Studies on the regulation of plasma cholesterol levels in squirrel monkeys of two genotypes

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Abstract Certain individual squirrel monkeys ("hyporesponders") are able to remain normocholesterolemic when fed diets containing cholesterol (0.5 mg/kcal). Other squirrel monkeys ("hyperresponders") when fed the same diet become hypercholesterolemic. The purpose of these studies was to identify the mechanisms which allow hyporesponders to compensate for dietary cholesterol.

Using formula diets and sterol balance techniques, we have compared cholesterol absorption, synthesis, excretion, and turnover in hypo- and hyperresponding monkeys. Cholesterol absorption was essentially identical in the two groups (about 55 mg/day). Cholesterol synthesis was likewise similar in the two groups (about 35 mg/day) and there was no evidence of feedback inhibition at the level of cholesterol fed. Hyporesponders had faster turnover rates and smaller body cholesterol pools than did hyperresponders. Excretion of neutral steroids was similar for hypo- and hyperresponders and did not change with cholesterol feeding. In contrast, hyporesponders increased bile acid excretion shortly after cholesterol feeding was begun. Hyperresponders responded more slowly and to a lesser degree. It is concluded that, in this species, the mechanism of control of plasma cholesterol levels is related to the rate of conversion of cholesterol to bile acids.

Supplementary key words sterol balance · hyper- and hyporesponding monkeys · neutral steroids · bile acids · cholesterol absorption · excretion and synthesis

We have recently established that genetic factors play a predominant role in enabling certain individual squirrel monkeys (*Saimiri sciureus*), termed hyporesponders, to regulate plasma cholesterol levels when fed diets containing cholesterol. Other individuals, termed hyperresponders, respond to exogenous cholesterol with marked hypercholesterolemia (1). From several feeding trials, we find that about one-fourth of any group of monkeys are hyporesponders and about one-fourth hyperresponders, with the remaining one-half having an intermediate response. Those animals that were either hypo- or hyperresponders are discernibly different on the basis of plasma cholesterol levels even when fed diets devoid of cholesterol. Even among hypercholesterolemic animals, the plasma is clear and triglyceride levels are not different from controls, generally 25–72 mg/100 ml. When plasma lipoprotein electrophoresis is carried out on paper (2), no pre-beta lipoprotein band is seen in samples from either hyper- or hyporesponders. In addition, as shown by selective breeding and genetic analysis, relatively few gene pairs, perhaps only one, are involved (1). These findings suggest that hyperresponding animals have at least some of the characteristics of the type II hyperbetalipoproteinemia of human beings described by Fredrickson, Levy, and Lees (3).

The genetically determined differences in control of plasma cholesterol levels could reside in any of several metabolic pathways, i.e., by the restriction of absorption, by control of hepatic or intestinal synthesis (or both), by regulation of the rates of excretion of neutral steroids or bile acids (or both), or by control of the rates of lipoprotein degradation. In addition, shifts of cholesterol to other body pools must be considered. Animal species appear to differ from each other in this regard. As pointed out by Beher et al. (4), rats fail to develop hypercholesterolemia when fed cholesterol because of their ability to enhance bile acid excretion, whereas rabbits lack this ability and develop extensive hypercholesterolemia and accumulate large quantities of cholesterol in most tissues and organs. Ho et al. (5) have

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

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shown that certain races of human beings, such as the Masai, regulate plasma cholesterol levels by negative feedback control of hepatic synthesis. In addition, Kaplan, Cox, and Taylor (6) and Wilson and Lindsey (7) showed that certain human beings had a limited ability to absorb cholesterol from the diet. More recently, Quintao, Grundy, and Ahrens (8) have shown that some individuals respond to dietary cholesterol by enhancing the rates of excretion of neutral steroids, but not of bile acids. Thus it is clear that a variety of mechanisms may be involved. We therefore designed the present experiments to allow us to study simultaneously, among genetically defined squirrel monkeys, rates of cholesterol absorption, synthesis, and excretion.

Another consideration affected the design of these experiments, and in addition, complicated them. When squirrel monkeys are fed cholesterol, plasma levels rise rapidly, and in a relatively short time a new steady state for plasma cholesterol is achieved, indicating that the compensatory events occur soon after the initiation of cholesterol feeding. Accordingly, the studies were designed to allow us to study steroid metabolism before and immediately after cholesterol feeding was begun, recognizing, however, that for part of the time the animals were not in the steady state in regard to steroid metabolism.

MATERIALS AND METHODS

Animals and diets

Seven male squirrel monkeys of the Brazilian type were chosen from our colony of animals originally caught from the wild in the vicinity of Leticia, Colombia, South America. The animals had been in our colony for approximately 6 yr and were mature adults. The monkeys were characterized as to their response to dietary cholesterol by feeding them, for a 3-month period, a semipurified diet (9) which contained 1 mg of cholesterol/kcal. During this time, the mean plasma cholesterol concentrations for the four hyperresponders were 1161, 1194, 1055, and 1153 mg/100 ml. Values for the three hyporesponders were 310, 203, and 304 mg/100 ml. The animals were then returned to an essentially cholesterol-free diet of Purina Special Monkey Chow-25 (Ralston-Purina Co., St. Louis, Mo.) for an additional 3 months, during which time plasma cholesterol values returned to their previous levels.

In order to carry out steroid balance studies, it was necessary to develop a liquid formula diet[1] which would maintain body weight. Cholesterol, when added, was dissolved in the warm corn oil, then blended with the other ingredients at a final concentration of 0.5 mg/kcal. The animals were adapted to handling and to the formula diet (without cholesterol) over a 5-month period, during which time frequent body weight measurements indicated no weight loss, and the animals remained healthy. During this and subsequent periods the monkeys were maintained individually in stainless steel metabolic cages with an arrangement for the drinking tubes which prevented the diet from becoming mixed with the feces. The latter were scraped completely from the bottom of the cage and kept frozen until analyzed. During the balance periods, food consumption was measured daily by weighing the drinking bottles before and after feeding.

Analytical methods

Plasma cholesterol was measured by the method of Block, Jarrett, and Levine (10) using an AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N.Y.). Radioactivity measurements were made using a Beckman DPM-100 liquid scintillation spectrometer (Beckman Instruments Corp., Fullerton, Calif.). Samples were dissolved in 10 ml of a solution of 6 g of diphenyloxazole/liter of toluene and were counted to a 2-sigma error of 3%. Correction for quenching was done using an external standard channels-ratio method. All radioactive materials ([^{14}C]cholesterol, [^{3}H]cholesterol, [^{14}C]cholic acid) were obtained from New England Nuclear Corp., Boston, Mass., and were checked for purity by TLC prior to use. All solvents used in the extractions were redistilled.

The methods used for analysis of fecal samples were the combination of isotopic and gas–liquid chromatography methods, essentially as recommended by Grundy and Ahrens (11).[2] In brief, they consisted of refluxing a sample of homogenized feces with alcoholic NaOH after adding [^{14}C]cholic acid as an internal standard for the acidic steroid fraction. The saponified mixture was extracted with Skellysolve B (Skelly Oil Co., Phillips, Okla.). After evaporation of the solvent, the residue was dissolved in ethyl acetate and an aliquot was taken for determination of radioactivity. Extraction of total neutral steroids by this procedure was essentially 100%. The remainder was separated by TLC into three fractions on plates coated with silica gel G, using ethyl ether–Skellysolve B 55:45 (v/v). The slowest-moving band contained cholesterol, campesterol, stigmasterol, β-sitosterol, and ring-saturated 5-α-sterols. Above this was a fraction containing coprostanol and ring-satu-

[1] Composition of the diet was: fat-free dry milk solids, 1680 g; complete vitamin mixture, 120 g; U.S.P. XIV salt mixture, 120 g; sucrose, 1200 g; gelatin, 80 g; corn oil, 800 g; cholesterol, when added, 9.1 g; and water, 12 liters.

[2] The authors are indebted to Drs. E. H. Ahrens, Jr. and S. M. Grundy for permitting a member of our group (RWS) to visit The Rockefeller University to learn details of their methodologies.

rated 5-β derivatives of plant steroids, and the fastest-moving fraction consisted of 3-ketocoprostanone and 3-keto derivatives of plant steroids (12). After TLC, the areas were eluted with ethyl ether and the two fastest-moving zones were combined. In squirrel monkey stool samples, only minimal amounts of radioactivity appear in these bands. Nonetheless, they were analyzed for radioactivity in every analysis. A known amount of 5-α-cholestane was added to the fraction containing cholesterol as a mass standard for GLC, and a portion of the eluted extract was counted. The remainder, after conversion to the trimethylsilyl derivatives by addition of 50 μl of Sil-Prep (Applied Science Laboratories, State College, Pa.), was separated by GLC. Separations were carried out on a 5-ft, $^1/_8$-inch, stainless steel column packed with 3% DC-560 on 100–200 Gas-Chrom Q, using a Micro-Tek model 2000R chromatograph equipped with a hydrogen flame detector (Micro-Tek Instruments, Inc., Baton Rouge, La.). The peaks were quantified using an Infotronics digital integrator (Infotronics Corp., Houston, Tex.).

After extraction of neutral steroids, 10 N NaOH was added to the residue and the mixture was saponified at 15 pounds pressure in an autoclave for 2 hr, then acidified to pH 2. Acidic steroids were extracted with chloroform–methanol 2:1, and portions of the chloroform phase were taken for radioactivity measurements. Using these methods as described, there was no "spill-over" of neutral steroids into the bile acid fraction, and by the use of internal standards and appropriate aliquots taken for radioactivity measurements both before and after TLC, we were able to correct the values obtained for losses incurred during extraction and TLC. All samples were analyzed in duplicate, and in the few instances where duplicates failed to agree by more than 5%, the analyses were repeated. In general there was excellent agreement among replicate samples. Recoveries of the isotopic internal standards ([1,2-^{3}H]-cholesterol and [26-^{14}C]cholic acid) ranged from 94 to 107% prior to TLC, and from 86 to 95% after TLC.

Grundy and Ahrens (11) have shown that there are at least two major potential sources of error in sterol balance studies in human beings. The first concerns the necessity of monitoring for fecal flow and completeness of stool collection. In preliminary experiments on squirrel monkeys, we administered chromic oxide by stomach tube and measured its appearance in the stool. Recoveries were always in excess of 90% in 24 hr. For this reason, we feel that our studies have no serious bias from this point of view. The second source of error lies in the degradation of the steroid ring, presumably by intestinal bacteria, to nonsteroidal products. When, however, [22,23-^{3}H]β-sitosterol was incorporated into the formula diet and administered to squirrel monkeys, recovery of the label in the neutral steroid fraction was complete. Thus it appears that, in squirrel monkeys, the transit time of intestinal contents is too rapid to allow extensive degradation of the steroid nucleus.

We have used the above observations as follows. It is impractical to administer chromic oxide to animals on formula diets since it settles out rapidly and the intake is uneven. The diets contain, however, significant amounts of corn oil, hence β-sitosterol. We have, accordingly, analyzed, as described above, each batch of diet prepared during the course of the experiments and have used the β-sitosterol content as a marker for completeness of recovery. For 15 balance periods, the mean fecal recovery of β-sitosterol was 104 $\pm$ 1.65% (SEM) of the amount calculated from the food consumption and β-sitosterol content of the diet. The mean cholesterol content of the 13 batches of diet to which it was added was 665 $\pm$ 6.08 μg/g.

Design of the experiment and treatment of the data

Balance studies were begun on the seven monkeys, four hyperresponders and three hyporesponders, after they had been fed the cholesterol-free formula diet for 5 months. On day 0 each animal received an intravenous injection of [1,2-^{3}H]cholesterol (1 ml, containing approximately 50 μCi, and 0.5 mg of cholesterol emulsified in 30% alcohol and one drop of Tween 20 per 10 ml of emulsion). Blood samples were drawn for measurement of plasma cholesterol specific activity on days 1, 3, 5, and 7, and at weekly intervals thereafter. Previous experiments had shown that by about day 21 the plasma specific activity reaches its final rate of exponential decay. Accordingly, between days 21 and 27, two 3-day stool collections were made and food consumption was measured. On day 28, cholesterol in the amount of 0.5 mg/kcal was added to the diet. Midway during the next week, and at weekly intervals thereafter for about 3 months, weekly 3-day stool collections were made, during which time the animals continued to consume the cholesterol-containing diet.

The data from the time-specific activity curve for plasma were analyzed as described by Gurpide, Mann, and Sandberg (13) and Goodman and Noble (14), in which cholesterol turnover is treated as a 2-pool model. From such treatment the turnover rate and the mass of the rapidly miscible pool can be determined, as can certain of the rate constants describing transfers to other body pools.

The calculation of rates of cholesterol absorption, synthesis, and excretion were made essentially as described by Grundy and Ahrens (11). Absorption was calculated by subtracting the unabsorbed dietary cholesterol from the cholesterol ingested. The former was calculated as the total fecal neutral steroid (determined

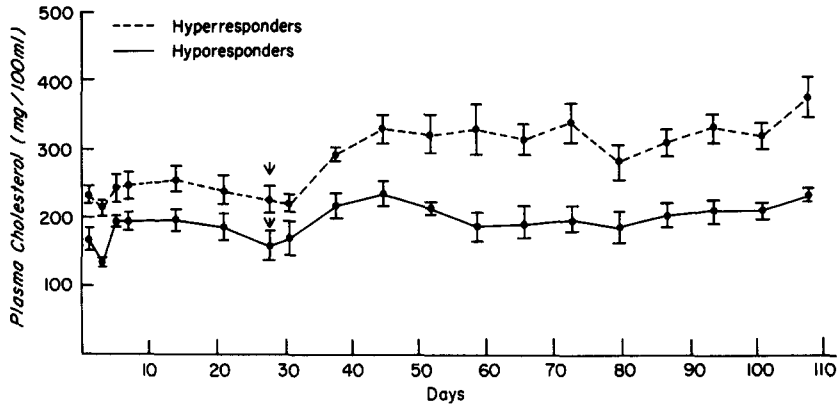

FIG. 1. Plasma cholesterol values for the two groups of monkeys during the experiment. Arrows indicate addition of cholesterol to the diet.

by GLC) minus the endogenous fecal neutral steroid. Endogenous fecal neutral steroid content was determined by dividing the total neutral steroid radioactivity by the plasma specific activity midway during the collection period. Excretion was calculated as the sum of the endogenous neutral steroids plus acidic steroids; the latter were determined by dividing the total radioactivity of the acidic steroid fraction by plasma specific activity midway during the collection period. Synthesis was calculated as the difference between excretion (endogenous neutral steroids plus bile acids) and absorption.

From these data, we were able to construct a balance sheet for 15 consecutive periods when the monkeys were first in a period of perturbation with dietary cholesterol, then finally in an apparent new plasma cholesterol steady state.

RESULTS

Plasma cholesterol

Fig. 1 depicts the plasma cholesterol values observed for 4 wk before addition of cholesterol to the diet and during the portion of the experiment after addition of dietary cholesterol. It is clear that the two classes of animals are different. There was a significant difference ($P < 0.01$) between the groups prior to the initiation of cholesterol feeding, as well as after. There appeared to be a transitory increase in both groups during the former period, which could, perhaps, reflect a response to the frequent handling and venipunctures attendant with beginning the isotope die-away part of the experiment. It can be seen that hyporesponders, after a slight initial increase, rapidly reach a plateau value which is essentially like the control period. Cholesterol values for hyperresponders also reach a plateau, but at a level that is distinctly hypercholesterolemic for this species. In both groups, however, the new steady state (at least for *plasma* cholesterol values) appears to be reached in a relatively short time.

Disappearance of labeled cholesterol from plasma

Fig. 2 is a semilogarithmic plot of plasma cholesterol specific activity vs. time after a single intravenous injection of [1,2-^{3}H]cholesterol. The values were fitted to the curve representing the sum of two exponentials

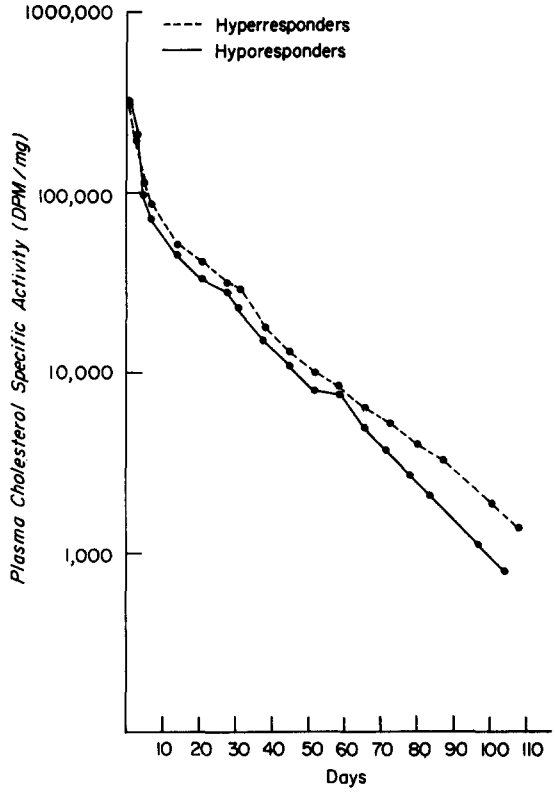

FIG. 2. Disappearance of [^{3}H]cholesterol from the plasma of hypo- and hyperresponding monkeys following a single intravenous injection.

(13) according to the formula: $SA = C_A e^{-\alpha t} + C_B e^{-\beta t}$, using an IBM 1620 computer.[3] The double exponential curves become erratic during the period when plasma cholesterol values were changing; they then become relatively linear for the remainder of the experimental period. The slope of the second exponential for the hyporesponders changed significantly ($P < 0.05$, t test) as a result of cholesterol feeding, whereas it did not for hyperresponders. The data were subjected to compartmental analysis essentially as described by Gurpide et al. (13) and by Goodman and Noble (14), in which the disappearance of isotopic cholesterol from plasma conforms to a 2-pool model, one a rapidly miscible pool (pool A), the other a series of pools having slower turnover rates (pool B). In addition, the "total traced mass" of cholesterol was calculated using the method described by Perl and Samuel (15). Hypo- and hyperresponding monkeys differ considerably in several regards: (*a*) in the mass of the rapidly miscible pool (317 ± 20 vs. 406 ± 7, $P < 0.01$); (*b*) in total traced mass (990 ± 62 vs. 1186 ± 53, $P < 0.01$); (*c*) in the rate at which new cholesterol appears in pool A (57 vs. 62 mg/day, a nonsignificant difference); and (*d*) in fractional turnover rates of cholesterol (0.179 ± 0.006 vs. 0.150 ± 0.003, $0.10 > P > 0.05$). The probability values were obtained using the t test. The findings are consonant with the hypothesis that hyporesponding squirrel monkeys have an accelerated rate of cholesterol metabolism which enables them to remain essentially normocholesterolemic.

Cholesterol absorption

In Fig. 3 the values obtained for absorption of dietary cholesterol are shown. It is immediately apparent that hyporesponders do not control plasma cholesterol levels by restricting intestinal absorption of cholesterol. Since absorption might, to some extent, be a function of the amount ingested, we compared the two groups on the basis of both intake of cholesterol and the percentage of ingested cholesterol that was absorbed. Hyporesponders ingested slightly more per day than did hyperresponders (102 ± 2.8 vs. 87 ± 2.3 mg/day). On the other hand, hyperresponders absorbed a slightly larger percentage of the amount ingested (62 ± 0.9 vs. 55 ± 1.3%). Thus, the two groups do not differ in regard to absorption, and the mean values obtained were 56 ± 1.9 and 54 ± 1.6 mg/day for hypo- and hyperresponders, respectively.

[3] SA = specific activity (dpm/mg cholesterol); C_A is the zero time intercept of the first exponential, C_B that of the second exponential. α and β are the slopes of the first and second exponentials, respectively; t = time; e = base of the natural logarithms.

Excretion of neutral steroids and bile acids

The values obtained for the excretion of neutral steroids and bile acids are shown graphically in Figs. 4 and 5. In neither group of monkeys is there a significant enhancement of excretion of neutral steroids in response to cholesterol feeding. In contrast are the data for the excretion of bile acids. Initially, bile acid excretion was less than that of neutral steroids (Figs. 4 and 5). Within 2–3 wk after cholesterol feeding was begun, the excretion of bile acids exceeded that of neutral steroids and continued to increase for the duration of the experiment. This phenomenon was especially marked among hyporesponders, and it seems likely that this mechanism is related to the ability of these animals to regulate plasma cholesterol levels. Since enhancement of bile acid excretion is seen only after a few weeks of cholesterol feeding, hyper- and hyporesponders were compared during the last seven weeks of the experiment, using analysis of variance. The difference between the two groups was significant ($P < 0.05$).

Synthesis of cholesterol

We attempted to estimate cholesterol synthesis in these experiments using the formula: excretion (endogenous neutral steroid + bile acids) − absorption = synthesis. Immediately after cholesterol feeding was begun, negative values for synthesis were obtained which ranged as high as −30 mg/day when the formula above was used to calculate rates of synthesis. It is apparent that the relationship expressed above is valid only when the animal is in the steady state. When the metabolic system is perturbed, as in the present experiment, this relationship should become: excretion − absorption = synthesis − Δ pool size. It seems probable, at least in the early weeks of cholesterol feeding, that the rate of change in the size of the body cholesterol pool exceeded the rate of synthesis and resulted in the negative values obtained for synthesis, rendering them essentially meaningless. On the other hand, it was of interest to observe the mean times at which hyper- and hyporesponders stopped having negative values for synthesis (or the times at which the rate of change in pool sizes slowed or stopped). For hyporesponders, this was observed to be after 6 wk of cholesterol feeding, for hyperresponders, only after 9 wk. These data point to the rapidity with which hyporesponders establish some degree of cholesterol homeostasis.

Inasmuch as the experiments described above provided no accurate estimate of rates of cholesterol synthesis, we attempted to gain this information in another way. After the 14-wk period of cholesterol feeding, the animals were restored to a diet of Purina Monkey Chow for a 6-month period, during which time plasma cho-

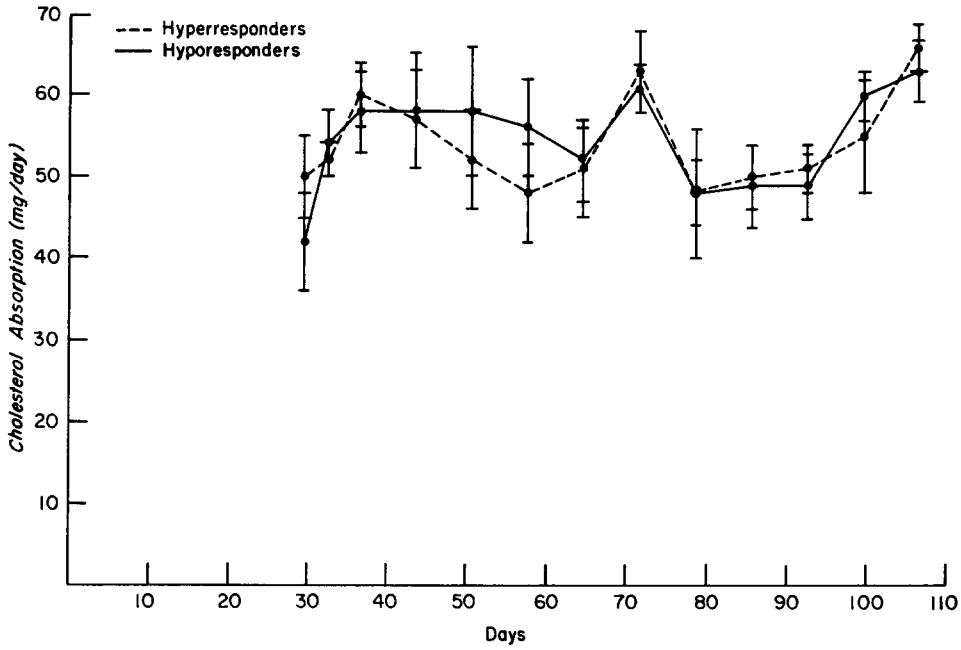

FIG. 3. Absorption of dietary cholesterol in hypo- and hyperresponding monkeys.

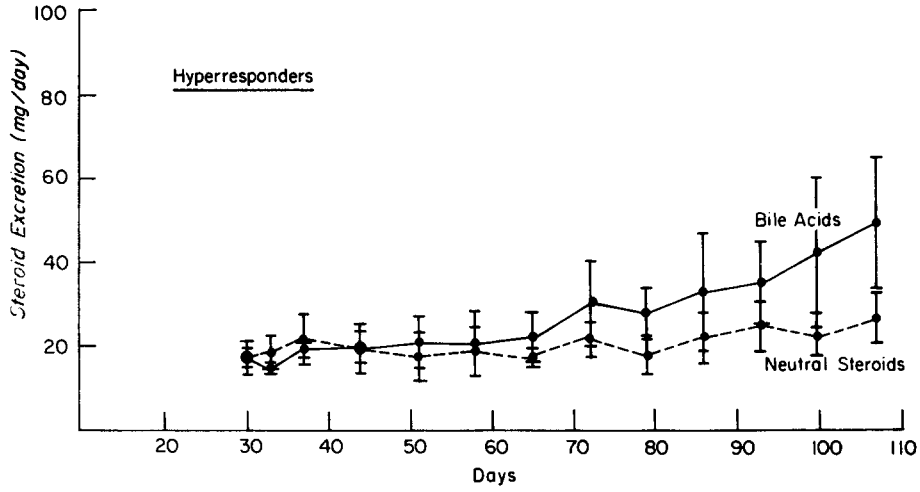

FIG. 4. Excretion of neutral steroids and bile acids in hyperresponders. Each value plotted represents the midpoint of a 3-day fecal collection period.

lesterol values returned to preexperimental levels. The animals were then injected with a single dose of [1,2-^{3}H]cholesterol, prepared as an emulsion as described previously. The size of the dose used for pulse labeling, in this case about 20 times the daily maintenance dose, was calculated as described by Zilversmit and Wentworth (16) to achieve the isotopic steady state in the shortest possible time. On the same day, the animals were placed on the formula diet used before (0.5 mg cholesterol/kcal), but in this case the diet contained [1,2-^{3}H]cholesterol (35,000 dpm/mg of cholesterol). For the next 80 days, the specific activity of plasma cholesterol was determined at weekly intervals. The results are shown in Fig. 6. The isotopic steady state was reached within about 17 days, and remained essentially constant thereafter. There was no significant difference in specific activity values for hyper- and hyporesponders, nor did the rate of synthesis appear to change as a result of cholesterol feeding. Thus, we conclude that regulation of rates of cholesterol synthesis is not the mechanism by which this primate species controls plasma cholesterol levels, at least when fed cholesterol at the relatively low levels used in this experiment. The actual rates of synthesis were calculated as follows. As shown by Morris et al. (17) by the continuous administration of isotopic cholesterol, the difference between

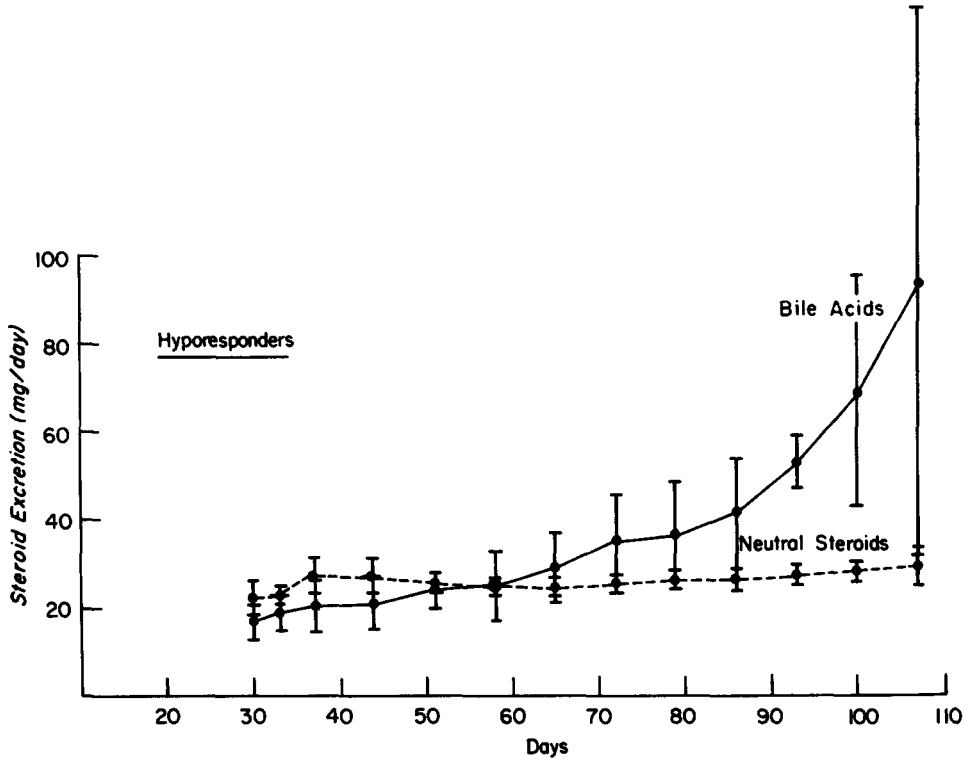

FIG. 5. Excretion of neutral steroids and bile acids in hyporesponders. Each value plotted represents the midpoint of a 3-day fecal collection period.

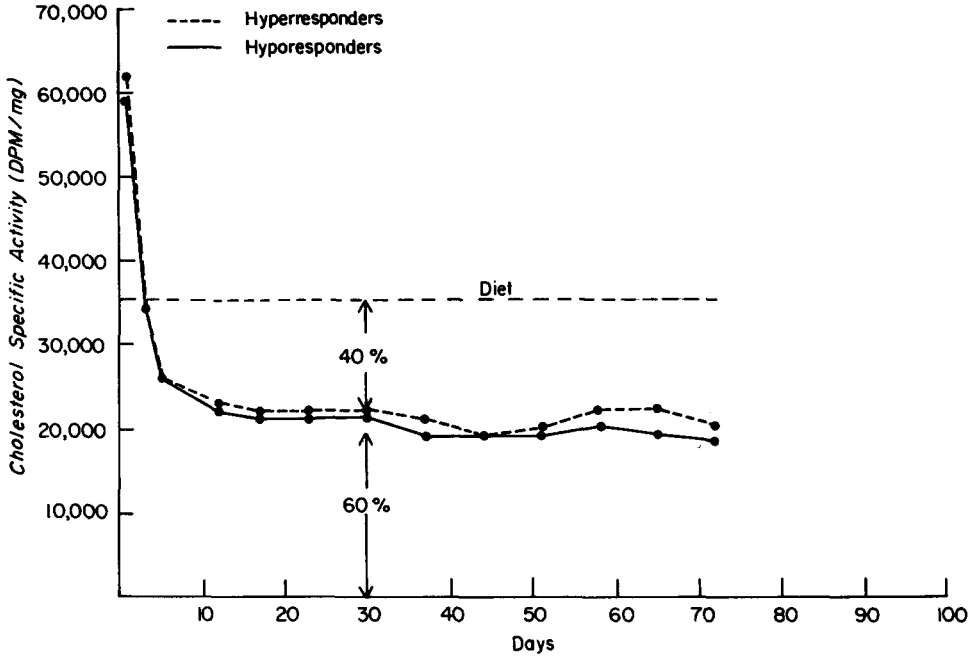

FIG. 6. Specific activity of the diet and of the plasma cholesterol of hypo- and hyperresponders when the animals were consuming a diet labeled with [^{3}H]cholesterol of constant specific activity.

plasma specific activity and that of the diet reflects the relative amount of new cholesterol appearing in the plasma as a result of synthesis. In squirrel monkeys, as is shown in Fig. 6, about 60% of the new cholesterol appearing in plasma was of dietary origin, the remainder from synthesis. From our observed values for absorption (Fig. 3), the mean value for both groups of animals was about 55 mg/day. If this value represents 60% of the

total, then about 90 mg of cholesterol was appearing each day, with approximately 35 mg/day from synthesis. This value appears to be reasonable, since the values for total steroid excretion (neutral steroids and bile acids) during the two collection periods prior to beginning cholesterol feeding, when all was coming from synthesis, ranged from 34 to 40 mg/day (Figs. 4 and 5). During the latter weeks of the experiment, excretion values should approach the 90 mg/day figure calculated above, and again, from Figs. 4 and 5, this is seen to be the case. We conclude that cholesterol synthesis is not inhibited by the levels of dietary cholesterol which were used here. This observation is generally in agreement with those of Dietschy and Wilson (18), who reported that feeding squirrel monkeys 0.2% cholesterol in the diet (a level comparable to that used here) resulted in incomplete suppression of hepatic cholesterol synthesis, on the basis of in vitro measurements.

DISCUSSION

The role played by various aspects of steroid metabolism, e.g., synthesis, absorption, and excretion, has recently been reviewed by Quintão et al. (8). A significant feature of their studies on human patients is the high degree of individual variability, especially in regard to control of excretion and synthesis. The present studies indicate that certain nonhuman primates, specifically squirrel monkeys, are also variable from individual to individual.

It seems clear from these studies that squirrel monkeys absorb cholesterol rather freely, about 60% of a dose of approximately 100 mg/day. Increased absorption is followed by an elevation of plasma levels and expansion of body cholesterol pools. That certain individuals (hyporesponders) are also able to control this expansion seems apparent.

These studies fail to provide evidence that the ability to regulate intestinal absorption is of importance to this species. Both hyper- and hyporesponding monkeys absorb to about the same extent, and no reduction of absorption was seen during the 14 wk that the animals were fed cholesterol.

Similarly, the mechanism of control does not appear to reside in the regulation of hepatic and/or intestinal synthesis. The two groups of animals were alike in this regard and, like rates of absorption, rates of synthesis failed to change in response to cholesterol feeding.

The possibility that hyporesponders maintain reduced plasma levels merely by shifting cholesterol to other tissue pools must be considered. This does not appear to be the case, since both the mass of the rapidly miscible pool, measured according to Goodman and Noble (14), and the total traced mass, measured as suggested by Perl and Samuel (15), appear to be smaller in hyporesponder monkeys. We recognize that this conclusion must be a guarded one, inasmuch as our turnover data derived from the disappearance of isotopic cholesterol were obtained, at least in part, when the animals were not in the steady state for plasma cholesterol. However, we have previously published studies on squirrel monkeys in which whole body pool sizes of cholesterol were determined by actual carcass analysis. In these studies, pool sizes were smaller in those monkeys that maintained lower levels of cholesterol in plasma (19).

Control of excretion appears to be the mechanism most likely to be responsible for the ability of hyporesponder squirrel monkeys to maintain lower plasma cholesterol levels. While the excretion of neutral steroids was not enhanced at these levels of cholesterol ingestion, there was a dramatic increase in the rate of bile acid excretion. The magnitude of this increase in hyporesponders would suggest that plasma cholesterol levels might even decrease, but this was not the case. We feel that this observation suggests some sort of "saturation" phenomenon, i.e., that during the early phases of cholesterol administration, body pools expand to some degree, but that the rate of transport of cholesterol from plasma to other pools eventually slows. The absorption and synthesis of cholesterol continues, however, and in this species further accumulation of cholesterol is prevented by the increased excretion of bile acids. Hyperresponder animals are less able to make this adjustment, or they make it more slowly. This, we feel, may explain our observation that hyperresponders, when fed cholesterol for long periods of time, accumulate sizeable amounts of cholesterol in the body and have lesions consistent with this increased accumulation, such as cutaneous and tendinous xanthomas, xanthelasma, and increased extent and severity of atherosclerosis (1).

From these studies we were unable to determine the exact site of metabolic control of plasma cholesterol levels. The data suggest that there may be, in hyporesponders, a marked enhancement of activity of the enzyme system(s) which controls the conversion of cholesterol to bile acids. There could, however, also be an increased rate of catabolism of plasma low density lipoproteins in hyporesponders. There is evidence that this may be the case in human beings having type II hyperbetalipoproteinemia (20).

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