the duodenum. Thus, both dogs A and B had completely intact enterohepatic circulations of bile acids and neutral steroids for the duration of the studies; however, in dog A the right kidney had been removed, and, since the common bile duct had been ligated, all bile passed from the liver to the duodenum via the cholecystoduodenostomy.

The cholesterol-fed animals C and D underwent balance studies in each of three periods (cholesterol-free, moderate- and high-cholesterol intakes). The high-cholesterol diet was labeled; during its administration, for 7–9 mo until the time of death, the animals received a constant daily oral intake of radioactive cholesterol.

Dogs E and F received the cholesterol-free diet throughout the entire experiment; they were subjected to complete bile diversion by means of a cholecystonephrostomy after a control period of 1 yr. Balance studies were carried out before and after bile diversion.

*General clinical status.* All animals remained in good health throughout the study. The high-carbohydrate content of the synthetic diet caused no dental problems. Body weight fluctuations averaged only 0.86–1.10 kg, or 3.1–3.9% of body weight, throughout the study; variations were minimized by periodic adjustment of daily caloric intakes.

TABLE 1

*Cholesterol Balance Data in Control Dogs A and B*

| | Dog A* Study 1 | Dog B Study 1 | Dog B Study 2 |
|---|---|---|---|
| Cholesterol intake, *mg/day* | 20 | 15 | 14 |
| Days of study in metabolic cage | 20 | 20 | 44 |
| No. of consecutive stool collections analyzed | 20 | 10 | 11 |
| Food intake, *g/day* | 618 | 459 | 425 |
| Daily caloric intake | 1,916 | 1,423 | 1,318 |
| Weight, *kg* | | | |
| Beginning | 31.5 | 26.0 | 26.5 |
| End | 32.5 | 25.5 | 26.5 |
| Plasma cholesterol, *mg/100 ml*±*SD*(*n*) | 251±13 (4) | 218±23 (3) | 210±16 (14) |
| Fecal neutral steroids, *mg/day*±*SD* | 320±80 | 157±50 | 118±14 |
| *mg/kg/day* | 10.0 | 6.0 | 4.5 |
| Fecal acidic steroids, *mg/day*±*SD* | 208±96 | 100±53 | 121±27 |
| *mg/kg/day* | 6.5 | 3.8 | 4.6 |
| Total fecal steroids, *mg/day* | 528 | 257 | 239 |
| *mg/kg/day* | 16.5 | 9.8 | 9.1 |
| Cholesterol balance (intake minus excretion), *mg/day* | −508 | −242 | −225 |
| *mg/kg/day* | −15.9 | −9.4 | −8.5 |

* A second study was carried out 10 wk after an unsuccessful attempt to perform a cholecystonephrostomy; in this 52-day study plasma cholesterol levels did not change, but fecal neutral steroids fell to 189 mg/day and fecal bile acids to 89 mg/day. The lower values of fecal steroid excretion may have been due to some peculiarity in gall bladder function (see Methods section, *Experimental plan*).

The caloric requirement of the dogs, when running freely, varied from 50 to 90 cal/kg body weight/day. During confinement to metabolism cages with its inevitable restriction of physical activity, caloric requirement fell by 3–11 cal/kg/day or 4–15% compared to the unrestricted state. An initial small increment in body weight during cage confinement was commonly seen in spite of caloric restriction.

*Diets.* Synthetic diets in pellet form were prepared by us every 2 wk in 50-kg batches; the food pellets showed no tendency to disintegrate. The dogs were trained to accept this diet over a period of 4 wk before the onset of the first balance studies. Each morning a weighed quantity of food was offered to each dog, and the intake was recorded; three of the animals ingested their ration within 1 h, and with few exceptions all food was consumed by the end of the day. Food wastage was minimal, as evidenced by the recovery in feces of the inert marker chromic oxide. Coprophagy and fur-licking were not observed.

The composition of the cholesterol-free diet was as follows (g/1,000 g, together with caloric composition): vitamin-free casein 175 (700 cal, 22.6% of calories as protein); dextrin 219 plus cerelose 307 (876 and 1,120 cal, giving 64.7% of calories as carbohydrates); corn oil 44 (396 cal, 12.8% of calories as fat); cellulose 88; salt mixture (USP XIV) 44; vitamin mixture ("Vitamin fortification mixture," General Biochemicals Div., Mogul Corp., Chagrin Falls, Ohio) 9; chromic oxide 1; water 113. The pelleted diet provided 3.1 cal/g; it was low in fat in order to minimize losses due to steatorrhea in the bile-diverted dogs. The corn oil used in these diets had been subjected to molecular distillation (by Distillation Products Industries, Div. of Eastman Kodak Co., Rochester, N. Y.) and steam deodorization (by Drew Chemical Corp., New York); these processes reduced the plant sterol content without impairing the palatability of the corn oil. Thus, the plant sterol content of the diet was derived entirely from sterols inherent in corn oil; each gram of diet contained β-sitosterol 289 μg, stigmasterol 17 μg, and campesterol 58 μg. Cholesterol, 33 μg/g diet, was derived entirely from casein.

Diets containing moderate and large amounts of cholesterol were prepared by adding crystalline cholesterol with a purity of 99% (Mann Research Labs, Inc., New York) in a homogenous aqueous dispersion to the mixed ingredients of the cholesterol-free diet before the pelleting process. Periodic analysis of the sterol content of these diets showed a consistent content of cholesterol with a concentration of 1.0 mg/g diet (322 mg/1,000 cal) and 6.0 mg/g diet (1,930 mg/1,000 cal) in the moderate- and high-cholesterol diets, respectively. The high-cholesterol diet contained [1,2-$^3$H]-cholesterol with a specific activity of 1190±103 dpm/mg ($n = 19$).

Fat-soluble vitamins (A, $D_2$, E, and K) were administered intramuscularly at weekly intervals to the two dogs subjected to biliary diversion. Each dose consisted of Vitamin A and $D_2$, 50,000 and 10,000 IU, respectively, and Vitamin E and K, 5 mg each. This was deemed necessary to prevent fat-soluble vitamin deficiency secondary to total absence of bile acids from the intestinal lumen. Parenteral vitamin supplements were not given to the control and cholesterol-fed dogs.

*Radioactive sterols.* [4-$^{14}$C]- and [1,2-$^3$H]cholesterols used in these experiments were purchased from New England Nuclear, Boston, Mass. The radiopurity of these compounds was determined by thin-layer chromatography on Florisil plates (Florisil Analytical Absorbents, Floridin Co., Tallahassee, Fla.) in diethyl ether-heptane (45:55, vol/vol); about 4% of radioactivity remained at the origin in all instances. Only the fraction of radiosterol with the same

TABLE II

*Cholesterol Balance Data on Low, Moderate, and*

| | Dog C | Dog C | | | |
|---|---|---|---|---|---|
| | Study 1 (low) | Study 2 (moderate) | Study 3 (moderate) | Study 4 (low)* | Study 5 (high) |
| Cholesterol intake, *mg/day* | 20 | 483 | 508 | 17 | 2,700 |
| Days after starting cholesterol diet | — | 28 | 175 | — | 1 |
| Days of study in metabolic cage | 20 | 20 | 28 | 20 | 152 |
| No. of consecutive stool collections analyzed | 10 | 5 | 7 | 3 | 15 |
| Food intake, *g/day* | 600 | 483 | 508 | 550 | 450 |
| Daily caloric intake | 1,860 | 1,497 | 1,575 | 1,705 | 1,395 |
| Weight, *kg* | | | | | |
| Beginning | 27.0 | 27.0 | 27.0 | 27.0 | 27.5 |
| End | 28.0 | 27.5 | 26.5 | 27.5 | 28.5 |
| Plasma cholesterol, *mg/100 ml*±*SD* (*n*) | 184±6 (3) | 203±26 (8) | 218±9 (9) | 292±17 (9) | 328±26 (76) |
| Fecal steroids, *mg/day*±*SD* | | | | | |
| Total neutral steroids | 220±39 | 213±21 | 254±54 | 236±44 | 1,588±180 |
| Endogenous neutral steroids | 220±39 | 160±18 | 106±12 | 236±44 | 215±49 |
| Unabsorbed dietary cholesterol | — | 53±5 | 149±57 | — | 1,392±167 |
| Absorbed dietary cholesterol | — | 430±34 | 359±96 | — | 1,308±119 |
| Acidic steroids | 146±61 | 243±71 | 416±126 | 306±87 | 1,204±146 |
| Total steroids (neutral and acidic) | 366 | 456 | 670 | 542 | 2,792 |
| Absorption, % *dietary cholesterol* | — | 89.0 | 70.1 | — | 48.5 |
| Cholesterol balance | −346 | +28 | −162 | −525 | −92 |
| (intake minus excretion), *mg/kg/day* | −12.6 | +1.0 | −6.1 | −19.3 | −3.3 |

* The elevated plasma cholesterol levels, increased fecal acidic steroid excretion, and more negative cholesterol balance in study 4 in dog C may have been due to the fact that it had consumed the cholesterol-free diet for only 7 wk before that study; for the previous 7 mo its diet contained 508 mg cholesterol/day.

$R_f$ value as a pure cholesterol standard was administered to the animals. [4-$^{14}$C]cholesterol was given intravenously (50–100 $\mu$Ci) as a single injection at the onset of each of 28 balance studies; the tracer, dissolved in 5 ml of ethanol, was dispersed in 150 ml of physiologic saline and infused over a period of 30–45 min. The infusion system was rinsed three times with about 30 ml of saline to minimize absorptive losses of radioactive tracer on the glassware and tubing. The extent of losses of radioactive cholesterol on the glassware was not measured systematically but on the basis of extensive other experience in man and in dogs was assumed to be 5%.

Except for transient mild salivation and irritability, no adverse effects were observed during the intravenous administration of these small amounts of ethanol. Specific activities of plasma total cholesterol were determined two or three times per week thereafter.

*Plasma lipids, blood chemistries and hematologic studies.* Blood was drawn after overnight starvation into tubes containing disodium ethylene-diaminetetra-acetic acid as an anticoagulant (about 1 mg/ml blood), and the separated plasma was stored at 4°C as long as 1 wk before analysis. Plasma cholesterol was measured by the method of Block, Jarrett, and Levine (5), and triglycerides were assessed by the method of Kessler and Lederer (6) on an AutoAnalyzer, model I (Technicon Instruments Corp., Tarrytown, N. Y.). A portion of the same plasma extract was used for the assay of radioactivity in a Packard Tri-Carb Scintillation Counter (Model 3003, Packard Instrument Co., Inc., Downers Grove, Ill.) as described previously (7). The qualitative lipoprotein pattern in the dogs' plasma was studied by paper-strip electrophoresis according to Lees and Hatch (8).

Serum electrolytes (including calcium and phosphorus), proteins, urea nitrogen, and glucose were measured monthly in all dogs in order to monitor the effects of long-term feeding of the synthetic diets and of bile diversion on their concentrations. Hemoglobin concentration, hematocrit, and white cell and platelet counts also were determined monthly. In both bile-shunted dogs, the hematologic indexes and levels of all serum electrolytes including calcium and phosphorus remained unchanged and were similar to those of control and cholesterol-fed dogs. There were no inflammatory complications and no stigmata of fat-soluble vitamin deficiency, although steatorrhea occurred in both dogs; in random stool samples the fecal fat losses varied from 65–85% on fat intakes of about 25 g/day. Serum bilirubin and alkaline phosphatase were measured monthly as indicators of cholestasis.

Except during the immediate postoperative period in dog F, there was no chemical evidence of jaundice in the bile-diverted dogs, and the postoperative levels of serum bilirubin did not differ from those before the shunting procedure. However, the serum alkaline phosphatase remained significantly elevated throughout the study in dog F (mean of 185 mIU/ml serum), and minimal elevation of this enzyme (mean of 97 mIU/ml) was observed in dog E; the control values in the six dogs were 33–79 mIU/ml. Since the level of 5'-nucleotidase in the serum of both dogs remained in the normal range, it is possible that a

*High Intake of Cholesterol in Dogs C and D*

| Dog D | | | | | | |
|---|---|---|---|---|---|---|
| Study 1 (low) | Study 2 (low) | Study 3 (moderate) | Study 4 (moderate) | Study 5 (moderate) | Study 6 (high) | Study 7 (high) |
| 20 | 16 | 500 | 500 | 500 | 3,000 | 3,000 |
| — | — | 62 | 214 | 283 | 307 | 487 |
| 26 | 32 | 36 | 24 | 24 | 56 | 40 |
| 13 | 8 | 6 | 6 | 3 | 7 | 5 |
| 600 | 474 | 500 | 500 | 500 | 500 | 500 |
| 1,860 | 1,470 | 1,550 | 1,550 | 1,550 | 1,550 | 1,550 |
| 21.0 | 23.5 | 23.5 | 22.5 | 23.5 | 23.5 | 22.0 |
| 22.0 | 24.0 | 23.5 | 23.5 | 23.5 | 24.5 | 23.0 |
| 154±12 (5) | 165±22 (10) | 214±14 (5) | 183±9 (8) | 181±6 (8) | 208±14 (21) | 253±30 (11) |
| 166±17 | 141±17 | 159±16 | 245±44 | 263±43 | 1,946±210 | 1,608±52 |
| 166±17 | 141±17 | 129±13 | 135±5 | 136±7 | 220±54 | 138±14 |
| — | — | 30±3 | 110±40 | 127±36 | 1,726±191 | 1,469±44 |
| — | — | 470±3 | 390±40 | 373±36 | 1,274±191 | 1,531±44 |
| 188±105 | 175±54 | 421±50 | 438±40 | 616±66 | 1,076±172 | 1,198±94 |
| 354 | 315 | 579 | 683 | 879 | 3,022 | 2,805±124 |
| — | — | 94.0 | 78.0 | 74.6 | 42.5 | 51.0 |
| −334 | −299 | −79 | −183 | −379 | −22 | +195 |
| −15.5 | −12.6 | −3.4 | −8.0 | −16.1 | −0.9 | +8.3 |

significant fraction of the elevated serum alkaline phosphatase originated in bone.

*Operative procedure for biliary diversion.* Complete bile diversion for total interruption of the enterohepatic circulation was achieved by shunting all bile into the urinary system. After resection and ligation of the distal choledochus, the fundus of the gallbladder was anastomosed to the pelvis of the right kidney as described by Kapsinow, Engle, and Harvey (9). The blood flow to the kidney was interrupted for about 30–45 min during performance of cholecystonephrostomy. The operations were carried out under halothane endotracheal anesthesia using aseptic technique.

*Fecal and urinary steroids.* The metabolism cages were designed to permit separate collection of feces and urine. During balance studies in the bile-diverted dogs, the spontaneously voided urine (actually a mixture of urine and bile) was always collected under ice in order to minimize degradative changes in the steroids.

Acidic and neutral steroids in urine and feces were isolated separately from 2- to 12-day pools by thin-layer and gas-liquid chromatographic procedures worked out in this laboratory (7, 10) that permit a separate quantitation of neutral and acidic steroids and their specific activities, as well as the essential distinction between plant sterols and cholesterol and their respective bacterial conversion products. β-sitosterol was used as an internal standard to correct for losses of cholesterol during intestinal transit as well as for variations in fecal flow (11, 12). Chromic oxide was employed as an internal standard to correct the excretion data for the bile acids for variations in fecal flow (13); it served also as a marker for completeness of recovery of feces.

In our analyses of a total of 259 specimens, stool recoveries averaged 91% (range 72–107%) as judged by the recovery of chromic oxide; β-sitosterol recovery averaged 88% (range 68–101%). The close approximation of the recovery figures for the two markers implies that there was no significant degradation of β-sitosterol and cholesterol during intestinal transit (11, 12). In most individual studies, the difference in recovery between chromic oxide and β-sitosterol was less than 10%. No internal standard was available for correction of losses of urine in the bile-shunted animals; copious amounts of distilled water were used to rinse the urine pan.

The daily excretion of bile acids in the urine during control periods was estimated isotopically in all six dogs. Since no mass of urinary bile acids was detectable by the chemical isolation procedures described above, a maximum estimate was obtained by dividing the daily urinary excretion of radioactivity (dpm) by the plasma cholesterol specific radioactivity (dpm/mg). This is essentially the "isotopic balance method" (reference 14, Eq. 5); it will overestimate "urinary bile acids," since the total urinary radioactivity is ascribable both to neutral and acidic steroids.

*Measurement of cholesterol synthesis, turnover, and pool size.* In the control and cholesterol-fed dogs, daily cholesterol synthesis was calculated from sterol balance data as described earlier (Eq. 8–13, reference 14). In the bile-diverted dogs, values for daily cholesterol synthesis were derived from the following formula:

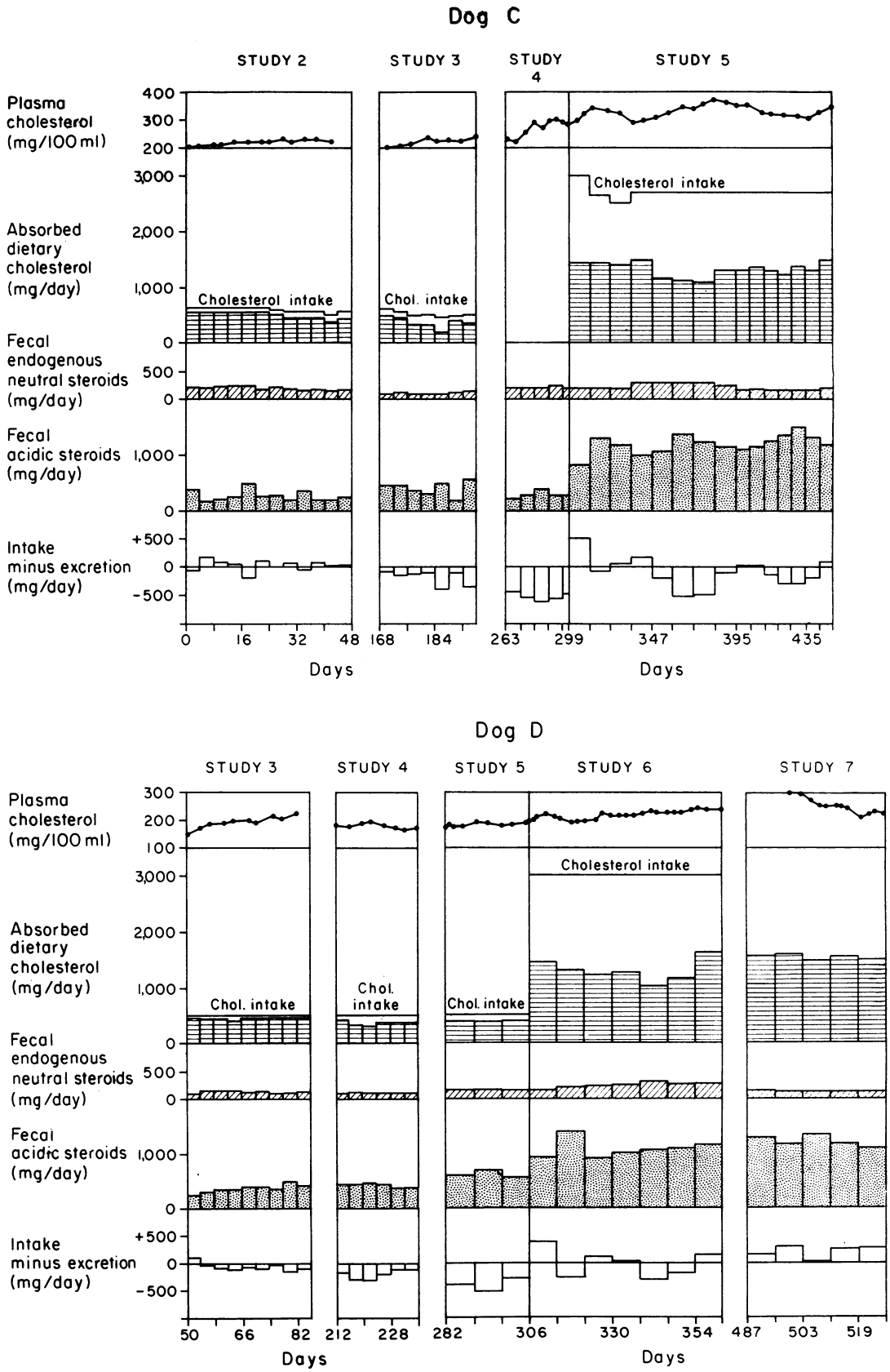
Dog C
STUDY 2
STUDY 3
STUDY 4
STUDY 5
Plasma cholesterol (mg/100 ml)
400
300
200
3,000
2,000
1,000
0
500
0
1,000
0
+500
0
-500
Absorbed dietary cholesterol (mg/day)
Cholesterol intake
Chol. intake
Cholesterol intake
Fecal endogenous neutral steroids (mg/day)
Fecal acidic steroids (mg/day)
Intake minus excretion (mg/day)
0
16
32
48
168
184
263
299
347
395
435
Days
Days
Dog D
STUDY 3
STUDY 4
STUDY 5
STUDY 6
STUDY 7
Plasma cholesterol (mg/100 ml)
300
200
100
3,000
2,000
1,000
0
500
0
1,000
0
+500
0
-500
Absorbed dietary cholesterol (mg/day)
Chol. intake
Chol. intake
Chol. intake
Cholesterol intake
Fecal endogenous neutral steroids (mg/day)
Fecal acidic steroids (mg/day)
Intake minus excretion (mg/day)
50
66
82
212
228
282
306
330
354
487
503
519
Days
Days

TABLE III

*Net Accumulation of Cholesterol in Tissue Pools*

| Dog | Cholesterol intake | Days of cholesterol intake | Total cholesterol intake | Total absorption of dietary cholesterol | Increment in total fecal steroids* | Total decrement in cholesterol synthesis | Net accumulation of cholesterol | Accumulation | Increment in plasma cholesterol‡ |
|---|---|---|---|---|---|---|---|---|---|
| | *g/day* | | *g* | *g* | *g* | *g* | *g* | *mg/day* | *g* |
| C | 0.48§ | 20 | 9.6 | 8.6 | 0.7 | 7.5 | 0.4 | 19 | 0.21 |
| | 0.51§ | 28 | 14.3 | 10.1 | 4.4 | 5.2 | 0.5 | 19 | 0.36 |
| | 2.70‖ | 152 | 410 | 198.8 | 133.3 | 62.9 | 2.6 | 17 | 1.90 |
| D | 0.50§ | 24 | 12 | 11.3 | 5.1 | 5.7 | 0.4 | 18 | 0.57 |
| | 0.50§ | 24 | 12 | 9.4 | 5.7 | 3.2 | 0.4 | 18 | 0.19 |
| | 0.50§ | 24 | 12 | 8.9 | 10.0 | −1.5 | 0.4 | 18 | 0.21 |
| | 3.00‖ | 56 | 168 | 71.3 | 53.8 | 16.6 | 1.0 | 18 | 0.53 |
| | 3.00‖ | 40 | 120 | 61.2 | 40.0 | 20.5 | 0.7 | 17 | 0.96 |

* Total fecal steroids = endogenous neutral + acidic steroids.

‡ Plasma volume X (increment in mean plasma cholesterol concentration during moderate and high cholesterol intakes) (Tables II, III). Plasma volume taken to be 46 ml/kg body weight (18).

§ Periods of moderate cholesterol intake were compared to those on cholesterol-free diet (Table II).

‖ Periods of high cholesterol intake compared to those on cholesterol-free diet (Table II).

Daily cholesterol synthesis (mg/day) = (daily excretion of fecal neutral steroids, corrected for neutral steroid losses and for variations in fecal flow, [mg/day] + urinary acidic steroids [mg/day] + urinary neutral steroids [mg/day]) − daily cholesterol intake (mg/day).

Daily cholesterol turnover rates and pool sizes of exchangeable cholesterol were obtained by input-output analysis (15) of specific activity-time-course curves of plasma cholesterol measured after a single intravenous dose of [4-$^{14}$C]cholesterol, as described above.

*Measurement of cholesterol absorption.* Absorption measurements could be made appropriately only in the cholesterol-fed dogs C and D. Absorption was calculated as the difference between dietary intake and unabsorbed dietary neutral steroids in feces, as previously described (Eq. 11, reference 14, method I). Values for unabsorbed dietary neutral steroids were derived as the difference between total fecal neutral steroids (measured by chromatographic methods) and fecal neutral steroids of endogenous origin (determined from isotopic data for plasma cholesterol after single intravenous labeling with radioactive cholesterol) (Eq. 10, reference 14).

## RESULTS

*Plasma lipids.* Plasma cholesterol levels during control and experimental periods in the six dogs are discussed below in relation to other measurements of cholesterol metabolism. No changes in plasma lipoproteins were noted by paper-strip electrophoresis when cholesterol was fed in large amounts or during total bile diversion: the lipoproteins migrated almost exclusively at the same rate as human α-lipoproteins.

Plasma triglycerides in dog A were: mean = 75.2 ±SD 22.6 ($n = 93$) in mg/100 ml plasma and in dog B 78.2±32.1 ($n = 135$). In the cholesterol-fed dogs C and D, triglyceride levels were 65.1±23.6 ($n = 50$) and 69.6±21.6 ($n = 55$), respectively, on the low-cholesterol diet; with feeding of moderate and large amounts of cholesterol, the triglyceride concentrations did not change significantly, 59.3±22.0 ($n = 84$) and 63.8±26.4 ($n = 76$). In dogs E and F, a highly significant reduction in plasma triglycerides was observed after bile diversion. In dog E, triglycerides were 78.5 ±21.3 ($n = 50$) before shunting of bile, and 50.5±19.8 ($n = 85$) after bile diversion, a reduction of 36%. In dog F, there was a fall from 73.8±24.6 ($n = 49$) to 42.6±17.9 ($n = 97$) after bile diversion, a reduction of 42%

*Control studies (dogs A and B).* Table I gives the cholesterol balance data for the control dogs A and B; the results of the control studies in the cholesterol-fed dogs C and D and in the bile-deprived animals E and

FIGURE 2 Sterol balance data on varying intakes of cholesterol in dogs C and D. Dog C ingested moderate amounts of cholesterol during studies 2 and 3; in study 4 the diet was cholesterol-free, while in study 5 the cholesterol intake was high. Dog D received a moderate-cholesterol diet during studies 3–5, and a diet high in cholesterol during studies 6 and 7. Plasma cholesterol levels increased during moderate- and high-cholesterol feeding in both dogs. Absorption in absolute terms increased substantially during high-cholesterol feeding; an equally striking rise in fecal bile acid excretion was sufficient to maintain zero sterol balance (intake minus excretion).

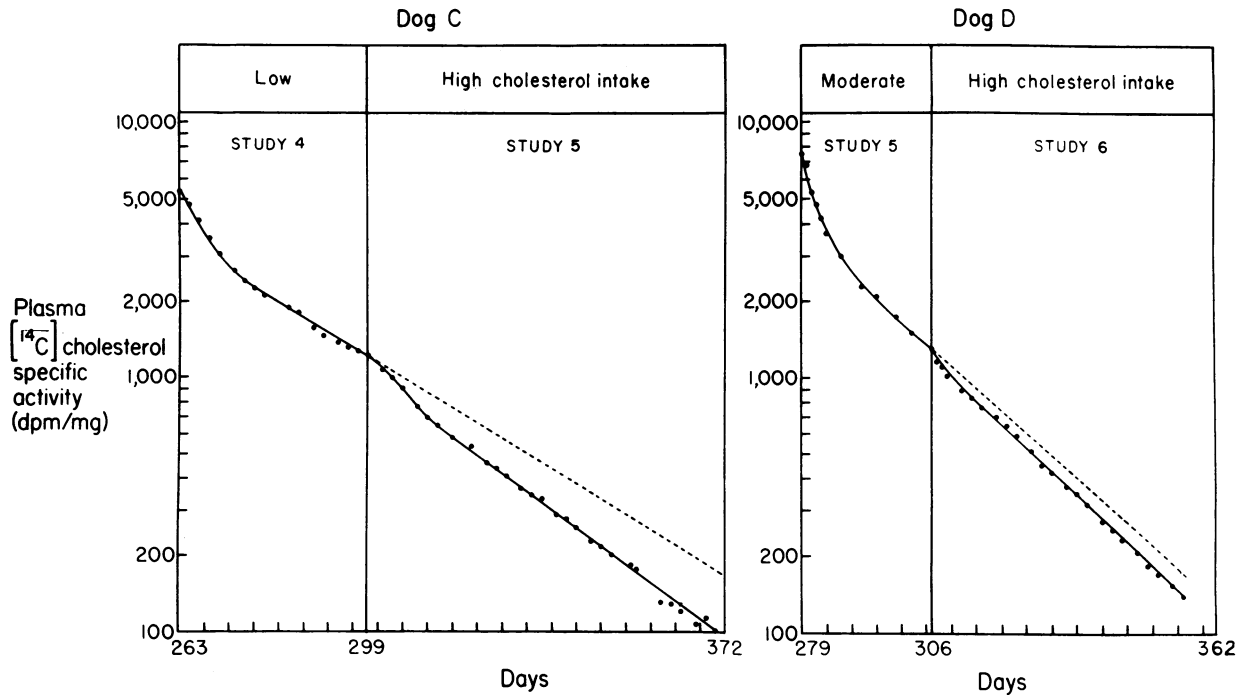


FIGURE 3 Specific activity-time curves for plasma cholesterol after single intravenous injection of [4-$^{14}$C]cholesterol on day 263 to dog C and on day 279 to dog D. In both animals an increase in cholesterol intake caused an immediate and significant acceleration in the rate of decay in specific activity of plasma cholesterol. A change from cholesterol-free to a high-cholesterol diet in dog C caused a more striking deflection of the fall-off curve than the switch from a diet of moderate cholesterol content to a cholesterol-rich diet in dog D. Since cholesterol synthesis was independently shown to be essentially totally suppressed, and since the total amount of exchangeable cholesterol was shown to be unreduced at sacrifice, the accelerated decay in plasma cholesterol specific activity can be considered to be due to increased absorption of dietary cholesterol not labeled with $^{14}$C.

F are shown in Tables II and III. Body weights were remarkably constant. Plasma cholesterol concentrations were significantly different in the two dogs but remained relatively constant in each dog.

The animal-to-animal variability in fecal neutral and acidic steroid excretion was substantial, but this variability was greatly reduced by expressing the results in terms of milligrams per kilogram per day. The ratio of neutral to acidic steroids averaged 1.4 (excluding study 4 of dog C, see footnote to Table II). In nine control studies in the six dogs the daily negative cholesterol balance (intake minus total steroid excretion) ranged from 225 to 508 mg/day, with a mean of 325 mg/day, or 8.5 to 15.9 mg/kg/day with a mean of 12.4 mg/kg/day; these figures represent rates of daily cholesterol synthesis (14).

Thin-layer and gas-liquid chromatographic analyses showed that the endogenous fecal neutral steroids were composed exclusively of cholesterol (except in dog E, which excreted significant amounts of coprostanol).

Daily excretions of urinary bile acids in the six dogs during the control period ranged from 0 to 9.5 mg/day ($n = 109$). (In view of the smallness of this quantity, it was not considered worthwhile to fractionate the urinary steroids into acidic and neutral fractions before counting their radioactivity.)

*Cholesterol feeding experiments (dogs C and D).* Table II and Fig. 2 present the sterol balance data in the cholesterol-fed dogs C and D.

When a moderate intake of cholesterol (500 mg/day) was fed, plasma cholesterol concentrations showed a small but significant rise compared with values during the cholesterol-free period. Absorption of cholesterol was high initially (89 and 94%) but diminished with time (70 and 75%). Excretion of endogenous fecal neutral steroids decreased, while the output of acidic steroids rose progressively as the cholesterol balance became increasingly negative.

With high intakes of cholesterol (2.7–3.0 g/day), further rises in plasma cholesterol levels (Table II and

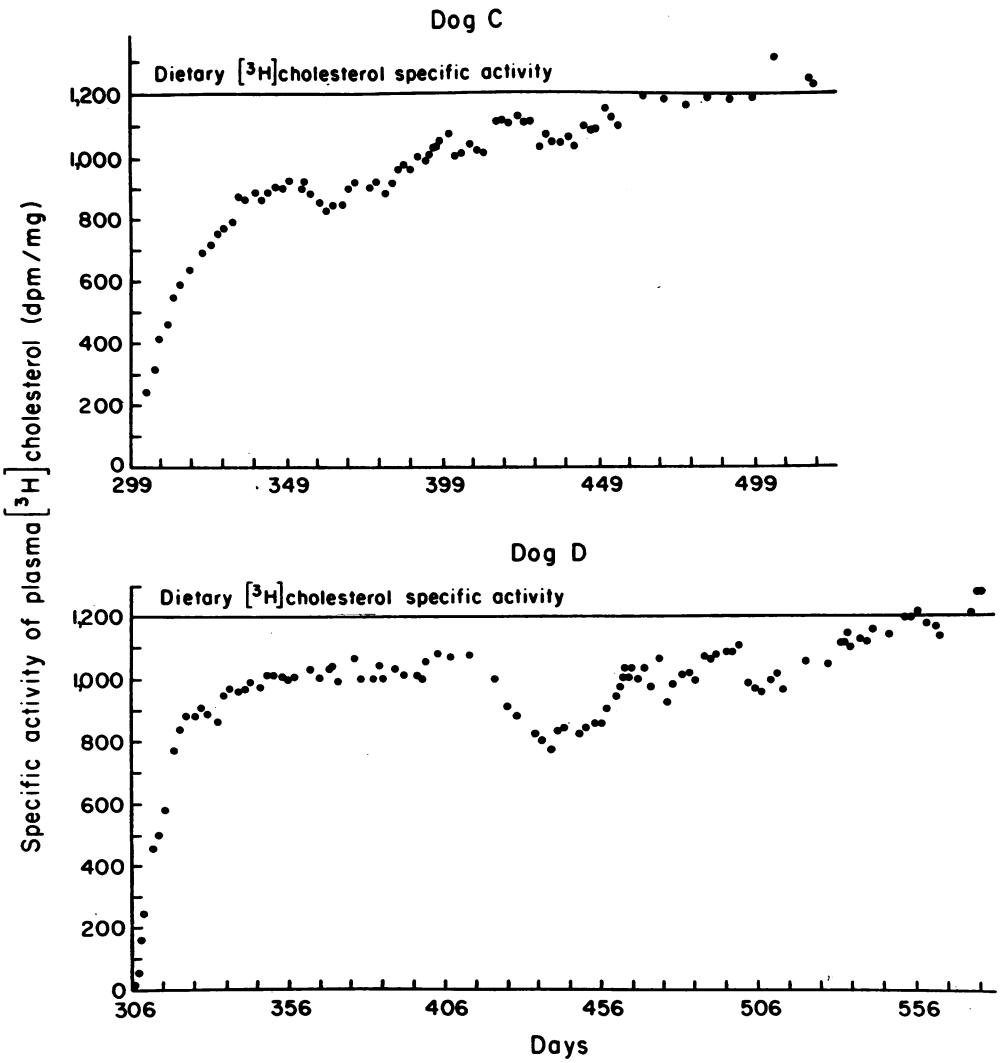


FIGURE 4 Change in plasma [$^3$H]cholesterol with time in dogs C and D fed [$^3$H]cholesterol daily (2.7 g/day and 3.0 g/day, respectively). The ratio of plasma and dietary [$^3$H]cholesterol specific activity eventually reached unity in both dogs; this reflects attainment of the isotopic steady state and derivation of all plasma cholesterol from the diet, i.e., almost total suppression of synthesis. No explanation can be offered for the depression of the curve between day 416 and 466 in dog D.

Fig. 2) were significant in both dogs. Almost half of the dietary cholesterol was absorbed. Fecal bile acid excretion rose substantially, but the increase in excretion of endogenous neutral steroids was variable and small. Thus, the net sterol balance (intake minus excretion) approached zero after an initial brief period (8–12 days), during which cholesterol balance was positive.

After intravenous administration of a single dose of [4-$^{14}$C]cholesterol the semilogarithmic plot of plasma specific activity against time became linear after about 10 days. Fig. 3 illustrates the effects of high-cholesterol feeding on the slopes of the die-away curves: they increased immediately in both cases, the change being greater in dog C. This deflection in slope was most likely due to increased absorption of dietary cholesterol not labeled with $^{14}$C, a conclusion that is supported by the calculations of absorption shown in Table II.

With daily oral feeding of 2.7–3.0 g/day of [$^3$H]-cholesterol, the specific activity of plasma [$^3$H]cholesterol eventually became equal to specific activity of labeled cholesterol in the diet (Fig. 4); convergence of the specific activities of plasma and dietary cholesterol indicated virtually complete repression of cholesterol synthesis. The fecal daily excretion of radioactive

TABLE IV
*Cholesterol Balance Data in Bile-Diverted*

| | Preoperative studies (mean of two studies) | Dog E Postoperative studies 1 | 2 | 3 |
|---|---|---|---|---|
| Period after bile diversion, *mo* | — | ½–1½ | 4–5 | 7½–8½ |
| Days of study in metabolic cage | 45 | 32 | 36 | 37 |
| No. of consecutive stool collections analyzed | 21 | 8 | 10 | 18 |
| Food intake, *g/day* | 600 | 490 | 538 | 478 |
| Daily caloric intake | 1,860 | 1,519 | 1,668 | 1,482 |
| Weight, *kg* | | | | |
| Beginning | 28.5 27.5 | 25.5 | 27 | 27.5 |
| End | 29.5 27.5 | 26 | 27 | 27.5 |
| Cholesterol intake, *mg/day* | 20 | 16 | 18 | 16 |
| Plasma cholesterol, *mg/100 ml*±*SD*(*n*) | 218±23 (13) | 185±7 (23) | 158±13 (7) | 191±26 (12) |
| Urinary steroids | | | | |
| Neutral steroids, *mg/day*±*SD* | 0 | 21±4 | 29±7 | 32.7±10 |
| Total bile acids, *mg/day*±*SD* | 3.1±3.5 (14) | 1,517±85 | 2,107±258 | 1,768±192 |
| *mg/kg/day* | — | 59.0 | 78.0 | 64.3 |
| Fecal steroids | | | | |
| Neutral steroids, *mg/day*±*SD* | 248±50 | 636±56 | 688±38 | 548±99 |
| *mg/kg/day* | 8.6 | 24.7 | 25.5 | 19.9 |
| Acidic steroids, *mg/day*±*SD* | 137±65 | — | — | — |
| *mg/kg/day* | 4.9 | — | — | — |
| Total steroid output, *mg/day* | 385 | 2,174 | 2,824 | 2,343 |
| *mg/kg/day* | 13.6 | 83.6 | 105 | 85.3 |
| Cholesterol balance (intake minus excretion), *mg/day* | −365 | −2,158 | −2,806 | −2,327 |
| *mg/kg/day* | −12.9 | −84.0 | −104 | −84.6 |

cholesterol became equal to the daily intake ($3.21 \times 10^6$ dpm) after 100 days of [$^3$H]cholesterol feeding in dog C (Fig. 5); during the subsequent 50 days (days 399–449 of Fig. 5) the mean daily excretion of [$^3$H]-cholesterol was 99.8% of the daily ingested dose. In dog D, $3.32 \times 10^6 \pm 0.21$ ($n = 7$) dpm or 93% of the daily intake of [$^3$H]cholesterol ($3.57 \times 10^6$ dpm) were recovered in the fecal neutral and acidic steroids each day during a 40-day isotopic balance study (days 470–510 of Fig. 4). By definition (16) the two dogs reached the isotopic steady state before their sacrifice.

Table III presents the estimated net accumulation of cholesterol during moderate- and high-cholesterol feeding in dogs C and D [the equations and rationale of these calculations were described by Quintão, Grundy, and Ahrens (17)]. These data suggested that no substantial accumulation of cholesterol in tissue pools had occurred during cholesterol feeding; repression of endogenous synthesis and increased excretion of bile acids compensated completely for increased absorption of dietary cholesterol, and hence any substantial accumulation of cholesterol in tissue stores appeared to have been prevented.

*Studies with bile diversion (dogs E and F).* Table IV shows a fall of 20 and 27% in plasma cholesterol in the two dogs, as well as a very large excretion of urinary bile acids and a substantial rise in fecal neutral steroid output after biliary shunting.

Urinary acidic steroids consisted exclusively of the two primary bile acids, cholic and chenodeoxycholic acids, with a ratio of 5.5/1 in dog E and 18/1 in dog F. Total absence of deoxycholic acid in both dogs indicated the completeness of bile diversion. The two primary bile acids were conjugated exclusively with taurine, as judged by thin-layer chromatography with silica-gel H in *n*-butanol-acetic acid-$H_2O$ (100:10:10 vol/vol). Urinary neutral steroids represented a very small fraction of the total urinary steroids (1.5%) and were composed mainly of cholesterol but also of a small quantity of cholesterol precursors. The latter were characterized by thin-layer and gas-liquid chromatography, and by mass spectrometry; the latter analyses (courtesy of Dr. Tatu Miettinen, Helsinki) identified lanosterol and dihydrolanosterol as the major components.

Fecal steroids were exclusively neutral; the absence of bile acids confirmed the completeness of interruption

| Dog E | | Dog F | | | |
|---|---|---|---|---|---|
| Postoperative studies | Preoperative study | Postoperative studies | | | |
| 4 | | 1 | 2 | 3 | 4 |
| 20½–21 | — | ½–1½ | 4–5 | 9–10 | 22–23 |
| 15 | 20 | 40 | 17 | 20 | 24 |
| 8 | 10 | 10 | 8 | 6 | 8 |
| 434 | 500 | 381 | 541 | 579 | 502 |
| 1,345 | 1,550 | 1,181 | 1,677 | 1,795 | 1,556 |
| 28.5 | 21 | 20 | 22 | 22 | 22.5 |
| 29 | 22 | 21 | 22.5 | 21.5 | 22.5 |
| 14 | 17 | 13 | 18 | 19 | 17 |
| 162±10 17 | 210±18 (4) | 267±41 (22) | 152±13 (11) | 166±12 (10) | 143±15 (14) |
| — | 0 | 20±6 | — | — | — |
| 1,553±275 | 4.9±3.7 (5) | 1,328±137 | 1,135±278 | 1,940±319 | 1,084±125 |
| 54.1 | — | 64.8 | 50.9 | 89.4 | 48.2 |
| 658±236 | 161±31 | 451±86 | 534±48 | 509±35 | 531±70 |
| 22.9 | 7.5 | 22.0 | 23.9 | 23.5 | 23.6 |
| — | 98±115 | — | — | — | — |
| — | (4.6) | — | — | — | — |
| 2,235 | 259 | 1,799 | 1,669 | 2,449 | 1,615 |
| 77.9 | 12.1 | 87.8 | 75.2 | 113 | 71.8 |
| −2,221 | −242 | −1,886 | −1,651 | −2,430 | −1,598 |
| −77.4 | −11.3 | −92.0 | −74.6 | −112 | −71.0 |

of enterohepatic circulation. In dog E, the neutral steroids were mainly cholesterol, but there were significant amounts of coprostanol and 5$\beta$- analogs of the dietary plant sterols; in dog F, cholesterol was the only endogenous neutral steroid in feces, and there were no 5$\beta$-analogs of the plant sterols. Thus, while the qualitative composition of fecal neutral steroids was not affected by diversion of bile, there was a substantial rise in fecal neutral steroid excretion in both animals (two- to threefold increases over control periods). The total net cholesterol balance became highly negative, suggesting enhanced cholesterol synthesis.

Specific activity-time curves of plasma cholesterol after a single intravenous dose of [4-$^{14}$C]cholesterol showed a much more rapid decline after biliary diversion. The half-life of the pool of labeled plasma cholesterol with slower turnover during a control study in dog E was 26 days; this was reduced to 7 days after biliary diversion. This is consistent with a more rapid dilution of the tracer by newly synthesized cholesterol. The specific radioactivities of fecal neutral steroids in dogs E and F after bile diversion were consistently 15–18% lower than those of plasma cholesterol.

*Cholesterol turnover by two methods.* Table V displays the turnover rates obtained in five of the six dogs by isotope kinetic and sterol balance methods. (In dog E the specific activity data were too few for satisfactory analysis.)

All time-course curves could be fitted with two exponentials; the respective $t_{\frac{1}{2}}$'s are shown in Table V. Note the varying cholesterol intakes in dog D, which explain the different turnover rates; in dog F the high turnover rates were due to the large excretion of urinary bile acids and fecal neutral steroids (Table IV).

There was reasonably good correspondence in the estimates of daily cholesterol turnover by the two methods used, except in dog B, but the data are too few to compare statistically. Previous clinical studies (14) have proven that in larger series of comparisons our estimates of turnover by the two different approaches are within 15% when averages are compared, but in individual patients the discrepancies can be much larger.

## DISCUSSION

The main objectives of these studies were to evaluate the feasibility and precision of long-term sterol balance

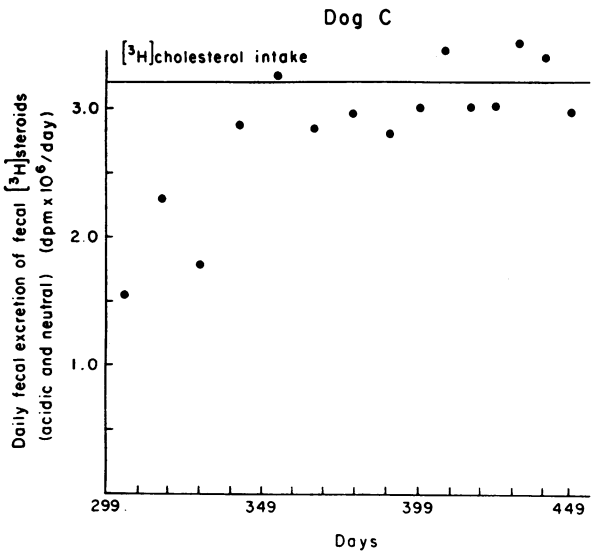


FIGURE 5 Changes in fecal excretion of tritiated cholesterol (neutral plus acidic steroids) with daily feeding of [$^3$H]cholesterol in dog C. Excretion of [$^3$H]cholesterol products became equal to daily intake, in dpm/day, after 100 days of [$^3$H]cholesterol feeding; this isotopic steady state was maintained for the subsequent 50 days (399–449) days in the above figure) before sacrifice.

studies in dogs and to determine the effects of cholesterol feeding and of prolonged bile diversion on synthesis rates, absorption, and storage of cholesterol in the intact animal. Measurements of these principal controlling factors by sterol balance and isotopic techniques were later confirmed by direct assay of total body and tissue cholesterol, as described in the accompanying paper.

*Estimation of synthesis on a cholesterol-free diet.* The rates of daily synthesis we have noted in these six dogs were substantially smaller than those previously observed in short-term studies in dogs by Abell, Mosbach, and Kendall (4) and in long-term isotopic experiments by Taylor and Gould (3). It is worth noting, however, that the levels of daily synthesis we have measured in man, using the same techniques described here (14), are surprisingly similar to those in these six dogs, when expressed per kilogram of body weight. Perhaps the discrepancies between our data and those of the earlier investigators are due to differences in methods as well as experimental design.

*Responses to cholesterol feeding.* The dog appears to absorb more exogenous cholesterol than man. Up to 1.5 g of cholesterol was absorbed per day when the dietary sterol load was increased to 2.7–3.0 g/day; absorption in man did not exceed 1 g/day on similar daily intakes (16, 17). In the experiments of Abell et al. (4) with smaller dogs weighing 2.2 and 4.8 kg, 77% (0.9 g/day) of a cholesterol load of 1,100–1,300 mg/day was absorbed.

In our experiments, the increased absorption of cholesterol was fully compensated by re-excretion of absorbed cholesterol, mainly in the form of bile acids, and by reduction in synthesis. Virtually total suppression of synthesis was indicated by close agreement of plasma and dietary cholesterol specific activities after continuous daily oral intake of radioactive cholesterol. Thus, the dog appears to respond rapidly to dietary cholesterol by acceleration of bile acid excretion, as was first documented by Abell et al. (4), as well as by inhibition of synthesis, as has been noted in the rat (19). In man, in contrast, excess absorbed cholesterol is re-excreted exclusively in the form of neutral steroids; in some patients, partial suppression of synthesis by exogenous cholesterol can be documented, but in others compensatory excretion and inhibition of synthesis may be sluggish (17). Thus, the lack of compensatory acceleration of bile acid production and excretion in man may be one of the factors contributing to cholesterol accumulation in body pools and possibly to the development of arteriosclerosis.

*Isotopic steady state.* Feeding of a high-[$^3$H]cholesterol diet for 7–9 mo led eventually to attainment of the isotopic steady state in both dogs, as indicated by the approximation of plasma and dietary [$^3$H]cholesterol specific activities and by finding that the daily intake of tritiated cholesterol and its daily excretion in the form of neutral plus acidic fecal steroids were essentially the same (11, 16). This observation confirms earlier work in dogs and rats that led to the conclusion that on cholesterol-rich diets plasma cholesterol was derived almost entirely from dietary cholesterol, and that endogenous cholesterol synthesis was almost totally repressed (20–23).

In man (21, 22) and the baboon (24) the response to prolonged feeding of isotopic cholesterol has been found to differ from that in the dog: in the isotopic steady state the specific activities of plasma cholesterol were found to be much lower than the specific activities of dietary cholesterol. However, in only a few studies has the attainment of the isotopic steady state been proved by equating isotopic sterol output with its intake (11, 16).

*Responses to bile diversion.* In the bile-diverted dogs, the sustained marked increase in excretion of bile acids in the urine can be explained as the result of marked acceleration of cholesterol synthesis, while the increase in neutral steroid excretion in feces may be due to transfer of plasma cholesterol across the intestinal wall or to enhanced synthesis of cholesterol in the intestinal mucosa.

Since repression of cholesterol synthesis in the liver by reabsorbed cholesterol and in the intestinal mucosa by bile acids is abolished by total diversion of bile, increased cholesterol synthesis would be expected, as previously reported in dogs, rats, and monkeys (19). However, the limits of maximal cholesterol synthesis have previously been defined clearly only in rhesus monkeys (25); the present study in dogs showed rather similar five to sevenfold increases over rates prevailing on cholesterol-free diets. Over a period of almost 2 yr, each bile-shunted dog excreted about 1 kg of cholesterol more than would have been predicted had the dogs not been operated upon. This amount of cholesterol was 20 times greater than the total body cholesterol of the animal measured at sacrifice and must have been derived mainly if not entirely from accelerated synthesis (see accompanying paper). In the presence of cholesterol pool sizes that were later found to be unaltered, the steeper slopes of specific activity-time curves of plasma cholesterol noted in the bile-diverted dogs must also have been due to accelerated synthesis.

A marked rise in fecal neutral steroid excretion after complete diversion of bile in dogs was noted in 1927 by Sperry (26). To explore whether this increase was due to the excretion in feces of cholesterol newly synthesized by the mucosa, we administered a single dose of radioactive cholesterol to the bile-diverted dogs intravenously and then repeatedly compared the specific activities of plasma cholesterol and fecal neutral steroids after the log-linear phase of the decay curve was reached (the dogs were on cholesterol-free diets). If all cholesterol excreted through the gut were derived from new local synthesis in the intestinal mucosa, the specific radioactivity of fecal neutral steroids would have approached 0, providing that there was no isotope exchange with labeled plasma cholesterol; whereas, if all fecal cholesterol were derived from the plasma by transfer across the intestinal wall, the specific activity of fecal neutral steroids would have been equal to that of the plasma. A 15–18% difference was found between fecal and plasma cholesterol specific activities. This suggests that, after prolonged deprivation of bile, fecal cholesterol could be predominantly of plasma origin. However, if there were rapid exchange in the mucosal cell between the labeled cholesterol of plasma and the unlabeled cholesterol newly synthesized in the mucosa, then any cholesterol secreted into the lumen would be as richly labeled as that in the plasma, and then, due to lack of luminal bile acids, this cholesterol would not be reabsorbed but excreted. In view of these considerations we cannot reach a firm conclusion as to the origin of the fecal neutral steroids in these dogs. What remains to be tested is (*a*) whether isotopic exchange in the mucosal cell is rapid under these conditions, and (*b*) whether increased cholesterol synthesis in the mucosa due to bile acid diversion persists indefinitely.

TABLE V

*Comparative Estimates of Daily Cholesterol Turnover Rates by Two Methods*

| Dog | Months before sacrifice | Kinetic data* $t_{\frac{1}{2}}$ (fast pool) | Kinetic data* $t_{\frac{1}{2}}$ (slow pool) | Kinetic data* Turnover | Turnover by sterol balance methods‡ |
|---|---|---|---|---|---|
| | | *days* | *days* | *mg/day* | *mg/day* |
| A | 1½ | 3.0 | 35 | 840 | |
| | 20 (study 1)§ | | | | 508 |
| B | 1½ | 2.7 | 22 | 510 | |
| | 26 (study 1) | | | | 242 |
| | 18 (study 2) | | | | 225 |
| C | 4 (study 5)‖ | 3.2 | 17.5 | 1,630 | 1,419 |
| D | 12 (study 4)¶ | 3.3 | 22 | 850 | 573 |
| | 4 (study 7)** | 3.0 | 14 | 1,730 | 1,336 |
| F | 3 | 1.6 | 12 | 1,140 | |
| | 1 (study 4) | 1.8 | 11.5 | 1,320 | 1,598 |

* Kinetic data were obtained by input-output analysis (15) of specific activity-time-course curves after intravenous injection of a single dose of [4-$^{14}$C]cholesterol shortly before or several months before sacrifice, as indicated.

‡ Turnover data obtained by sterol balance methods (7, 10) represent the sum of fecal neutral steroids of endogenous origin plus fecal bile acids.

§ Study numbers refer to the various metabolic studies carried out in each dog and presented in Tables I, II, and IV.

‖ Daily cholesterol intake = 2,700 mg/day.

¶ Daily cholesterol intake = 500 mg/day.

** Daily cholesterol intake = 3,000 mg/day

The production rates of bile acids after bile diversion in two dogs were almost identical to those in two other dogs fed a high-cholesterol diet. This observation suggests that these two manipulations may have resulted in maximal bile acid synthesis and that feeding still larger amounts of cholesterol might exceed the dogs' capacities to compensate: the result would be the production of still more marked hypercholesterolemia.

*Accumulation and depletion of body cholesterol.* Any increase in cholesterol absorption must result in expansion of body pools unless compensated by an increase in excretion of cholesterol or bile acids, or by inhibition of cholesterol synthesis, or both. Expansion of tissue pools is regularly seen in cholesterol-fed rabbits (27), but in rats fed high-cholesterol diets the accumulation of cholesterol is restricted to the liver (27). Our studies in cholesterol-fed dogs showed a substantial rise in plasma cholesterol concentrations (37–44%), but there was virtually total suppression of endogenous synthesis and zero cholesterol balances were attained. Calculations of cholesterol accumulation on high-cholesterol diets indicated the absence of significant storage, confirming once again that plasma cholesterol concentrations are, at times, poor indicators of events in tissues:

in human cholesterol balance studies, marked accumulations of cholesterol have been observed with surprisingly small increments in plasma cholesterol concentrations (17).

Depletion of tissue cholesterol stores must occur with total bile diversion unless compensatory increases in synthesis counteract the marked increase in cholesterol excretion through the urine and feces. Reduction of plasma cholesterol levels by 20–27% over a period of nearly 2 yr in bile-diverted dogs suggested that some reduction in tissue stores might have occurred. However, we know of no way by which to calculate how much of the excreted sterols was due to increased synthesis as compared to flux from the tissues, and we had to rely on data obtained at autopsy for this information.

The extent of cholesterol accumulation due to cholesterol feeding and the extent of cholesterol depletion due to bile diversion were found at autopsy to be negligible. Those findings, described in detail in the accompanying report, indicated the completeness of compensation in these two experimental extremes. To cholesterol feeding: increased excretion of bile acids and virtually total suppression of cholesterol synthesis. To bile diversion with large outputs of neutral steroids in feces and of bile acids through the urine: an increase in synthesis sufficiently large to account for all losses.

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