Role of liver in the synthesis of cholesterol and the clearance of low density lipoproteins in the cynomolgus monkey

Stephen D. Turley,[1] David K. Spady, and John M. Dietschy

Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-8887

Abstract The suitability of the adult male cynomolgus monkey as a model for investigating genetic mechanisms that regulate dietary cholesterolemic response was evaluated by carrying out a systematic characterization of the major aspects of cholesterol metabolism in this species. In monkeys maintained on a diet enriched with saturated fat but low in cholesterol (0.019%, wt/wt), plasma total and low density lipoprotein cholesterol (LDL-C) concentrations were 118 ± 6 and 45.3 ± 3.4 mg/dl, respectively. Intestinal cholesterol absorption averaged 54.0 ± 2.5%, and the rate of whole body sterol synthesis was 10.8 ± 0.6 mg/day per kg body weight. Only 11.2 ± 2.6% of this synthesis occurred in the liver. In contrast, the liver was the major site for low density lipoprotein clearance accounting for almost 80% of LDL-C degradation in these animals. The liver, which represented 1.5% of whole body mass, had a total and esterified cholesterol concentration of 4.95 ± 0.29 and 2.05 ± 0.30 mg/g, respectively. When challenged with a matching high cholesterol diet (0.19%, wt/wt), the monkeys developed marked hypercholesterolemia that was accounted for mainly by a 7-fold increase in the LDL-C levels. There was, however, wide individual variation among the monkeys in the magnitude of their cholesterolemic response. Hepatic total and esterified cholesterol levels increased 2.5- and 4.6-fold, respectively. Comparative experiments showed that while several of the metabolic characteristics of this species of monkey were similar to those found in the hamster, they were generally very different from those seen in the rat. Thus, the male cynomolgus monkey has many characteristics in common with humans and represents an attractive model for further delineating the genetic mechanisms that dictate variable responsiveness to dietary cholesterol and triacylglycerol.—**Turley, S. D., D. K. Spady, and J. M. Dietschy.** Role of liver in the synthesis of cholesterol and the clearance of low density lipoproteins in the cynomolgus monkey. *J. Lipid Res.* 1995. **36:** 67–79.

Supplementary key words cholesterol synthesis • cholesteryl esters • dietary cholesterolemic response • bile acid • genetic regulation

The relationship between cholesterol synthesis and the concentration of cholesterol carried in low density lipoproteins (LDL-C) is now fairly well understood in most experimental animals and humans. The rate of cholesterol synthesis in the whole animal varies inversely with the weight of that animal in such a way that synthesis decreases by approximately 10 mg/day per kg body weight (bw) for each 10-fold increase in the weight of the adult animal (1). Under circumstances where the dietary intake of cholesterol is equal to about 25–50% of the daily synthesis rate, the vast majority of this endogenous synthesis takes place in the extrahepatic organs (1, 2). Similarly, the rate at which LDL-C is removed from the plasma is also determined by the weight of the adult animal. The rate of clearance of LDL-C decreases by about 1.8 ml/h per kg bw for each 10-fold increase in body mass (1). However, in contrast to its minor role in cholesterol synthesis, the liver accounts for the great majority of this LDL-C transport activity in most species including, apparently, humans (1, 3–5). Despite these major quantitative differences in rates of cholesterol synthesis and LDL-C transport, however, the steady-state LDL-C concentration in virtually all species is well below 50 mg/dl when these animals are maintained on diets relatively low in cholesterol and triacylglycerol.

When additional amounts of these lipids are added to the diets of these experimental animals or humans, this concentration of LDL-C invariably increases, and there are predictable changes in the rates of both cholesterol synthesis and LDL-C transport. Progressively increasing the cholesterol content of the diet, for example, increases the cholesteryl ester content of the liver, suppresses hepatic, but not extrahepatic, sterol synthesis, reduces hepatic LDL receptor activity, and increases LDL-C production (6–8). As a result of these changes, the steady-state plasma LDL-C level increases in proportion to the dietary cholesterol challenge. The addition of triacyl-

Abbreviations: LDL-C, cholesterol carried in low density lipoproteins; bw, body weight; EDTA, ethylenediamine tetraacetic acid; DPS, digitonin-precipitable sterols; TCB, tyramine cellobiose; GLC, gas–liquid chromatography.

[1]To whom correspondence should be addressed.

glycerol containing predominantly saturated fatty acids to such diets reduces the level of cholesteryl esters in the liver, further suppresses hepatic LDL receptor mRNA levels and activity, and further increases LDL-C production rates (7, 9–14). As a result of these changes, the plasma LDL-C concentration becomes still more elevated. In contrast, if the dietary triacylglycerol contains predominantly unsaturated fatty acids, cholesteryl ester levels in the liver remain constant or increase, hepatic LDL receptor mRNA levels and activity are restored, production rates are reduced, and the LDL-C concentration is lowered (11–14).

While these general principles appear to be applicable to most species, including humans, it is now also clear that individual members of any outbred species may respond in a quantitatively different manner to challenge by a constant amount of dietary cholesterol and triacylglycerol (14). Thus, in many species, there are hypo- and hyperresponsive individuals where the steady-state plasma LDL-C concentration will increase by either a small or large amount, respectively, when these individuals are challenged with a constant load of dietary lipid (15–22). Presumably, these differences in response are genetically determined inasmuch as hyperresponsive individuals usually give rise to hyperresponsive offspring (19). Despite the fact that such hypo- and hyperresponsive individuals have been identified in many animal species, the polymorphisms that must exist in the function of a single transporter or enzyme that may account for these different phenotypes have, as yet, not been identified.

In selecting a particular species in which to study this genetically determined response to dietary lipids, it was clear that it would be desirable to meet a number of criteria. Presumably, it would be preferable to use a nonhuman primate where the characteristics of cholesterol and LDL metabolism were similar to those in humans. Furthermore, there would have to be adequate numbers of hypo- and hyperresponsive animals available. Ideally, the rates of cholesterol synthesis in such animals would be in the range of 10–20 mg/day per kg. Most of this synthetic activity should occur in the extrahepatic organs, but the liver should play the predominant role in LDL-C metabolism. Finally, it would also be useful if the response of the steady-state plasma LDL-C level to changes in dietary cholesterol intake was similar to that seen in humans. Based on several previous publications (23–29) and considerable advice from investigators at the Primate Center at Bowman Gray Medical School, we chose to use the cynomolgus monkey for these studies. The purpose of this initial publication, therefore, is twofold: first, we report investigations carried out to establish whether the quantitative methods necessary to measure organ-specific cholesterol and LDL metabolism can be applied to this relatively large primate, and second, we describe studies that characterize the major aspects of cholesterol and LDL metabolism in a group of these primates and compare these findings to those seen in other species, including hamsters and humans.

MATERIALS AND METHODS

Animals and diets

Feral adult male cynomolgus monkeys (*Macaca fascicularis*) were obtained from either the primate facility at the Bowman Gray School of Medicine (Winston-Salem, NC), or from the Winters Institute (Winters, CA). They were housed individually in stainless steel cages in a light-cycled room with equal periods of light (7:00 AM to 7:00 PM) and darkness (7:00 PM to 7:00 AM). The room temperature was kept at 24–26°C. The monkeys had free access to drinking water at all times and were fed a fixed quantity of a custom diet (16 g per kg bw) twice each day at about 9 AM and 4 PM. On average, 11% of each of these meals was wasted. This custom-made, pelleted diet was enriched with saturated fat and was made to contain cholesterol at either a low basal level, or at a 10-fold higher level. In most of the experiments the monkeys were fed only the low cholesterol diet. The high cholesterol or challenge diet was used mainly to characterize the extent of individual variability in the cholesterolemic response of this species of primate and to compare this response to that of the hamster and rat fed the same diet. In all experiments, except one initial study in which a large group of randomly selected monkeys was fed the challenge diet over 9 weeks, all monkeys were fed their respective diets for a minimum of 20 weeks before any metabolic measurements were made. On the day of the experiments the monkeys were not given their morning meal. For the comparative studies young adult virus-free male Golden Syrian hamsters (Charles River Laboratories, Montreal, Canada) and Sprague-Dawley rats (Sasco, Inc., Omaha, NE) were fed ad libitum the same low or high cholesterol diets that the monkeys were given. The hamsters and rats were housed in separate light-cycled rooms and were kept in plastic colony cages with free access to water.

The low and high cholesterol diets were prepared by Teklad Diets (Madison, WI), using formulations developed at the Bowman Gray School of Medicine. The low cholesterol diet consisted of high protein monkey chow (Ralston Purina Co., St. Louis, MO) (76.4%), lard (14.1%), brewer's yeast (4.0%), an egg yolk substitute (3.4%), raw wheat germ (2.0%), and ascorbic acid (0.1%). The egg yolk substitute was prepared by mixing the following ingredients in the proportions indicated: casein (18.0 g), soybean lecithin (13.1 g), sucrose (2.1 g), and a mineral mix (Hegsted IV, 1.3 g). The composition of the high cholesterol diet was exactly as described earlier (27). It consisted of high protein monkey chow (75.7%), lard (10.0%), egg yolk powder (8.2%), brewer's yeast (4.0%),

raw wheat germ (2.0%), and ascorbic acid (0.1%). Both diets had the same caloric density (4.2 kcal/g) and total lipid content (17.5 g/100 g diet). Hence each diet contained 38% of calories as fat. The low and high cholesterol diets had a cholesterol content of 0.19 mg/g (0.044 mg/kcal) and 1.9 mg/g (0.044 mg/kcal), respectively. The proportions of the major fatty acids in the low and high cholesterol diets were, respectively: oleic acid, 39.2 and 40.9%; palmitic acid, 27.5 and 27.8%; linoleic acid, 17.1 and 15.9%; stearic acid, 11.7 and 11.0%; palmitoleic acid, 2.5 and 2.7%; and myristic acid, 2.0 and 1.7%. The diets were stored at 4°C and were used within 12 weeks of preparation. All experiments were approved by the Institutional Animal Care and Research Advisory Committee.

Measurement of rates of sterol synthesis in vivo

The monkeys were tranquilized by the intramuscular administration of a mixture of tiletamine hydrochloride and zolazepham hydrochloride (Telazol, Aveco Co., Inc., Fort Dodge, IA) at a dose of 3 mg/kg bw and weighed. They were then fitted with a saphenous vein catheter and placed under a well-ventilated fume hood. Each monkey then received a bolus intravenous injection of [^{3}H]water (ICN Radiochemicals, Irving, CA) at a dose of 440 mCi/kg bw, and remained tranquilized for 1 h during which time they were covered with a blanket. After exactly 1 h an overdose of sodium pentobarbital (Nembutal, Abbott Laboratories, Chicago, IL) (75 mg/kg bw) was administered intravenously and the animal was bled from the abdominal aorta into an evacuated glass bottle containing EDTA as anticoagulant. The volume of blood removed was recorded. The gallbladder bile was drawn into a syringe and the liver was removed, rinsed in 0.9% NaCl solution, and weighed. The entire small bowel was also removed and cut into two sections which were then rinsed and weighed. Aliquots of whole blood and liver, as well as the entire intestine segments and residual carcass, were added to ethanolic KOH for saponification, after which the digitonin-precipitable sterols (DPS) were isolated and their ^{3}H content was determined (30). The .tissue [^{3}H]DPS contents were corrected for the [^{3}H]sterol contained in residual blood. This was calculated from the blood [^{3}H]DPS content (cpm/ml) and the residual blood volume (μl/g) for each tissue which was determined in separate experiments using an ^{131}I-labeled protein as a volume marker. The specific activity of the plasma water in each animal was measured and averaged about 10 cpm/nmol of water. An aliquot of the liver was also placed in chloroform–methanol 2:1 (v/v) for the determination of unesterified and esterified cholesterol levels. The rates of sterol synthesis were expressed in terms of tissue [^{3}H]DPS content, and represent the nmol or μmol of [^{3}H]water incorporated into DPS per h per g of tissue (nmol/h per g) or per h per whole organ (μmol/h per whole organ). The rates of whole body sterol synthesis were calculated as the sum of the whole organ [^{3}H]DPS contents normalized per kg bw (μmol/h per kg bw).

In the sterol synthesis experiments with hamsters and rats, the animals were anesthetized with diethyl ether and given a bolus intravenous injection of [^{3}H]water (hamsters, 65 mCi/100 g bw; rats, 15 mCi/100 g bw) directly into a femoral vein. They were then allowed to recover and after 1 h were anesthetized again and bled from the abdominal aorta. The liver was removed and weighed. Aliquots of liver and the entire remaining carcass were taken for the measurement of their labeled sterol content (30). The rates of hepatic and whole body sterol synthesis in the hamsters and rats were determined and calculated in the same way as for the monkeys. For all three species the sterol synthesis experiments were carried out at about 1 to 4 h after the commencement of the light period.

Measurement of rates of LDL clearance in vivo

The rates of LDL clearance by the liver and all the major extrahepatic organs were measured using homologous LDL preparations labeled with ^{125}I-labeled tyramine cellobiose (TCB) or with ^{131}I directly (3, 31, 32). The LDL was isolated from the plasma of normocholesterolemic monkeys by preparative ultracentrifugation in the narrow density range of 1.020–1.055 g/ml. After measurement of its protein concentration, the LDL was labeled, dialyzed, and filtered. Each labeled LDL preparation was used within 48 h. The monkeys were tranquilized with telazol (3 mg/kg bw given intramuscarly), weighed, and fitted with a saphenous vein catheter. Each monkey was then given a bolus injection of ^{125}I-labeled TCB-LDL (about 20 μCi/kg bw). Ten minutes later, and at several time points over the following 6 h, about 3 ml of blood was withdrawn from the saphenous vein in the other leg for the measurement of the plasma ^{125}I-labeled TCB-LDL specific activity. Throughout this 6-h period the monkeys were placed under a warm blanket and were kept continuously tranquilized with telazol. At exactly 6 h each monkey was given a bolus intravenous injection of ^{131}I-labeled LDL (about 5 μCi/kg bw) as a volume marker followed 10 min later by an intravenous dose of sodium pentobarbital (2 mg/kg bw). The animals were then bled from the abdominal aorta into an evacuated glass bottle containing EDTA as anticoagulant. The liver and all of the major extrahepatic organs, as well as pieces of skeletal muscle, abdominal adipose tissue, skin, stomach, colon, esophagus, and abdominal aorta, were removed. The gallbladder bile was drawn into a syringe and the volume was measured. All of the tissues were rinsed in 0.9% NaCl solution and whole organ weights were recorded. Multiple aliquots of all the tissues, as well as of plasma and bile, were taken for the measurement of their ^{125}I and ^{131}I content in a gamma counter. Aliquots of liver were also taken for the determination of unesterified and esterified cholesterol levels (33). Plasma from each monkey was fractionated by

density gradient ultracentrifugation for the measurement of LDL-C levels. In this case the LDL was isolated in the conventional density range of 1.020–1.063 g/ml. The rates of LDL clearance by the tissues were calculated using the mean plasma ^{125}I-labeled TCB-LDL specific activity over the 6-h period, and were expressed as the μl of plasma cleared of its LDL content per h per g of tissue (μl/h per g) (5, 34). These data, along with the directly measured or derived whole organ weights (35), were used to calculate the rates of LDL clearance per h per whole organ normalized per kg bw (μl/h per whole organ per kg bw).

Measurement of percent cholesterol absorption in vivo

Cholesterol absorption was measured by a fecal isotope ratio method using a modification of a recently described protocol (36). Over 6 successive days, the monkeys were dosed with a mixture of [1,2-^{3}H]cholesterol (Amersham Corp., Arlington Heights, IL) and β-[4-^{14}C]sitosterol (Amersham). The daily dose of labeled sterols (approximately 7 μCi of [^{3}H]cholesterol and 1 μCi of β-[^{14}C]sitosterol) was contained in 25 μl of ethanol that was placed on a slice of apple. This was fed to the monkeys with their usual morning meal. From the fourth to the seventh day after commencement of dosing, all of the stools from each animal were collected and mixed to form a slurry. The levels of ^{3}H and ^{14}C in aliquots of the slurry and the initial dosing mixture were then determined (37). The percentage of cholesterol absorption was calculated as follows:

$$\text{Cholesterol absorption (\%)} = \frac{(^3\text{H}/^{14}\text{C})\text{dose} - (^3\text{H}/^{14}\text{C})\text{feces}}{(^3\text{H}/^{14}\text{C})\text{dose}} \times 100$$

Analytical procedures

Plasma total cholesterol concentrations were measured enzymatically using a kit (No. 1127578) from Boehringer Mannheim (Indianapolis, IN). The various plasma lipoprotein fractions and aliquots of liver and gallbladder bile were saponified and extracted, and their cholesterol concentration was then measured by gas liquid chromatography (GLC) using stigmastanol (Sigma Chemical Company, St. Louis, MO) as an internal standard (38). The esterified and unesterified cholesterol fractions in the liver were separated on silicic acid/celite columns and their cholesterol content was then measured by GLC (33). Dietary cholesterol levels and fatty acid composition were determined as described elsewhere (38, 39). The total lipid content of the diets was determined gravimetrically. Biliary phospholipid and bile acid concentrations and the bile acid composition of the gallbladder bile were determined as previously described (40–42).

Analysis of data

The equations for calculating rates of sterol synthesis in vivo (30) and LDL uptake by the various tissues (34) are published elsewhere. Where appropriate the mean ± 1 SEM values for groups of data are given. For correlating the blood [^{3}H]DPS content with that of the tissues, a linear regression line with the form $y = a + bx$ was fitted by the method of least squares to the data from individual animals. Differences between mean values for the groups fed the low and high cholesterol diets were tested for statistical significance using the two-tailed unpaired Student's t-test.

RESULTS

The first set of measurements made in these animals consisted of determining the distribution of body mass among the various major organ systems. In several of the metabolic experiments using monkeys fed the low cholesterol diet, the liver and various other whole organs were excised and weighed. These organ weights are summarized in **Table 1.** It is particularly noteworthy that the liver accounted for only 1.5% of the whole body weight. While this proportion is similar to that found in other large animals including humans (~2.0%), it is less than half the relative weight of the liver found in smaller animal models such as the hamster, rat, and mouse (3.5–5%) (3, 35, 43).

In the second set of studies, rates of cholesterol synthesis were measured in vivo in individual organs as well as in the whole animal. In these experiments, the rates of incorporation of [^{3}H]water into sterols by the liver and the extrahepatic tissues were measured in a total of 12 mon-

TABLE 1. Absolute and relative weights of the liver and several extrahepatic organs in the adult male cynomolgus monkey

Organ	Number of Animals	Absolute Weight	Percent of Body Weight
		g	%
Liver	27	90.4 ± 2.5	1.50 ± 0.03
Small intestine	27	53.6 ± 1.7	0.90 ± 0.03
Kidney	15	17.8 ± 0.6	0.29 ± 0.01
Spleen	15	9.7 ± 1.7	0.15 ± 0.02
Lung	15	26.1 ± 1.1	0.42 ± 0.02
Testis	15	34.5 ± 2.0	0.56 ± 0.04
Adrenal	27	0.57 ± 0.02	0.01 ± 0.001

These data were derived from a series of studies in which the rates of sterol synthesis and LDL clearance were measured in vivo in adult male cynomolgus monkeys fed a low cholesterol, high triacylglycerol chow diet. While a total of 27 animals with a mean body weight of 6.00 ± 0.18 kg were studied, complete organ weights were not obtained in every monkey. The values represent the mean ± 1 SEM of data from the number of animals indicated.

keys maintained on the low cholesterol diet for greater than 20 weeks. The content of [^{3}H]DPS in each g of the major tissues is shown in **Fig. 1.** The highest rates of sterol synthesis in this species were found in the small intestine (185 ± 7 nmol/h per g), adrenal gland (165 ± 32 nmol/h per g), and liver (95 ± 29 nmol/h per g). The mean rate of synthesis in the remaining organs included in the carcass was only 12 ± 0.5 nmol/h per g, and only a small amount of newly synthesized cholesterol had been exchanged into the blood compartment (5 ± 1 nmol/h per g).

The presence of labeled sterol in the blood raised the question of whether it was of hepatic or extrahepatic origin. This was resolved by plotting the [^{3}H]DPS content of the blood in each animal as a function of the corresponding [^{3}H]DPS content in the liver, carcass, and small intestine. As shown in **Fig. 2,** the amount of labeled sterol in the blood correlated directly with the [^{3}H]DPS content of the liver (A), but not with that of the carcass (B) or small intestine (C). Thus, as has been previously established in smaller animals (30, 44), the [^{3}H]DPS that appears in whole blood 1 h after administration of the [^{3}H]water comes almost exclusively from the liver. If the liver is surgically removed or if hepatic synthesis is suppressed, virtually no [^{3}H]DPS appears in blood at 1 h. Therefore, the labeled sterol content of the whole blood volume was included with that of the liver in the calculation of whole organ [^{3}H]DPS content.

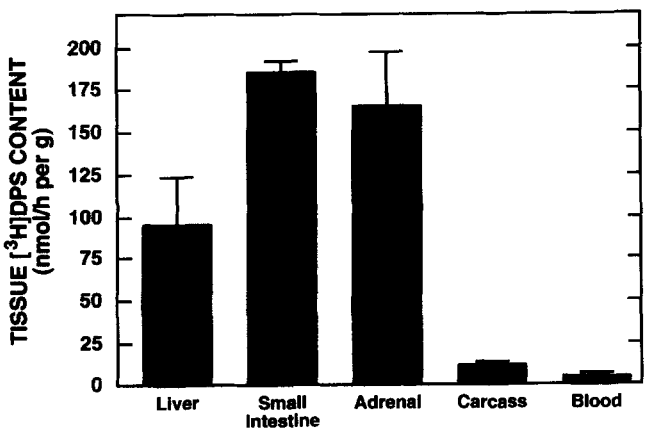

Fig. 1. Rates of sterol synthesis in the liver and various extrahepatic tissues measured in vivo in male cynomolgus monkeys fed the low cholesterol diet. Adult male cynomolgus monkeys that had been fed a chow diet enriched with saturated fat but containing a low level of cholesterol (0.019%, wt/wt) for at least 20 weeks were tranquilized and then given an intravenous bolus injection of [^{3}H]water (440 mCi/kg bw). The animals remained tranquilized for the next 60 min at which time they were given an overdose of sodium pentobarbital. They were then bled from the abdominal aorta, and the whole liver, small intestine, and adrenal glands were removed and weighed. Aliquots of whole blood, liver, and adrenal tissue, as well as the whole small intestine and the entire remaining carcass, were saponified in alcoholic KOH and their content of labeled digitonin-precipitable sterols ([^{3}H]DPS) was determined. The rate of sterol synthesis in each organ was expressed in terms of the tissue [^{3}H]DPS content which represents the nmol of [^{3}H]water incorporated into DPS per h per g of tissue. Values are the mean ± 1 SEM of data obtained from 12 monkeys.

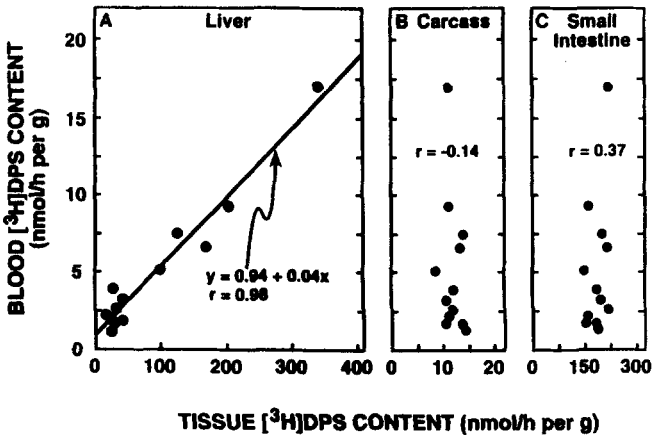

Fig. 2. Relationship between the [^{3}H]DPS content of whole blood and that in the liver, carcass, and small intestine in male cynomolgus monkeys fed the low cholesterol diet. Linear regression lines were fitted to the data for the blood and tissue [^{3}H]DPS contents (from Fig. 1) in 12 monkeys. Each point represents data from a single animal. The slope of the regression line for the liver (A) is significantly different from zero ($P < 0.05$), while there is no significant ($P > 0.05$) relationship between the content of [^{3}H]DPS in the blood and in the carcass (B) or small intestine (C).

These data, along with those in Table 1, were used to next calculate the rates and relative importance of sterol synthesis in each organ to whole body cholesterol synthesis. As shown in panel A of **Fig. 3,** when the mass of each organ is taken into account, there was much more sterol synthesis in the organs of the residual carcass than in either the liver or small intestine. The proportion of whole body synthesis that occurred in each of these three major tissue compartments is shown in panel B. The liver accounted for only 11.2 ± 2.6% of the total and the small intestine made a similar contribution (12.8 ± 0.9%). However, the major proportion (76.0 ± 2.1%) of cholesterol synthesis in the whole animal occurred in the remaining extrahepatic organs. The insert in panel A gives the whole body [^{3}H]DPS content which represents the sum of the values for the liver, intestine, and carcass, normalized per kg bw. As the number of protons from water that are incorporated into the cholesterol molecule has been established (30, 44), it can be calculated that this whole body [^{3}H]DPS content of 14.48 μmol/h per kg bw is equivalent to the synthesis of 10.8 mg of cholesterol/day per kg bw.

The third major parameter measured in these animals was the rate of total LDL clearance by the liver and extrahepatic organs. These data are given in **Fig. 4** and represent measurements in a total of 12 monkeys fed the low cholesterol diet. In these studies, each animal was given a bolus injection of ^{125}I-labeled TCB-LDL and the rate of LDL clearance into each organ was measured 6 h later using the mean value for the plasma ^{125}I-labeled TCB-LDL specific activity. The data in panel (A) show the μl of plasma cleared of its LDL-C content per h per

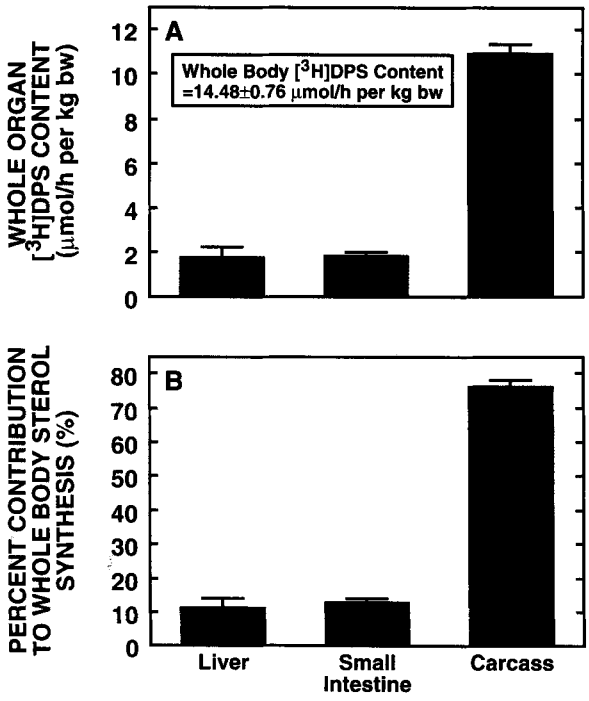

Fig. 3. Rates of sterol synthesis in the whole liver, small intestine, and carcass, and the percent contribution of each of these organs to whole body sterol synthesis in male cynomolgus monkeys fed the low cholesterol chow diet. These data were derived from the same study that is described in Fig. 1. The tissue [^{3}H]DPS content (nmol/h per g) was multiplied by the whole organ weight to obtain the whole organ [^{3}H]DPS content (A). As essentially all of the [^{3}H]DPS in blood was of hepatic origin, the value for whole liver [^{3}H]DPS content includes all of the labeled sterol found in the blood. The [^{3}H]DPS content of the adrenal glands is included in that for the carcass. The whole organ [^{3}H]DPS content represents the μmol of [^{3}H]water incorporated into DPS per h per whole organ normalized per kg body weight (μmol/h per kg bw). The sum of these values equals the whole body [^{3}H]DPS content shown in the insert. The percent contribution of each of the three major organ compartments to whole body sterol synthesis (B) was calculated by expressing the whole organ [^{3}H]DPS content as a percentage of that for the whole body. Values are the mean ± 1 SEM of data obtained from 12 monkeys.

g of tissue. The adrenal gland (265 ± 36 μl/h per g) and liver (71 ± 5 μl/h per g) had the highest rates of clearance while most of the extrahepatic organs manifested very low rates of LDL uptake. It should be noted that the value for the liver includes a correction for the label that was present in the gallbladder bile when these measurements were made. When these clearance data are expressed on a whole organ basis and normalized per kg bw (Fig. 4B), the calculated whole body LDL clearance rate equalled approximately 1516 μl/h per kg bw. Given a plasma volume of 37 ml/kg (29), this corresponds to a fractional catabolic rate of approximately 1.0 pool/day. As is evident in panel B, the liver was the predominant site for this uptake and accounted for about 80% of whole body LDL degradation. Taken together, the results of these initial quantitative studies revealed the strikingly divergent role that the liver plays in its contribution to whole body cholesterol synthesis and LDL metabolism in this species.

As the enterohepatic flux of bile acids and cholesterol profoundly influences the rates of hepatic sterol synthesis and LDL metabolism, the characterization of this primate model was extended to include the measurement of the efficiency of intestinal cholesterol absorption and an analysis of the composition of the bile acid pool. The percent of dietary sterol absorption was determined in a total of seven monkeys fed the low cholesterol diet using a fecal isotope ratio method that employed β-[^{14}C]sitosterol as a nonabsorbable marker. Although the data are not shown, the level of cholesterol absorption averaged 54.0 ± 2.5% over 4 consecutive days of measurement. Thus, it can be calculated that when fed the low cholesterol diet, these monkeys absorbed about 2.9 mg of cholesterol from their diet each day per kg bw.

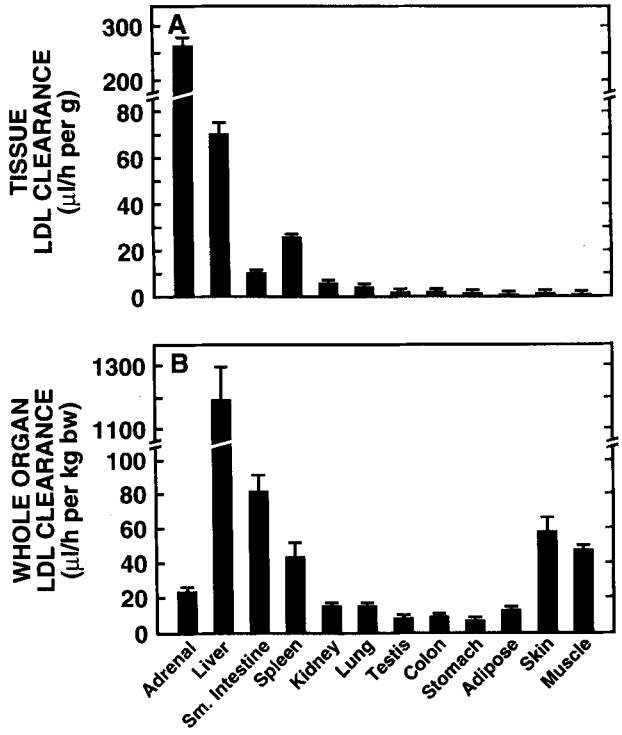

Fig. 4. Rates of LDL clearance by the liver and extrahepatic organs measured in vivo in male cynomolgus monkeys fed a low cholesterol diet. Adult male cynomolgus monkeys that had been fed a diet enriched with saturated fat but containing a low level of cholesterol (0.019%, wt/wt) for at least 20 weeks were tranquilized and then given an intravenous bolus injection of ^{125}I-labeled tyramine cellobiose-labeled, homologous LDL as described in Materials and Methods. The monkeys were kept tranquilized over the next 6 h. At 6 h they were given an intravenous bolus injection of ^{131}I-labeled LDL followed 10 min later by an overdose of sodium pentobarbital. The animals were then bled from the abdominal aorta and the whole liver and most of the extrahepatic organs were removed and weighed. Aliquots of all the tissues, plasma, and gallbladder bile were taken for the measurement of their ^{125}I and ^{131}I content. These data were used to calculate the clearance rate of LDL-C either in terms of the μl of plasma cleared of its LDL content per h per g of tissue (μl/h per g) (A), or of the μl of plasma cleared per h per whole organ, normalized per kg bw (μl/h per kg bw) (B). Values are the mean ± 1 SEM of data obtained from 12 monkeys.

The determination of biliary bile acid and lipid composition was carried out on gallbladder bile taken from the monkeys that were used for the sterol synthesis experiments. As shown in **Fig. 5**, cholic acid was the predominant bile acid in the pool (about 55%) followed by deoxycholic acid (30%) and chenodeoxycholic acid (13%). The proportion of the bile acids conjugated to taurine and glycine was about 82% and 18%, respectively. The data in **Fig. 6** give the concentrations of bile acid, phospholipid, and cholesterol in gallbladder bile in absolute (A) and relative (B) terms. The low molar percentage of cholesterol (1.7 ± 0.2%) indicates that the bile of these monkeys is relatively undersaturated, at least when they are fed a low cholesterol diet.

The remaining series of experiments was undertaken to examine the response of the plasma cholesterol concentration in these monkeys to a high dietary cholesterol challenge and to compare this response to that seen in the rat and hamster. When maintained on the basal diet, the monkeys had an average plasma total cholesterol concentration of 134 ± 4 mg/dl. After the 9 weeks of dietary challenge this average had increased to 366 ± 25 mg/dl. However, there was marked individual variation among the monkeys in the extent of their cholesterolemic response. While some of the animals showed very little change in their plasma cholesterol concentration, others had levels above 500 mg/dl.

Although the cynomolgus monkey, like many other species, is highly variable in its response to dietary cholesterol, the hypercholesterolemia that develops in this primate model is due almost entirely to an increase in

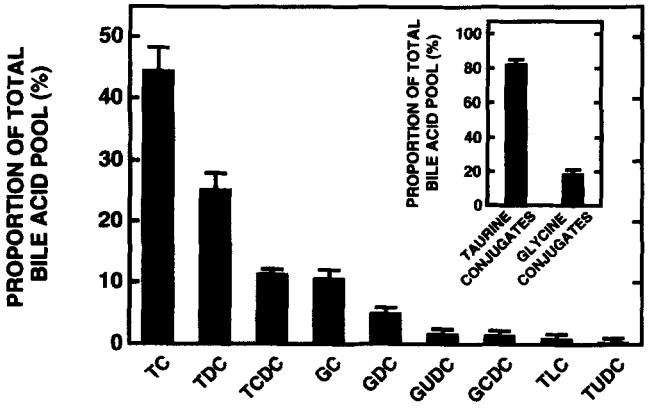

Fig. 5. Bile acid composition of gallbladder bile in male cynomolgus monkeys fed a low cholesterol chow diet. The bile acid composition of gallbladder bile obtained from the monkeys in which measurements of whole body sterol synthesis were made (Fig. 3) was determined as described in Materials and Methods. The bile acids detected were taurocholic (TC), glycocholic (GC), taurochenodeoxycholic (TCDC), glycochenodeoxycholic (GCDC), taurodeoxycholic (TDC), glycodeoxycholic (GDC), tauroursodeoxycholic (TUDC), glycoursodeoxycholic (GUDC), and taurolithocholic (TLC). Values are the mean ± 1 SEM of data obtained from 12 monkeys.

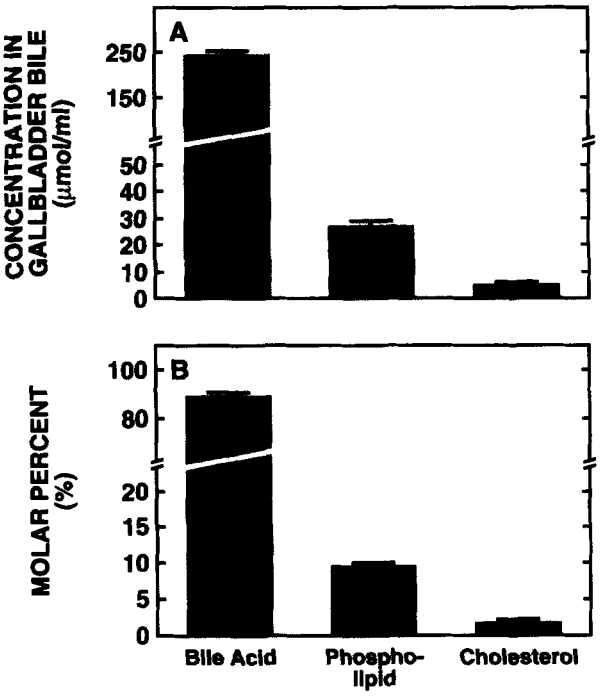

Fig. 6. Absolute and relative concentrations of bile acid, phospholipid, and cholesterol in the gallbladder bile of male cynomolgus monkeys fed a low cholesterol chow diet. The concentrations of bile acid, phospholipid, and cholesterol in the gallbladder bile from the monkeys in which whole body sterol synthesis was measured (Fig. 3) were determined as described in Materials and Methods. The absolute concentrations of each lipid (A) were also expressed on a molar percentage basis (B). Values are the mean ± 1 SEM of data obtained from 12 monkeys.

LDL-C levels. This is well illustrated in **Fig. 7** which shows the plasma lipoprotein cholesterol levels (A) and the unesterified and esterified hepatic cholesterol concentrations (B) in monkeys fed the low and high cholesterol diets. It is evident from Fig. 7A that most of the increase in plasma total cholesterol levels was due to a dramatic rise in the LDL-C concentration. In these monkeys about 81% of the plasma cholesterol appeared in LDL compared to 38% in the plasma of monkeys fed the low cholesterol diet. The data in Fig. 7B show that essentially all of the increase in hepatic cholesterol levels that occurred in response to feeding the high cholesterol diet was in the cholesteryl ester fraction. It is noteworthy that in the monkeys fed the low cholesterol diet, the total and esterified cholesterol levels were 4.95 and 2.05 mg/g, respectively. These concentrations, particularly that for the esterified fraction, are higher than those found in small animal models maintained on a similar low cholesterol diet but nearly identical to values reported in humans (6, 8, 45, 46).

For comparative purposes the rates of hepatic and whole body sterol synthesis were measured in vivo in adult male hamsters and rats that had been fed this same low cholesterol primate diet. At the time of study the ham-

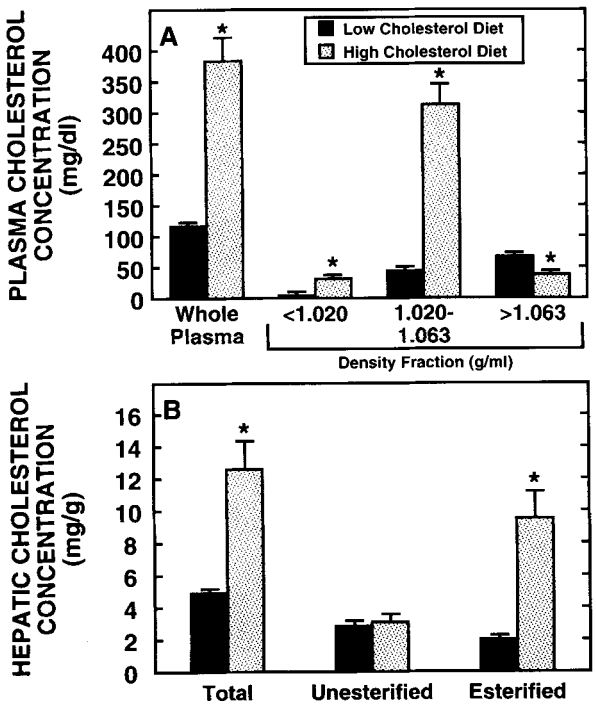

Fig. 7. Plasma and hepatic cholesterol concentrations in male cynomolgus monkeys fed either low or high levels of cholesterol. Adult male cynomolgus monkeys were fed a chow diet that was enriched with saturated fat and that contained either a low (0.019%, wt/wt) or a high (0.19%, wt/wt) level of cholesterol for 20 weeks. They were then used for the measurement of various parameters of cholesterol metabolism including the concentration of total cholesterol in the plasma lipoprotein fractions (A) and the levels of unesterified and esterified cholesterol in the liver (B) as described in Materials and Methods. Values are the mean ± 1 SEM of data obtained from 27 and 17 monkeys fed the low and high cholesterol diets, respectively. An asterisk indicates that the value is significantly different ($P < 0.05$) from the corresponding value for the group fed the low cholesterol diet.

sters and rats weighed 162 ± 6 g and 365 ± 5 g, respectively. The data in **Fig. 8A** show the whole body [^{3}H]DPS content normalized per kg body weight for these two species along with the corresponding value for the cynomolgus monkey (from Fig. 3A insert). While both rodent species, and particularly the rat, manifested higher rates of whole body synthesis than the monkeys, the proportion of this synthesis that occurred in the liver of the rat (51.0 ± 7.8%) was 10-fold more than in the hamster (5.0 ± 0.4%). A comparison of these data with the corresponding value for the cynomolgus monkey (11.2 ± 2.6%) points up the inverse correlation that appeared to exist in these three species between their basal rates of hepatic cholesterol synthesis and their general responsiveness to dietary cholesterol. This question was specifically examined in another study in which plasma LDL-C levels and hepatic total cholesterol concentrations were measured in hamsters and rats that had been fed the low and high cholesterol primate diets for 6 weeks. These data are given in **Fig. 9** which includes values for the cynomolgus monkey (from Fig. 7). While the plasma LDL-C level (A) and hepatic cholesterol content (B) remained essentially unchanged in the rats, both increased substantially in the hamsters and monkeys fed the high cholesterol diet.

DISCUSSION

From the quantitative measurements made in this study it is possible to calculate the absolute rates of sterol synthesis and cholesterol flux through the major tissue compartments of the male cynomolgus monkey. These rates are summarized in **Fig. 10** where the circled numbers represent mg of cholesterol/day per kg bw. In this formulation, the body has been divided into three functionally distinct groups of organs. The small intestine (A) is the site of entry of dietary cholesterol into the body, and

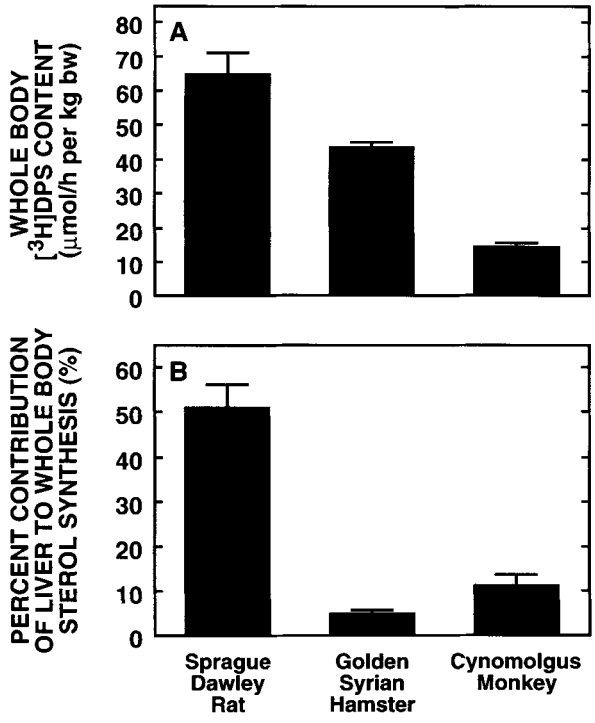

Fig. 8. Contribution of the liver to whole body sterol synthesis in male rats, hamsters, and cynomolgus monkeys fed a low cholesterol diet. Adult male Sprague Dawley rats, Golden Syrian hamsters, and cynomolgus monkeys that had been fed a diet enriched with saturated fat but containing a low level of cholesterol were given an intravenous bolus injection of [^{3}H]water and killed 1 h later. The [^{3}H]DPS content of the liver and the remaining carcass was determined as described in Materials and Methods. The whole body [^{3}H]DPS content (A) represents the μmol of [^{3}H]water incorporated into DPS by the whole body per h normalized per kg body weight (μmol/h per kg bw). For each species the percent contribution of the liver to whole body sterol synthesis (B) includes the labeled sterol content of blood. The values for the rat and hamster each represent the mean ± 1 SEM of data obtained from 6 animals. The values for the monkeys correspond to those given in Fig. 3 and represent the mean ± 1 SEM of data obtained from 12 monkeys.

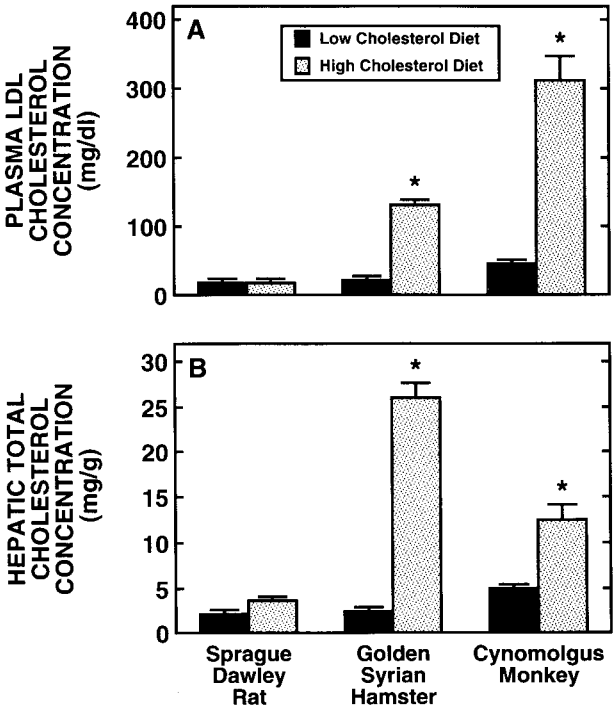

Fig. 9. Plasma LDL-C and hepatic total cholesterol concentrations in male rats, hamsters, and cynomolgus monkeys fed a chow diet containing low or high levels of cholesterol. Adult male Sprague Dawley rats, Golden Syrian hamsters, and cynomolgus monkeys were all fed the same diet that was enriched with saturated fat and that contained either a low (0.019%, wt/wt) or a high (0.19%, wt/wt) level of cholesterol for either 6 weeks (rats and hamsters) or 20 weeks (monkeys). The plasma LDL-cholesterol and hepatic total cholesterol concentrations were then measured. The values for the rat and hamster each represent the mean ± 1 SEM of data obtained from 6 animals. The values for the monkeys correspond to those given in Fig. 7 and represent the mean ± 1 SEM of data from 27 and 17 animals fed the low and high cholesterol diets, respectively. An asterisk indicates that the value is significantly different ($P < 0.05$) from the corresponding value for the group fed the low cholesterol diet.

this process is mediated by the chylomicron particle (CM) and the remnant receptor in the liver (47–49). Cholesterol from the other extrahepatic tissues (B) also must be transported to the liver, but this process is presumably mediated by high density lipoproteins (HDL) with transfer of the sterol esters to the apoB-containing lipoproteins and, hence, to the liver (50, 51). The liver (C) plays a central role in two separate, but related processes. First, in the steady state it must excrete an amount of sterol equal to that which is absorbed from the intestine and synthesized in all of the organs. Second, this organ is the sole site for the synthesis of very low density lipoproteins (VLDL) and the major tissue for removing LDL-C from the plasma (1). The relative velocities of these two processes are major determinants of the steady-state plasma LDL-C concentration.

In these studies the monkeys were hand-fed 32 g per kg bw of the low cholesterol diet each day during the 5-month experiments. After accounting for 11% wastage, therefore, each animal had a constant dietary cholesterol intake equal to 5.4 mg/day per kg. Because, on average, 54% of this dietary sterol was absorbed, 2.9 mg/day per kg must have entered the body pools while the remainder became part of the sterols excreted in the feces. At the same time these animals synthesized 10.8 mg/day per kg, 76% of which (8.2 mg/day per kg) occurred in the extrahepatic, extraintestinal tissues (Fig. 3). The liver accounted for only 11% (1.2 mg/day per kg) of endogenous sterol synthesis. Thus, the dietary intake of cholesterol (5.4 mg/day per kg) equaled about 50% of the daily rate of endogenous synthesis (10.8 mg/day per kg) in these animals fed the low cholesterol diet. These figures are nearly identical to those seen in humans on western diets where endogenous synthesis is commonly in the range of

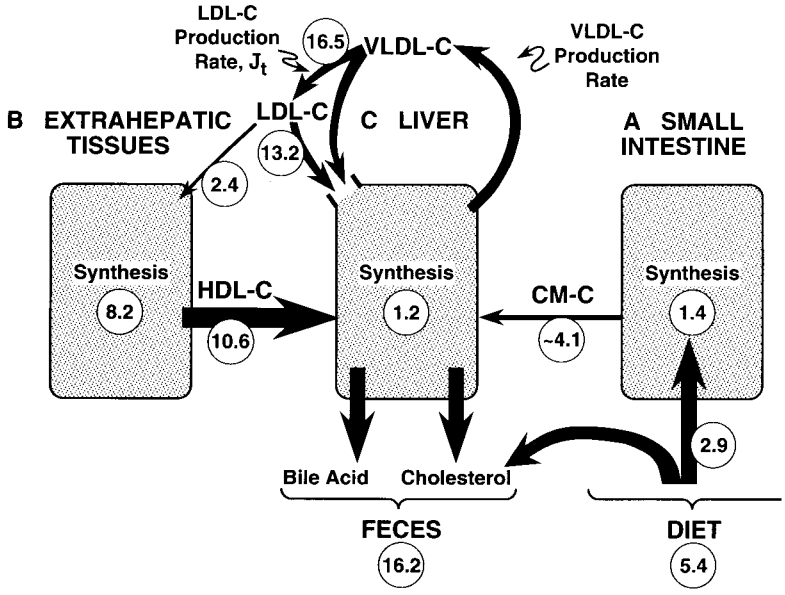

Fig. 10. Quantitation of rates of cholesterol synthesis and net sterol flux in the male cynomolgus monkey fed the low cholesterol diet. The numbers in the circles represent mg of cholesterol per day per kg of body weight (mg/day per kg). These values were all calculated from the experimental data described in the text. To simplify this diagram, the small amount of LDL-C taken up by the small intestine (0.9 mg/day per kg) is not shown. Thus, the net amount of cholesterol, either taken up from the plasma or synthesized in the small intestine, equalled 2.3 mg/day per kg. Assuming that about 54% of this sterol was also incorporated into the CM, then the net delivery of cholesterol to the liver from the intestinal epithelial cell and the diet equalled approximately 4.1 mg/day per kg.

9–11 mg/day per kg, and dietary sterol intake usually equals 4–6 mg/day per kg or about 40–60% of the endogenous synthesis rate (1). Furthermore, in the steady state the concentration of cholesterol in the liver of humans under these conditions equals 4–5 mg/g (45, 46) compared to the value observed in these monkeys of 5 mg/g (Fig. 7). Finally, the limited data available suggest that the liver is also a minor site for cholesterol synthesis in humans, as it is in these monkeys (1).

Under these steady-state conditions, the monkey is also synthesizing and degrading an amount of LDL-C equal to 1.0 pool of plasma LDL each day. In the present study this figure was obtained by summing the rates of clearance in all of the organs (Fig. 4), but it is essentially identical to that derived in this species using classical turnover techniques (26). Thus, the net amount of cholesterol flowing from VLDL to LDL, i.e., the LDL-C production rate (J_t), must equal about 16.5 mg/day per kg (Fig. 10). Fully 80% of this LDL-C (13.2 mg/day per kg) is cleared from the plasma by the liver while the remainder is distributed to the various organs in the extrahepatic compartment (2.4 mg/day per kg) and to the small intestine (0.9 mg/day per kg, not shown). As these same extrahepatic tissues synthesize 8.2 mg/day per kg, it follows that there must be a net movement of 10.6 mg/day per kg of cholesterol to the liver for excretion, as there is no accumulation of sterol in these extrahepatic organs in the steady state. Presumably, this unidirectional net flow of cholesterol from the extrahepatic tissues to the liver is accomplished by the intervention of HDL and cholesteryl ester transfer protein (51). Again, these figures are similar to those found in humans where the LDL-C production rate equals approximately 13 mg/day per kg and, in so far as data are available, the liver is also the major site for LDL-C degradation (1, 52). It should be noted in Fig. 10 that these studies provide no quantitation of the VLDL-C production rate or of that fraction of the remnants of the VLDL-C pool that is cleared directly into the liver.

When additional amounts of cholesterol are added to the diet of these monkeys, there are changes in both the rate of LDL-C production and hepatic LDL-C clearance so that when a new steady state is achieved, the plasma cholesterol concentration is increased. In the cynomolgus monkey, as in humans, this increase is almost entirely accounted for by a rise in the LDL-C fraction (Fig. 7). Furthermore, from these data it can be calculated that the increase in the plasma LDL-C concentration would equal ~24 mg/dl when the animals are challenged with an additional amount of dietary cholesterol equal to 50% of their daily sterol synthetic rate (5.4 mg/day per kg). When either newborn (53, 54) or older humans (55, 56) are challenged with similar amounts of dietary sterol, i.e., an amount equal to 50% of their respective daily synthetic rates, their observed increases in the plasma LDL-C concentration equal ~30 mg/dl and ~14 mg/dl, respectively. Thus, the increase seen in these young, sexually mature monkeys is intermediate between the increases observed in the newborn and older humans and, presumably, is similar to the values that would be anticipated in young, sexually mature human subjects.

The rate constants in Fig. 10 illustrate the remarkable similarity with respect to cholesterol metabolism and LDL-C turnover in the cynomolgus monkey and in humans. These studies also point out certain differences, however, particularly with respect to bile acid metabolism. In contrast to human bile, in the monkey the predominant bile acid found in the gallbladder is cholic acid and the great majority of these compounds is conjugated to taurine, rather than glycine (Fig. 5). Furthermore, the relative concentration of cholesterol in the bile is only 1.7 molar percent and is well below the near saturation concentrations observed in humans and, under certain dietary conditions, the African green monkey (57, 58). Even when challenged with the high cholesterol diet, this value rose to only ~2.6 molar percent. Thus, the cynomolgus monkey may not be a useful model for studying cholesterol gallstone formation.

On the basis of these observations, there are four major points that should be emphasized concerning sterol metabolism in the cynomolgus monkey. First, these studies demonstrate that it is technically feasible to make detailed, physiological measurements of cholesterol turnover and LDL-C metabolism in this primate using techniques that previously have been applied only to smaller animals like the rat, hamster, guinea pig, and rabbit (1–8). One technical problem that should be emphasized, however, is that significantly longer periods of time are required to reach a new steady state once a dietary or other physiological manipulation has been initiated. Previous studies have established that a minimum of 30 days is required in 100–200 g animals for all rate constants related to cholesterol metabolism to achieve new, constant values. During this period an animal like the hamster, which has an LDL-C fractional catabolic rate of about 4 pools/day, will turn over ~120 pools. A minimum of 120 days is required to reach a similar new steady state in a species like the cynomolgus monkey that turns over only 1 pool of LDL-C per day, while > 300 days would be required in humans that degrade only ~0.35 pools/day.

Second, these measurements confirm in the primate, for the first time, several general principles that previously have been elucidated in detail only in small animals like the hamster. In the cynomolgus monkey, as in the hamster and, apparently, humans, the rate of cholesterol synthesis in the liver is relatively low and accounts for only about 11% of whole body synthesis. The great majority of de novo sterol synthesis occurs in the extrahepatic organs where it presumably meets the needs of those tissues for daily cholesterol turnover. In contrast, in the monkey, as again in the hamster and human, the liver is the major

site for the clearance of LDL-C from the plasma (1, 3, 52). Thus, it appears that the biological function of chylomicrons and VLDL is similar. The chylomicron functions primarily to move triacylglycerol from the intestine to the extrahepatic tissues of utilization, but the remnants of these particles are cleared almost exclusively by the liver. Similarly, the VLDL particles function to move triacylglycerol from the liver to these same extrahepatic sites, and the remnants of these particles, including LDL, are also predominantly cleared by the liver. As is also evident in Fig. 10, the major function of HDL is to promote the essentially unidirectional movement of cholesterol from the sites of synthesis in the extrahepatic organs to the liver. Use of the term "reverse cholesterol transport" to describe this process is clearly inappropriate as the great majority of the sterol being moved to the liver comes from de novo synthesis in the peripheral organs.

Third, the relatively low rate of cholesterol synthesis in the liver of the cynomolgus monkey largely determines the response of the circulating LDL-C concentration to challenge by an additional load of dietary cholesterol. In the rat where the rate of hepatic cholesterol synthesis is more than 20-fold greater than that found in the monkey, hamster, or human, challenge with the high cholesterol diet used in these studies is compensated for by a marked reduction in the high rate of hepatic cholesterol synthesis and, possibly, by an increase in bile acid production (59, 60). As a consequence of this near perfect adaptation, no excess sterol flows into the ester pool, there is no suppression of hepatic LDL receptor activity, and no increase in the plasma LDL-C concentration. In contrast, the low basal level of hepatic cholesterol synthesis found in the cynomolgus monkey makes it impossible for this species to compensate for the same increase in dietary sterol load by down-regulating synthesis. As a consequence, when the new steady state is achieved, the hepatic ester pool is expanded, receptor activity is suppressed, and the plasma LDL-C concentration becomes significantly elevated (Fig. 9). Thus, because of the low basal rate of hepatic cholesterol synthesis, the cynomolgus monkey behaves very much like the hamster and human with respect to the response of the plasma LDL-C concentration to an additional challenge by dietary cholesterol.

Finally, these studies also confirm a technical observation made earlier in smaller animals that may make it possible to quantitate the rate of hepatic sterol synthesis in humans. As found in the present studies in the cynomolgus monkey and described in detail earlier in the rat and hamster (30), the amount of ^{3}H-labeled, newly synthesized cholesterol that appears in the blood 1 h after administration of the [^{3}H]water is a linear function of the rate of synthesis in the liver (Fig. 2). This relationship presumably reflects the rapid rate of molecular exchange of cholesterol between the newly synthesized pool in the liver and the pool of sterol in the blood. During this first hour, the remaining organs of the body apparently exchange little or no radiolabeled cholesterol into the blood as the level of radioactivity in this compartment decreases to virtually zero when the liver is surgically removed or hepatic synthesis is suppressed prior to administration of the [^{3}H]water. Similar relationships have recently been described in humans administered deuterated water (53, 54). With recent technical advances in the ability to detect deuterium enrichment in blood cholesterol over much shorter time intervals, the possibility exists that stable isotopes can be used to repeatedly quantitate rates of sterol synthesis specifically in the liver of human infants and adults.

In summary, these studies provide detailed, quantitative data on the metabolism of cholesterol and LDL-C in the male cynomolgus monkey. The liver plays a minor role in cholesterol synthesis in this species, but is essential for maintenance of net sterol balance in the whole animal and for the clearance of LDL-C from the plasma. Thus, in this species, as in the hamster and human, the metabolic processes within the liver dictate the response of the plasma LDL-C concentration to the intake of dietary cholesterol and fatty acids. In addition, in these out-bred animals, there is significant variability in the response of the plasma LDL-C level to a fixed dietary lipid challenge, as is also evident in human populations. Thus, this primate represents an excellent model for examining in detail the effects of specific fatty acids on hepatic LDL receptor activity and for exploring the mechanisms of action of a variety of pharmaceutical agents. In addition, this animal is probably a particularly good species in which to begin to explore the polymorphisms in specific enzymes or transporters that account for the variable response of the plasma LDL-C concentration to challenge by a fixed amount of dietary lipids.

This work was supported by U. S. Public Health Service Grants HL 09610 and HL 38074, and by a grant from the Moss Heart Fund. The authors thank Mark Herndon, Russell Nelson, Janet Hainline, and Anne Bell for their excellent technical assistance and Marty Parkey for preparation of the manuscript. We also thank Dr. Tom Clarkson, Mary Anthony, and other staff at the Bowman Gray School of Medicine for their invaluable help with the selection of these monkeys and the formulation of their diets.

Manuscript received 23 March 1994 and in revised form 8 July 1994.

REFERENCES

1. Dietschy, J. M., S. D. Turley, and D. K. Spady. 1993. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J. Lipid Res.* **34:** 1637–1659.
2. Spady, D. K., and J. M. Dietschy. 1983. Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster, and rat. *J. Lipid Res.* **24:** 303–315.
3. Spady, D. K., D. W. Bilheimer, and J. M. Dietschy. 1983. Rates of receptor-dependent and -independent low density

lipoprotein uptake in the hamster. *Proc. Natl. Acad. Sci. USA.* **80:** 3499–3503.
4. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1985. Receptor-independent low density lipoprotein transport in the rat in vivo. Quantitation, characterization, and metabolic consequences. *J. Clin. Invest.* **76:** 1113–1122.
5. Spady, D. K., M. Huettinger, D. W. Bilheimer, and J. M. Dietschy. 1987. Role of receptor-independent low density lipoprotein transport in the maintenance of tissue cholesterol balance in the normal and WHHL rabbit. *J. Lipid Res.* **28:** 32–41.
6. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1985. Rates of low density lipoprotein uptake and cholesterol synthesis are regulated independently in the liver. *J. Lipid Res.* **26:** 465–472.
7. Spady, D. K., and J. M. Dietschy. 1985. Dietary saturated triacylglycerols suppress hepatic low density lipoprotein receptor activity in the hamster. *Proc. Natl. Acad. Sci. USA.* **82:** 4526–4530.
8. Spady, D. K., and J. M. Dietschy. 1988. Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. *J. Clin. Invest.* **81:** 300–309.
9. Bonanome, A., and S. M. Grundy. 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *N. Engl. J. Med.* **318:** 1244–1248.
10. Woollett, L. A., D. K. Spady, and J. M. Dietschy. 1989. Mechanisms by which saturated triacylglycerols elevate the plasma low density lipoprotein-cholesterol concentration in hamsters. Differential effects of fatty acid chain length. *J. Clin. Invest.* **84:** 119–128.
11. Woollett, L. A., D. K. Spady, and J. M. Dietschy. 1992. Saturated and unsaturated fatty acids independently regulate low density lipoprotein receptor activity and production rate. *J. Lipid Res.* **33:** 77–88.
12. Daumerie, C. M., L. A. Woollett, and J. M. Dietschy. 1992. Fatty acids regulate hepatic low density lipoprotein receptor activity through redistribution of intracellular cholesterol pools. *Proc. Natl. Acad. Sci. USA.* **89:** 10797–10801.
13. Horton, J. D., J. A. Cuthbert, and D. K. Spady. 1993. Dietary fatty acids regulate hepatic low density lipoprotein (LDL) transport by altering LDL receptor protein and mRNA levels. *J. Clin. Invest.* **92:** 743–749.
14. Spady, D. K., L. A. Woollett, and J. M. Dietschy. 1993. Regulation of plasma LDL-cholesterol levels by dietary cholesterol and fatty acids. *Annu. Rev. Nutr.* **13:** 355–381.
15. Lofland, H. B., Jr., T. B. Clarkson, R. W. St. Clair, and N. D. M. Lehner. 1972. Studies on the regulation of plasma cholesterol levels in squirrel monkeys of two genotypes. *J. Lipid Res.* **13:** 39–47.
16. St. Clair, R. W., L. L. Wood, and T. B. Clarkson. 1981. Effect of sucrose polyester on plasma lipids and cholesterol absorption in African green monkeys with variable hypercholesterolemic response to dietary cholesterol. *Metabolism.* **30:** 176–183.
17. Bhattacharyya, A. K. and D. A. Eggen. 1981. Feedback regulation of cholesterol biosynthesis in rhesus monkeys with variable hypercholesterolemic response to dietary cholesterol. *J. Lipid Res.* **22:** 16–23.
18. Poledne, R. 1986. Cholesterol metabolism in Prague hereditary hypercholesterolemic rat. *In* Lipid Metabolism and Its Pathology. M. J. Halpern, editor. Elsevier Science Publishers, New York, NY. 185–188.
19. Beynen, A. C., M. B. Katan, and L. F. M. Van Zutphen. 1987. Hypo- and hyperresponders: individual differences in the response of serum cholesterol concentration to changes in diet. *Adv. Lipid Res.* **22:** 115–171.
20. Miettinen, T. A., and Y. A. Kesäniemi. 1989. Cholesterol absorption: regulation of cholesterol synthesis and elimination and within-population variations of serum cholesterol levels. *Am. J. Clin. Nutr.* **49:** 629–635.
21. Overturf, M. L., S. A. Smith, D. Hewett-Emmett, D. S. Loose-Mitchell, M. R. Soma, A. M. Gotto, Jr., and J. D. Morrisett. 1989. Development and partial metabolic characterization of a dietary cholesterol-resistant colony of rabbits. *J. Lipid Res.* **30:** 263–273.
22. Kushwaha, R. S., K. S. Rice, D. S. Lewis, H. C. McGill, Jr., and K. D. Carey. 1993. The role of cholesterol absorption and hepatic cholesterol content in high and low responses to dietary cholesterol and fat in pedigreed baboons (*Papio* species). *Metabolism.* **42:** 714–722.
23. Rudel, L. L., L. L. Pitts, II., and C. A. Nelson. 1977. Characterization of plasma low density lipoproteins of nonhuman primates fed dietary cholesterol. *J. Lipid Res.* **18:** 211–222.
24. Rudel, L. L., and L. L. Pitts, II. 1978. Male-female variability in the dietary cholesterol-induced hyperlipoproteinemia of cynomolgus monkeys (*Macaca fascicularis*). *J. Lipid Res.* **19:** 992–1003.
25. Malinow, M. R., W. E. Connor, P. McLaughlin, C. Stafford, D. S. Lin, A. L. Livingston, G. O. Kohler, and W. P. McNulty. 1981. Cholesterol and bile acid balance in *Macaca fascicularis:* effects of alfalfa saponins. *J. Clin. Invest.* **67:** 156–162.
26. Goldberg, I. J., N-A. Le, H. N. Ginsberg, J. R. Paterniti, Jr., and W. V. Brown. 1983. Metabolism of apoprotein B in cynomolgus monkey: evidence for independent production of low-density lipoprotein apoprotein B. *Am. J. Physiol.* **244:** E196–E201.
27. Adams, M. R., T. B. Clarkson, D. R. Koritnik, and H. A. Nash. 1987. Contraceptive steroids and coronary artery atherosclerosis in cynomolgus macaques. *Fertil. Steril.* **47:** 1010–1018.
28. Goldberg, I. J., N-A. Le, H. N. Ginsberg, R. M. Krauss, and F. T. Lindgren. 1988. Lipoprotein metabolism during acute inhibition of lipoprotein lipase in the cynomolgus monkey. *J. Clin. Invest.* **81:** 561–568.
29. Melchior, G. W., C. K. Castle, T. J. Vidmar, H. G. Polites, and K. R. Marotti. 1990. ApoA-I metabolism in cynomolgus monkeys: male-female differences. *Biochim. Biophys. Acta.* **1043:** 97–105.
30. Turley, S. D., J. M. Andersen, and J. M. Dietschy. 1981. Rates of sterol synthesis and uptake in the major organs of the rat in vivo. *J. Lipid Res.* **22:** 551–569.
31. Glass, C. K., R. C. Pittman, G. A. Keller, and D. Steinberg. 1983. Tissue sites of degradation of apoprotein A-I in the rat. *J. Biol. Chem.* **258:** 7161–7167.
32. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
33. Andersen, J. M., and J. M. Dietschy. 1978. Relative importance of high and low density lipoproteins in the regulation of cholesterol synthesis in the adrenal gland, ovary, and testis of the rat. *J. Biol. Chem.* **253:** 9024–9032.
34. Spady, D. K., J. B. Meddings, and J. M. Dietschy. 1986. Kinetic constants for receptor-dependent and receptor-independent low density lipoprotein transport in the tissues of the rat and hamster. *J. Clin. Invest.* **77:** 1474–1481.
35. 1981. Units of measurement, body fluids, composition of

the body, nutrition. In Geigy Scientific Tables. Vol. 1. C. Lenter, editor. Ciba-Geigy Corp, West Caldwell, NJ. 217–225.
36. Parks, J. S., and J. R. Crouse. 1992. Reduction of cholesterol absorption by dietary oleinate and fish oil in African green monkeys. *J. Lipid Res.* **33:** 559–568.
37. Turley, S. D., B. P. Daggy, and J. M. Dietschy. 1994. Psyllium augments the cholesterol-lowering action of cholestyramine in hamsters by enhancing sterol loss from the liver. *Gastroenterology.* **107:** 444–452.
38. Turley, S. D., M. W. Herndon, and J. M. Dietschy. 1994. Reevaluation and application of the dual-isotope plasma ratio method for the measurement of intestinal cholesterol absorption in the hamster. *J. Lipid Res.* **35:** 328–339.
39. Lepage, G., and C. C. Roy. 1986. Direct transesterification of all classes of lipids in a one-step reaction. *J. Lipid Res.* **27:** 114–120.
40. Chalvardjian, A., and E. Rudnicki. 1970. Determination of lipid phosphorus in the nanomolar range. *Anal. Biochem.* **36:** 225–226.
41. Turley, S. D., and J. M. Dietschy. 1978. Re-evaluation of the 3α-hydroxysteroid dehydrogenase assay for total bile acids in bile. *J. Lipid Res.* **19:** 924–928.
42. Turley, S. D., B. P. Daggy, and J. M. Dietschy. 1991. Cholesterol-lowering action of psyllium mucilloid in the hamster: sites and possible mechanisms of action. *Metabolism.* **40:** 1063–1073.
43. Stange, E. F., and J. M. Dietschy. 1984. Age-related decreases in tissue sterol acquisition are mediated by changes in cholesterol synthesis and not low density lipoprotein uptake in the rat. *J. Lipid Res.* **25:** 703–713.
44. Dietschy, J. M., and D. K. Spady. 1984. Measurement of rates of cholesterol synthesis using tritiated water. *J. Lipid Res.* **25:** 1469–1476.
45. Kwiterovich, P. O., Jr., H. R. Sloan, and D. S. Fredrickson. 1970. Glycolipids and other lipid constituents of normal human liver. *J. Lipid Res.* **11:** 322–330.
46. Salen, G., G. Nicolau, S. Shefer, and E. H. Mosbach. 1975. Hepatic cholesterol metabolism in patients with gallstones. *Gastroenterology.* **69:** 676–684.
47. Herz, J., U. Hamann, S. Rogne, O. Myklebost, H. Gausepohl, and K. K. Stanley. 1988. Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. *EMBO J.* **7:** 4119–4127.
48. Kowal, R. C., J. Herz, J. L. Goldstein, V. Esser, and M. S. Brown. 1989. Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. *Proc. Natl. Acad. Sci. USA.* **86:** 5810–5814.
49. Hussain, M. M., F. R. Maxfield, J. M. Oliva, I. Tabas, Z-S. Ji, T. L. Innerarity, and R. W. Mahley. 1991. Clearance of chylomicron remnants by the low density lipoprotein receptor-related protein/α_2-macroglobulin receptor. *J. Biol. Chem.* **21:** 13936–13940.
50. Breslow, J. L. 1989. Familial disorders of high density lipoprotein metabolism. *In* The Metabolic Basis of Inherited Disease. 6th ed. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 1251–1266.
51. Tall, A. R. 1993. Plasma cholesteryl ester transfer protein. *J. Lipid Res.* **34:** 1255–1274.
52. Bilheimer, D. W., J. L. Goldstein, S. M. Grundy, T. E. Starzl, and M. S. Brown. 1984. Liver transplantation to provide low-density-lipoprotein receptors and lower plasma cholesterol in a child with homozygous familial hypercholesterolemia. *N. Engl. J. Med.* **311:** 1658–1664.
53. Cruz, M. L., F. Mimouni, W. Wong, D. L. Hachey, P. Klein, and R. C. Tsang. 1991. Effects of infant nutrition on cholesterol synthesis rates in infancy. *Clin. Res.* **39:** 689A.
54. Wong, W. W., D. L. Hachey, W. Insull, A. R. Opekun, and P. D. Klein. 1993. Effect of dietary cholesterol on cholesterol synthesis in breast-fed and formula-fed infants. *J. Lipid Res.* **34:** 1403–1411.
55. Grundy, S. M., E. Barrett-Connor, L. L. Rudel, T. Miettinen, and A. A. Spector. 1988. Workshop on the impact of dietary cholesterol on plasma lipoproteins and atherogenesis. *Arteriosclerosis.* **8:** 95–101.
56. Spady, D. K., L. A. Woollett, and J. M. Dietschy. 1993. Regulation of plasma LDL-cholesterol levels by dietary cholesterol and fatty acids. *Annu. Rev. Nutr.* **13:** 355–381.
57. Scobey, M. W., F. L. Johnson, and L. L. Rudel. 1991. Plasma HDL cholesterol concentrations are correlated to bile cholesterol saturation index in the African green monkey. *Am. J. Med. Sci.* **301:** 97–101.
58. Scobey, M. W., M. S. Wolfe, and L. L. Rudel. 1992. Age- and dietary fat-related effects on biliary lipids and cholesterol gallstone formation in African green monkeys. *J. Nutr.* **122:** 917–923.
59. Wilson, J. D. 1964. The quantification of cholesterol excretion and degradation in the isotopic steady state in the rat: the influence of dietary cholesterol. *J. Lipid Res.* **5:** 409–417.
60. Spady, D. K., and J. A. Cuthbert. 1992. Regulation of hepatic sterol metabolism in the rat. *J. Biol. Chem.* **267:** 5584–5591.