# Effects of dietary protein on turnover, oxidation, and absorption of cholesterol, and on steroid excretion in rabbits

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**Abstract** Rabbits fed a low fat, cholesterol-free, semi-purified diet containing casein became hypercholesterolemic ($\approx$300 mg/dl) after 5 weeks on diet. Rabbits on a similar diet containing soy protein isolate had low plasma cholesterols comparable to those on commercial feed (40–60 mg/dl). Cholesterol turnover, which conformed to a two-pool model, was determined by analysis of the decay of plasma cholesterol specific activity after a single intravenous injection of [26-$^{14}$C]cholesterol. Rabbits on the soy protein diet or commercial feed showed a much faster rate of cholesterol turnover and a reduced size of pool A compared to rabbits on the casein diet. They also oxidized [26-$^{14}$C]-cholesterol to respiratory $^{14}CO_2$ at much faster rates. Analysis of fecal steroids by gas–liquid chromatography indicated that bile acid and neutral steroid excretion was increased on the soy protein and commercial diets, relative to the casein diet. Cholesterol was absorbed to a greater extent on the casein diet. Addition of 15% (w/w) butter to the semipurified diets had little effect on the above parameters of cholesterol metabolism. Comparison of cholesterol turnover measured by kinetic analysis, combined sterol balance, or analysis of fecal steroids by gas–liquid chromatography, showed that all three methods gave similar results. Measurement of bile acid production by oxidation of [26-$^{14}$C]cholesterol to respiratory $^{14}CO_2$ also gave results comparable to those obtained by analysis of fecal bile acids. —**Huff, M. W., and K. K. Carroll.** Effects of dietary protein on turnover, oxidation, and absorption of cholesterol, and on steroid excretion in rabbits. *J. Lipid Res.* 1980. **21:** 546–558.

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Hypercholesterolemia and atherosclerosis can be produced in rabbits by feeding cholesterol-free, semi-purified diets (1–3), whereas rabbits on commercial feed maintain low levels of plasma cholesterol and are free of atherosclerosis. Studies in our laboratory have shown that the protein component of the semipurified diets is primarily responsible for these effects (4, 5). Casein and other proteins from animal sources produced a hypercholesterolemia while soy protein

isolate or other plant proteins gave low levels of plasma cholesterol (4–7). This hypercholesterolemia is characterized by an increase in the cholesterol content of intermediate density lipoproteins and the atherosclerosis associated with long-term feeding of the casein semipurified diet can be completely prevented by replacing the casein with soy protein isolate (7).

A number of attempts have been made to elucidate the mechanisms by which cholesterol-free, semi-purified diets induce a hypercholesterolemia in rabbits. Hellström, Sjövall, and Wigand (8) and Hellström (9) reported that rabbits fed semipurified diets containing casein excrete less neutral steroid and produce less bile acid than rabbits fed commercial feed. Studies in our laboratory (10) showed that liver cholesterol biosynthesis, as measured by acetate incorporation into cholesterol by liver slices, was reduced in rabbits fed casein, semipurified diets compared to those on commercial feed. These studies suggest that the decreased excretion of neutral steroids and bile acids by rabbits on casein-containing, cholesterol-free, semipurified diets may lead to an increase in plasma cholesterol and a decrease in hepatic cholesterogenesis.

Since semipurified diets containing plant proteins result in low levels of plasma cholesterol comparable to those in rabbits on commercial feed, it was of interest to determine whether plant protein also increased the rate of metabolism and excretion of cholesterol relative to animal protein. This was investigated in the present studies by feeding rabbits cholesterol-free, semipurified diets containing either casein or isolated soy protein.

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Diets low in fat or containing 15% (w/w) butter were used for the experiments. Cholesterol oxidation and turnover kinetics were studied following injection of [26-$^{14}$C]cholesterol. Excretion of fecal neutral steroids and fecal and urinary bile acids was measured by GLC. These determinations made it possible to compare rates of cholesterol turnover, catabolism, and excretion by different methods. Cholesterol absorption was also measured following oral administration of [4-$^{14}$C]cholesterol and [22,23-$^3$H]β-sitosterol.

## MATERIALS AND METHODS

### Experimental animals and diets

Male New Zealand White rabbits, initially weighing about 1.5 kg, were used for these experiments. The rabbits were housed individually in metal cages with wire mesh bottoms, as described earlier (4, 5). Rabbits were fed ground Purina Rabbit Pellets (Ralston Purina Co., St. Louis, MO) for one week after arrival and were transferred gradually to the experimental diets over a 3–4 day period. Groups of six rabbits were fed either commercial diet or one of four semi-purified diets: casein-low fat; casein-high fat (15% w/w butter); soy protein isolate-low fat; and soy protein isolate-high fat (15% w/w butter). The composition of these diets, shown in **Table 1** was similar to those used in earlier experiments (4–6). Rabbits maintained on commercial diet throughout the experiments were fed Purina chow as pellets. Diets and water were provided ad libitum. Animals were weighed weekly and feed intake was measured routinely.

Blood samples from fed animals were obtained in heparinized syringes, by cardiac puncture from un-anesthetized rabbits. Slanted stainless steel trays were fitted to the bottom of the cages for collection of feces. Urine drained into a collection beaker and the feces remained on the tray.

### Isotopes

[26-$^{14}$C] and [4-$^{14}$C]cholesterol were obtained from Amersham-Searle, Oakville, Ont. [1,2-$^3$H]cholesterol, [2,4-$^3$H]cholic acid and [22,23-$^3$H]β-sitosterol were obtained from New England Nuclear, Lachine P.Q. All isotopes were shown to be greater than 98% pure by TLC on Silica Gel H. The solvent system used for cholesterol and β-sitosterol was hexane–diethyl ether–acetic acid 60:40:1 (v/v/v) and for cholic acid, toluene–1,4-dioxane–acetic acid 75:20:2 (v/v/v). All samples were counted in a Searle Mark III liquid scintillation counter. Appropriate corrections for quenching were made for both single and dual labeled samples.

TABLE 1. Composition of semipurified diets[a]

| Ingredient[b] | Low Fat | High Fat |
|---|---|---|
| | % | |
| Casein or soy protein isolate | 27 | 30 |
| Dextrose | 60 | 41 |
| Celluflour | 5 | 5 |
| Salt mixture | 4 | 5 |
| Molasses (diluted 1:1 with water) | 3 | 3 |
| Butter | 0 | 15 |
| Corn oil | 1 | 1 |
| Sterols[c] | | |
| Cholesterol | 0 | 0.02 |
| β-sitosterol | 0.002 | 0.002 |

[a] The protein content of the high-fat diet was increased to compensate for the higher caloric value of the fat. The diets also contained supplements of water-soluble and fat-soluble vitamins, as reported previously (4). The fat soluble vitamins were dissolved in the corn oil to provide a source of essential fatty acids.

[b] High nitrogen casein was obtained from ICN, Nutritional Biochemicals Division, Cleveland, OH. The casein was analyzed according to methods described previously (54) and was found to contain: protein, 93% (N × 6.25); <0.5% lipid and cholesterol, <1 mg/100 g of protein. The soy protein isolate (Promine-R) was generously donated by Dr. E. W. Meyer of Central Soya, Chicago, IL. According to the manufacturer the isolate contained (moisture-free basis): protein, 92% (N × 6.25); lipid, 0.5%; carbohydrate, 2.5% (includes crude fiber); crude fiber, 0.5%, and ash, 4.5%. The values for protein, lipid, and sterol content were confirmed by us using previously described methods (54). Butter was purchased in one lot from a local creamery and was analyzed saparately for sterols. The sources of all other dietary ingredients were as reported previously (5).

[c] Sterols were extracted from the diets (14) and the trimethylsilyl derivatives were analyzed by GLC as described in the text. The coefficient of variation for the analysis of the cholesterol and β-sitosterol content of the diets was approximately 2%. In the high fat diets the cholesterol was derived from butter. The source of β-siterosterol was the corn oil used to dissolve the fat-soluble vitamins.

### Analyses of neutral steroids and bile acids

Plasma total cholesterol was determined after saponification and extraction into petroleum ether (11) and assayed by the method of Zlatkis and Zak (12). Liver lipids were extracted as described by Folch, Lees, and Sloane Stanley (13) and analyzed for cholesterol as described for plasma. Cholesterol radioactivity was measured by evaporating aliquots of the petroleum ether extract to dryness under nitrogen and counting them in a toluene scintillator (0.55% PPO and 0.022% POPOP).

Fecal neutral steroids were extracted essentially by the methods of Miettinen, Ahrens, and Grundy (14) and bile acids by slight modifications of the methods described by Grundy, Ahrens, and Miettinen (15). The recovery of neutral steroids was determined by the addition of [1,2-$^3$H]cholesterol and of bile acids by the addition of [2,4-$^3$H]cholic acid.

Briefly, a 2 g aliquot of freeze-dried, ground feces was saponified by refluxing in 50 ml 1 N KOH in 90%

ethanol for 1.5–2 hr. The neutral steroids were extracted with petroleum ether after dilution with water and then backwashed with ethanolic KOH. The steroids were separated into three major bands by TLC as described by Miettinen et al. (14), and were analyzed by GLC as their trimethylsilyl derivatives. 5α-cholestane was used as an internal standard. Recovery of added [³H]cholesterol was usually greater than 90%. The cholesterol was counted in PCS™ Scintillator (Amersham-Searle).

The lower aqueous phase, after extraction of neutral steroids, was autoclaved for 3 hr at 120°C and 15 p.s.i. The sample was acidified and the bile acids were extracted with chloroform. The bulk of the fatty acids was removed on a 5 g Florisil column. The bile acid fraction was methylated by refluxing for 1.5 hr in dry methanolic HCl, prepared by adding 1 vol of acetyl chloride dropwise to 9 vol of dry methanol (16). The methyl esters were extracted with diethyl ether after the addition of water. The efficiency of this methylation procedure was determined by the recovery of added [2,4-³H]cholic acid as its methyl ester and was found to be 98 ± 1%. The remaining fatty acid methyl esters were separated from the methylated bile acids on 500 μm alumina TLC plates as described by Grundy et al. (15). The methyl cholanoates with free hydroxyl groups were analyzed by GLC, using 5α-cholestane-3β-ol as an internal standard. The identity of the bile acids was determined by comparison to known standards and was confirmed by GLC–mass spectrometry as described elsewhere (17). Total bile acids were accurately quantitated following conversion by mild chromic acid oxidation to their ketonic derivatives (18). 5α-cholestane-3-one was used as an internal standard. Recovery of added [³H]cholic acid, also counted in PCS, was usually greater than 75%.

Urine was freeze-dried and aliquots were analyzed for bile acids as described for feces. Cholesterol and β-sitosterol were extracted from the diets as described by Miettinen et al. (14) and the trimethylsilyl derivatives were analyzed by GLC. Standards for GLC analysis were obtained from Supelco Inc., Bellefonte, PA.

All steroids were analyzed on a Beckman GC-45 equipped with a hydrogen flame ionization detector. The stainless steel column (3/16 in × 4 ft) was packed with Supelcoport 100/120, coated with SP2250 (3% w/w). The packing materials were obtained from Supelco, Inc., Bellefonte, PA. Nitrogen was used as the carrier gas at a flow rate of 60 ml/min. The column temperature for neutral steroids was 260°C, with the injector and detector temperatures set at 275°C and 290°C respectively. All bile acid derivatives were chromatographed at a column temperature of 280°C, with the injector set at 295°C and the detector at

310°C. All samples for injection were dissolved in carbon disulfide. A disc integrator was used to quantitate the peaks. Appropriate detector response corrections were made for each of the different bile acids. Corrections were unnecessary for the neutral steroids.

## Cholesterol oxidation and turnover

To examine the oxidation and turnover of plasma cholesterol, rabbits were transferred to experimental diets, and plasma cholesterol, body weight, and feed consumption were monitored for 4 weeks. At the beginning of the fifth week, each rabbit was injected via the marginal ear vein, with 12 μCi of [26-¹⁴C]-cholesterol in a single dose, prepared as described previously (19). Rabbits were bled from the opposite ear daily (2 ml per day) for the following 7 days, on day 10, day 14, and then every 7 days up to 42 days postinjection. After the decay of plasma cholesterol specific activity became log-linear, feces and urine were collected for 5 days (days 20 through 25). In order to obtain complete fecal collections the following procedure was used. The cages and slanted stainless steel collecting trays were washed before the collection periods. Each day feces were carefully transferred to tared beakers. At the end of the 5-day collection period, the fecal samples were freeze-dried in the tared beakers and weighed. All feces were well formed pellets, therefore there was little difficulty in complete collection or separating the feces from the small amount of spilled diet. With these precautions it is highly unlikely that more than a few percent of feces could have been lost. The fecal samples were then used for the GLC determination of neutral and acidic steroids. Feed consumption was measured accurately from day 15 through day 25 in order to determine the exact daily intake of β-sitosterol. These values were used to correct the recoveries of fecal neutral steroids due to bacterial degradation (20).

On day 21 post-injection, rabbits were placed in metabolism cages and respiratory ¹⁴CO₂ was collected for two 20-min periods. All collections were made at the same time of day. The metabolism cages, constructed of clear Plexiglass, were cylindrical in shape (internal diameter, 28 cm; height, 20 cm) and had a total volume of approximately 12.5 liters. Rabbits were supported on a perforated Plexiglass disc, fitted 3 cm above the bottom of the cage, which allowed urine and feces to drop through. The methods for collection of respiratory $CO_2$ and measurement of radioactivity were as reported previously (21). The rate of oxidation of cholesterol was calculated as the amount of radioactivity expired as $^{14}CO_2$ per unit time, divided by the specific activity of total plasma cholesterol on the day of collection. Collections of $CO_2$ were made on day 21 post-injection during the log-linear portion of the

die-away curve. Errors in calculations would result if $^{14}CO_2$ was derived from cholesterol of a specific activity higher than observed in plasma on the day of collection. These errors were effectively avoided by collecting at a time when the plasma cholesterol specific activity was changing slowly. Times later than day 21 were not normally used for $CO_2$ collections because on some diets the level of radioactivity in expired $CO_2$ became a limiting factor. However, in some animals $^{14}CO_2$ was also collected on days 28 and 35, and values for cholesterol oxidation were found to remain constant with time.

Kinetic parameters of cholesterol turnover were calculated from semilogarithmic plots of plasma cholesterol specific activity versus time, as reported previously (22, 23). The die-away curves that visually appeared to best fit a two-pool model were fitted to a two-exponential function and the parameters calculated by computer, utilizing a non-linear least squares technique (24). The data were also fitted to a three-exponential function as described for man by Goodman, Noble, and Dell (25). The Fisher F statistic was used to test the decrease in the residual mean square when changing from a two-pool model to a three-pool model. The addition of a third exponential did not result in a significant reduction of the residual mean square.

After blood samples were taken on day 42 post-injection, animals were killed by an intracardiac injection of sodium pentobarbital. Livers were removed and analyzed for cholesterol concentration and specific activity.

## Cholesterol absorption

In a separate experiment, cholesterol absorption was examined in rabbits after 8 weeks on diet. The method was essentially the same as described by Borgström (26) and Quintão, Grundy, and Ahrens (27), and adapted for rabbits by Ross and Zilversmit (28). Rabbits were given an oral dose by stomach tube of [4-$^{14}$C]cholesterol and [22,23-$^3$H]$\beta$-sitosterol (1 $\mu$Ci each) dissolved in corn oil. Each dose, containing 10 mg of cholesterol, recrystallized from ethanol, was dissolved in 1 ml of corn oil. This amount of corn oil contained 3 mg of $\beta$-sitosterol. Feces were collected as described above, every 24 hours for the following 14 days. Neutral steroids were extracted and saponified from aliquots of freeze-dried, ground feces (14). An aliquot of the petroleum ether extract was evaporated under nitrogen and counted in PCS. Calculation of cholesterol absorption was carried out as described by Ross and Zilversmit (28). The absorption of $\beta$-sitosterol was assumed to be negligible (28).

Although the determination of cholesterol absorption by this method has been used for rats (29), rabbits (28, 30), and guinea pigs (31), it is possible that inaccurate results may be obtained in copraphagic species due to the reingestion of labeled steroids. As the purpose of this study was to determine the absorption of cholesterol in the rabbit under our normal laboratory conditions, the animals were not prevented from practicing coprophagy. It has been shown that the percentage of cholesterol absorbed was similar in rats that did and did not have access to their feces (32). In the present experiments it is doubtful that our results were affected by coprophagy. If in fact these rabbits were reingesting significant amounts of labeled steroids we would have expected a steady decline in the fecal [$^{14}$C]cholesterol/[$^3$H]$\beta$-sitosterol ratio as progressively more cholesterol is absorbed with each passage through the gut. However, in these experiments the ratio remained relatively constant until 7 to 10 days after the oral dose was administered. In previous experiments, it was found that the prevention of coprophagy, had no effect on the hypercholesterolemia produced by the casein semipurified diet (4).

## Statistics

The data were analyzed for variance using a 2 × 2 factorial design containing two sources of protein and two sources of fat (33). Statistical significance was determined using Duncan's Multiple Range Test (34). Significant differences between the various methods used in this study were also determined by Duncan's Multiple Range Test.

## RESULTS

### Condition of animals, plasma and liver lipids

Rabbits fed the semipurified diets and those fed commercial diet showed no significant differences in either average feed consumption or average body weight during the experimental period (**Table 2**). The fur of all animals was in good condition throughout, and there were no outward signs of any nutritional deficiency.

As in earlier studies (4–6), rabbits fed either a high or low fat, casein, semipurified diet developed significant hypercholesterolemia (**Fig. 1**). By 35 days, plasma cholesterol levels averaged 275–350 mg/dl. During this period, rabbits fed similar diets containing isolated soy protein or commercial feed maintained low plasma cholesterol levels. On day 35, an intravenous dose of [26-$^{14}$C]cholesterol was administered and the disappearance of radioactivity from plasma cholesterol was followed for 42 days. Changes in body weight and plasma cholesterol concentration were used as measures of metabolic

TABLE 2. Effect of dietary protein on concentration and specific activity of cholesterol in rabbits[a]

| Diet | Feed Consumption[b] | Body Weight[b] | Plasma Cholesterol[b] | Plasma Cholesterol Specific Activity[c] | Liver Cholesterol[c] | Liver Cholesterol Specific Activity[c] |
|---|---|---|---|---|---|---|
| | (g/day) | (kg) | (mg/dl) | (dpm × 10³/mg) | (mg/g wet wt) | (dpm × 10³/mg) |
| Casein | | | | | | |
| Low fat | 110 ± 4 | 2.7 ± 0.1 | 335 ± 36 | 5.6 ± 0.5 | 4.8 ± 0.4 | 5.9 ± 0.5 |
| High fat | 95 ± 6 | 2.8 ± 0.2 | 289 ± 38 | 4.5 ± 0.5 | 5.6 ± 0.4 | 4.2 ± 0.4 |
| Soy Protein | | | | | | |
| Low fat | 90 ± 3 | 2.8 ± 0.2 | 42 ± 5 | 2.0 ± 0.2 | 2.4 ± 0.2 | 2.0 ± 0.3 |
| High fat | 86 ± 7 | 3.0 ± 0.2 | 67 ± 7 | 2.4 ± 0.1 | 2.5 ± 0.2 | 2.5 ± 0.3 |
| Commercial | 105 ± 10 | 3.1 ± 0.1 | 63 ± 6 | 1.8 ± 0.3 | 2.9 ± 0.1 | 1.7 ± 0.2 |

[a] Results are expressed as mean ± S.E. for six rabbits in each dietary group.
[b] Mean of each dietary group from days 0 to 42 after injection of [26-¹⁴C]cholesterol.
[c] Measured on day 42 after injection of [26-¹⁴C]cholesterol.

steady state. From the time of injection until the termination of the experiment, plasma cholesterol remained constant for all dietary groups. During this same period the small increase in body weight for each dietary group (average 3%) was found to be statistically non-significant. Therefore, all animals were considered to be in a steady state with respect to cholesterol metabolism. Plasma cholesterol levels in rabbits fed the casein diets were significantly higher ($P < 0.01$) than those in rabbits fed either the soy protein diets or commercial feed. There were no significant differences between the two casein diets or between the soy protein diets and commercial feed.

On day 42 post-injection, the animals were killed and the concentration and specific activity of cholesterol were determined for liver and plasma. Table 2 shows that in rabbits fed the casein diets, the cholesterol content and specific activity of both liver and plasma were significantly higher than in rabbits fed the soy protein diets or commercial feed ($P < 0.01$). The amount of fat in the diet made little difference to the results obtained. No differences between liver and plasma cholesterol specific activity were observed on day 42 post-injection.
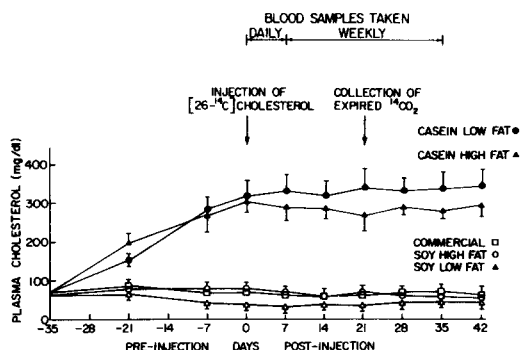
## Kinetic parameters of cholesterol turnover

Specific activity-time curves of plasma cholesterol were found to conform most closely to a two-exponential function (two-pool model) as shown in **Fig. 2.** The decay of cholesterol specific activity in the plasma of rabbits fed either commercial feed or the soy protein diets was much more rapid than in rabbits fed either casein diet. Kinetic parameters, summarized in **Table 3,** show that the plasma cholesterol concentration was highly correlated ($r = 0.84$) with the mass of pool A ($M_A$), of which the plasma is a major component. The $M_A$ in rabbits fed the casein diets was significantly higher ($P < 0.05$) than in rabbits on the soy protein diets or commercial feed. The metabolic turnover rate ($R_A$) of cholesterol was significantly



**Fig. 1.** Plasma cholesterol levels in rabbits during cholesterol turnover study, for six animals per dietary group. Values expressed as mean ± S.E.
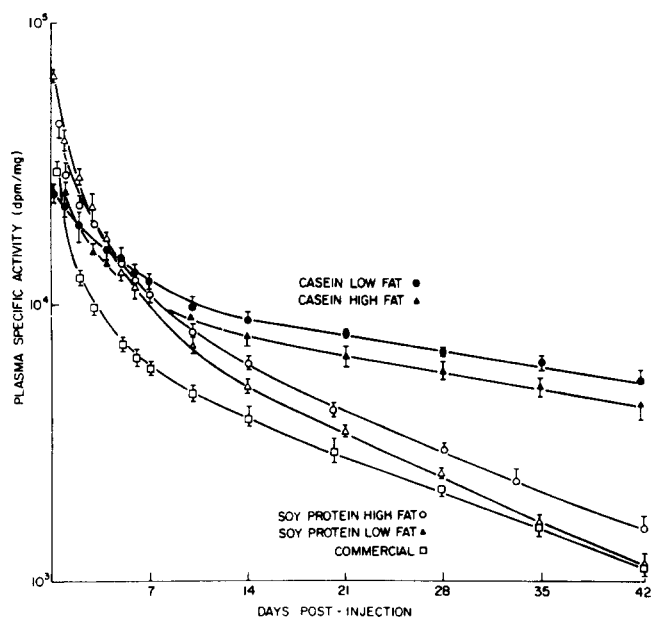


**Fig. 2.** Decay of plasma cholesterol specific activity following injection of [26-¹⁴C]cholesterol to rabbits fed semipurified diets, for six animals per dietary group. Values expressed as mean ± S.E.

TABLE 3. Effect of dietary protein on cholesterol oxidation and kinetic parameters of cholesterol turnover[a]

| Diet | $M_A$ | $R_A$ | $R_{AB}$ | $M_B$ (min) | Plasma Cholesterol[b] | Cholesterol Oxidized to $^{14}CO_2$[c] |
|---|---|---|---|---|---|---|
| | (mg) | (mg/day) | (mg/day) | (mg) | (mg/dl) | (mg/day) |
| *Casein* | | | | | | |
| Low fat | 1116 ± 99 | 46 ± 6 | 191 ± 40 | 971 ± 112 | 335 ± 36 | 13 ± 2 |
| High fat | 1283 ± 195 | 57 ± 8 | 170 ± 20 | 1392 ± 177 | 289 ± 38 | 20 ± 4 |
| *Soy protein* | | | | | | |
| Low fat | 726 ± 108 | 90 ± 5 | 153 ± 30 | 677 ± 79 | 42 ± 5 | 32 ± 2 |
| High fat | 671 ± 79 | 96 ± 14 | 110 ± 12 | 585 ± 79 | 67 ± 7 | 36 ± 2 |
| *Commercial* | 784 ± 95 | 75 ± 8 | 132 ± 32 | 478 ± 107 | 63 ± 6 | 54 ± 5 |

[a] Results expressed as mean ± S.E. for six rabbits in each dietary group. Parameters are calculated as described by Goodman and Noble (22) and Nestel, Whyte, and Goodman (23). $M_A$, mass of cholesterol in pool A; $R_A$, the metabolic turnover of cholesterol; $R_{AB}$, the transfer of cholesterol from pool A to pool B; and $M_B$, the minimum mass of pool B, assuming that cholesterol synthesis in tissues of pool B is negligible.
[b] Mean of each dietary group from days 0 to 42 after injection [26-$^{14}$C]cholesterol.
[c] Calculated as expired $^{14}CO_2$ (dpm) divided by the specific activity of plasma cholesterol, measured the same day as $CO_2$ collection, as described previously (21).

higher ($P < 0.05$) in rabbits fed commercial chow and the soy protein diets, relative to the casein diets. The transfer of cholesterol from pool A to pool B ($R_{AB}$) and the estimated size of pool B ($M_B$) were both higher for the casein diets ($P < 0.05$).

## Cholesterol oxidation

On day 21 post-injection, when a log-linear decay of plasma cholesterol specific activity was achieved (Fig. 2), animals were placed in metabolic cages for the collection of expired $CO_2$. The rates of cholesterol oxidation, calculated from the expired $^{14}CO_2$, and the plasma cholesterol specific activity, are shown in Table 3. Rabbits fed the soy protein diets oxidized cholesterol at a much faster rate ($P < 0.01$) than animals fed the casein diets. Commercial feed produced even higher rates of cholesterol oxidation ($P < 0.01$). Addition of fat to the semipurified diets produced a slight but non-significant increase in the rate of cholesterol oxidation. Comparison of the results in Table 3 showed that rates of cholesterol oxidation were inversely correlated ($r = -0.92$) with plasma cholesterol concentrations in rabbits on the different diets.

## Fecal and urinary steroid analysis by GLC

Neutral and acidic steroids were determined in feces collected from day 20 to day 25 post-injection (**Table 4**). Rabbits fed the casein diets had significantly lower neutral steroid output than those fed the soy protein diets ($P < 0.01$). Commercial feed gave an intermediate value which was not significantly different from the other dietary groups.

Fecal bile acids were identified and their relative amounts determined by GLC analysis of the methyl cholanoates with free hydroxyl groups (**Fig. 3**). The presence of non-bile acid peaks in samples from rab-

bits fed soy protein diets made quantitative measurements difficult. These peaks (Fig. 3A, peaks 3, 6, 7, 8) were analyzed by GLC–mass spectrometry and the resulting spectra indicated that these compounds were not steroids or bile acids. The methylated bile acids were converted to their ketonic derivatives, which removed most of the unidentified peaks and permitted accurate quantitation (Fig. 3B). Rabbits fed the casein diets excreted significantly lower amounts of bile acids than those fed the soy protein diets ($P < 0.01$) (Table 4). The amount of fat in the soy protein diets had little effect, but addition of butter to the casein diet significantly increased bile acid excretion ($P < 0.05$). Commercial diet produced the highest rate of bile acid excretion. The bile acids excreted in the urine were determined by GLC and the amounts were found to be unaffected by the type of dietary protein (Table 4). However, as a percentage of total bile acids, the casein-fed rabbits excreted approximately 25% of their bile acids in the urine, whereas on the soy protein diets or commercial feed, only 10% of bile acids were found in the urine.

## Cholesterol absorption

In a separate experiment, cholesterol absorption was measured after an oral dose of [4-$^{14}$C]cholesterol and [22,23-$^3$H]$\beta$-sitosterol (Table 4). A different set of rabbits was used in this experiment to avoid cross contamination of label, but these rabbits did not differ statistically from those used in the other experiments with respect to age, weight, plasma cholesterol, and length of time on diet.

Absorption was calculated as the $^{14}C/^3H$ ratio in fecal neutral steroids, divided by the $^{14}C/^3H$ ratio in the administered dose (28). Except for day 1, which gave somewhat variable results, the fecal isotope ratio was

TABLE 4. Effect of dietary protein on sterol balance in rabbits[a]

| Diet | β-Sitosterol | | | Cholesterol Intake[b] | Cholesterol Absorption[d] | | Steroids excreted[c] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Intake[b] | Fecal Excretion[c] | Fecal Recovery | | Cholesterol Absorbed[e] | Recovery of Oral [³H]β-Sitosterol[f] | Fecal Neutral Steroids[g] | Fecal Bile Acids | Urinary Bile Acids |
| | (mg/day) | | (%) | (mg/day) | (%) | | (mg/day) | | |
| *Casein* | | | | | | | | | |
| Low fat | 2.2 ± 0.03 | 1.2 ± 0.01 | 55 ± 2 | 0 | 86 ± 2 | 52 ± 2 | 20 ± 3 | 7 ± 1 | 2.2 ± 0.8 |
| High fat | 1.9 ± 0.02 | 1.3 ± 0.03 | 70 ± 4 | 19 ± 1 | 85 ± 3 | 67 ± 5 | 29 ± 3 | 18 ± 3 | 3.5 ± 0.1 |
| *Soy Protein* | | | | | | | | | |
| Low fat | 1.8 ± 0.01 | 1.2 ± 0.01 | 67 ± 1 | 0 | 74 ± 4 | 61 ± 4 | 53 ± 5 | 36 ± 3 | 2.3 ± 0.4 |
| High fat | 1.7 ± 0.01 | 1.2 ± 0.02 | 75 ± 3 | 17 ± 1 | 68 ± 4 | 70 ± 4 | 41 ± 4 | 41 ± 2 | 3.7 ± 0.5 |
| *Commercial* | 61 ± 1 | 53 ± 2 | 87 ± 2 | trace[b,h] | 73 ± 2 | 88 ± 1 | 34 ± 2 | 47 ± 4 | 5.3 ± 0.3 |

[a] Results expressed as mean ± S.E. Fecal and urinary steroids were determined for four of six rabbits (per dietary group) used in the cholesterol turnover study.

[b] Calculated from feed consumption data (Table 2) and the sterol content of the diet determined by GLC.

[c] Determined by GLC.

[d] Results expressed as mean ± S.E. for four rabbits for each dietary group.

[e] Measured after simultaneous oral administration of [³H]β-sitosterol and [¹⁴C]cholesterol dissolved in corn oil (28). Percent absorption is calculated as:

$$\left[ 1 - \frac{^{14}C/^3H \text{ in fecal neutral sterols}}{^{14}C/^3H \text{ in the administered dose}} \right] \times 100.$$

[f] Cumulative ³H recovery in fecal neutral steroids up to 14 days after the dose was administered.

[g] Neutral steroid excretion was corrected for dietary β-sitosterol recovery and values for the high fat diets were corrected for unabsorbed dietary cholesterol.

[h] Cholesterol intake for rabbits fed commercial diet was less than 1 mg/day.

initially relatively constant for all animals. However, this ratio increased in rabbits fed commercial feed after 6 days and in those fed semipurified diets after 12 days. This change was assumed to be due to excretion of absorbed, labeled cholesterol (30). The average fecal ratios from days 2 to 5 for commercial feed and days 2 through 12 for the semipurified diets were used to calculate cholesterol absorption. In order to determine the cumulative recovery of [³H]β-sitosterol, fecal collections were continued until day 14, at which time less than 0.2% of the oral dose was being excreted daily.

No differences in absorption were observed between rabbits on commercial feed or the soy protein diets (Table 4). However, significantly higher values ($P < 0.05$) were obtained for the casein diets. Little effect of dietary fat was observed. The cumulative recovery of [³H]β-sitosterol was essentially the same as the recovery of dietary β-sitosterol as determined by GLC (Table 4). The recovery was significantly lower for the semipurified diets relative to commercial feed ($P < 0.01$) indicating increased bacterial degradation of neutral steroids. A small percentage of this loss could occur during the extraction of neutral steroids from feces but in the previous experiment the recovery of added [³H]cholesterol after saponification was always greater than 98%. Inasmuch as the high fat diets contained 0.02% cholesterol, the values obtained for cholesterol absorption were used to correct

the fecal neutral steroid data, measured by GLC, for unabsorbed dietary cholesterol.

## Relative proportions of fecal steroids

The fecal neutral steroids were easily separated by TLC into three groups for analysis of GLC as described by Miettinen et al. (14). The casein-fed rabbits excreted 80–90% of their neutral steroids of endogenous origin as cholesterol (Table 5). In contrast, rabbits fed the soy protein diets or commercial feed excreted approximately 60% of their neutral steroid as coprostanol and only 30–40% as cholesterol. Coprostanone and corresponding 3-keto homologues of the three plant steroids were minor components on all diets.

Analysis of the free-hydroxy methyl cholanoates showed that rabbits fed the semipurified diets excreted lithocholic, deoxycholic and 12-keto-lithocholic (3α-hydroxy-12-keto-5β-cholanoic acid) (Table 5). In contrast, the only detectable bile acid in rabbits on commercial feed was deoxycholic. Rabbits fed the low fat, semipurified diets excreted the three bile acids in approximately equal quantities, whereas those fed the high fat diets showed an increase in deoxycholic and a corresponding decrease in lithocholic. Diet had no effect on the distribution of urinary bile acids. Deoxycholic was the major bile acid, making up 70–80% of the total, and lithocholic accounted for the remainder. Urinary neutral steroids accounted for less

than 1% of the fecal neutral steroid excretion on all diets.

## Comparison of methods for measuring cholesterol catabolism, excretion, and turnover

In steady state conditions, the loss of bile acids through excretion is replaced by oxidation of cholesterol to bile acids. Since both cholesterol oxidation and bile acid excretion were determined in this study, the results can be compared. To obtain accurate comparisons, feces and urine were collected for 5 days, during which time cholesterol oxidation rates were measured. **Table 6** shows that values for cholesterol oxidation, as determined by the $^{14}CO_2$ method, gave essentially the same results as those obtained for bile acid excretion, determined directly by GLC. The degree of similarity is remarkable despite the conceptual differences between the two methods.

In addition to determining excretion of neutral steroids directly by GLC, an isotopic sterol balance method was also used (35). Endogenous fecal neutral steroid excretion was calculated as dpm/day in the fecal neutral steroid extract, divided by the plasma cholesterol specific activity, measured 2 days prior to the midpoint of the fecal collection period. A period of 2 days was chosen because radioactivity excreted in the absorption experiments peaked 2 days after the oral dose was administered, for all diets. This provided a measure of intestinal transit time for neutral steroids (30). The fecal neutral steroids remain labeled since the isotopic carbon is only lost when the terminal carbons are removed from the [26-$^{14}$C]cholesterol. The isotopic sterol balance method gave values similar to those obtained directly by GLC only for the casein diets (Table 6). Thus, endogenous neutral steroid excretion was significantly lower than total neutral steroid excretion ($P < 0.05$) for both commercial- and soy-fed rabbits.

Table 6 shows the results obtained for total cholesterol turnover determined by GLC (column 5), combined sterol balance (column 6), and kinetic analysis (column 7). All three determinations show that the low fat, casein diet significantly reduced the rate of cholesterol turnover ($P < 0.01$), relative to the soy diet or commercial feed. The protein effect was still observed for the high fat diets ($P < 0.01$), despite the increased rate, when butter was added to the casein diet. Table 6 also shows that values for all three methods agreed well with respect to total cholesterol turnover. However, in rabbits fed the low fat, casein diet, the metabolic turnover rate determined from the kinetic analysis (column 7) gave a higher value ($P < 0.05$) than the other two methods (columns 5 and 6). The fact that
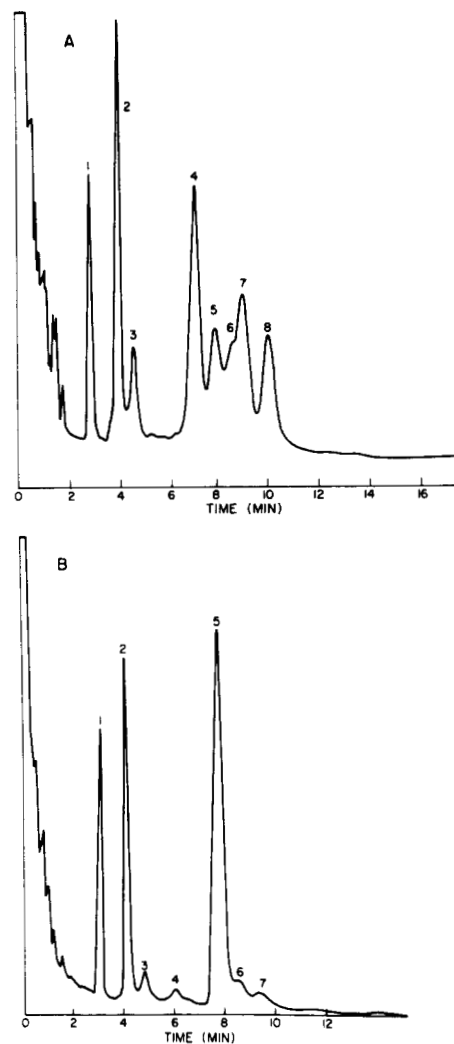


**Fig. 3.** Gas–liquid chromatography of the bile acids in feces of rabbits fed the low-fat soy protein diet. A: chromatographed as free-hydroxyl bile acid methyl esters. Peak identification: 1, 3β-hydroxy, 5α-cholestane (standard); 2, lithocholic; 3, not a bile acid (unknown); 4, deoxycholic; 5, 12-keto-lithocholic (3α-hydroxy, 12-keto, 5β-cholanic acid); 6, 7, 8, not bile acids (unknowns). B: chromatographed as ketonic derivatives of bile acid methyl esters. Peak identifications: 1, 3-keto, 5α-cholestane (standard); 2, 3-keto, 5β-cholanic acid; 3, 4, not bile acids (unknowns); 5, 3, 12-diketo, 5β-cholanic acid; 6, 7, not bile acids (unknowns). GLC conditions are as described in the text.

concordant results were obtained by all three methods, that are so different in approach, indicates that dietary protein has a real effect on the rate of cholesterol turnover in rabbits.

## DISCUSSION

The hypercholesterolemia which develops in rabbits fed cholesterol-free, semipurified diets is associated

TABLE 5. Effect of dietary protein on the relative proportions of fecal steroids in the rabbit[a]

| | Relative Proportions of Fecal Steroids | | | | | |
|---|---|---|---|---|---|---|
| | % of Total Fecal Neutral Steroids[b] | | | % of Total Fecal Bile Acids[c] | | |
| Diet | Cholesterol | Coprostanol | Coprostanone | Lithocholic | Deoxycholic | 12-Keto-Lithocholic[d] |
| *Casein* | | | | | | |
| Low fat | 90 ± 2 | 9 ± 2 | 1.0 ± 0.1 | 34 ± 4 | 37 ± 2 | 29 ± 2 |
| High fat | 82 ± 5 | 17 ± 5 | 1.2 ± 0.7 | 29 ± 3 | 44 ± 2 | 27 ± 1 |
| *Soy Protein* | | | | | | |
| Low fat | 40 ± 5 | 58 ± 5 | 2.0 ± 0.7 | 31 ± 4 | 38 ± 3 | 31 ± 2 |
| High fat | 32 ± 2 | 66 ± 2 | 2.4 ± 0.3 | 21 ± 3 | 44 ± 2 | 35 ± 2 |
| *Commercial* | 41 ± 1 | 57 ± 1 | 1.4 ± 0.1 | trace[e] | 100 | n.d.[e] |

[a] Results expressed as mean ± S.E. Fecal steroids were determined for four of six rabbits (per dietary group) used in the cholesterol turnover study.

[b] Neutral steroids were analyzed by GLC.

[c] Bile acids were analyzed by GLC as the free hydroxy methylated derivatives.

[d] 3α-Hydroxy, 12-keto-5β-cholanoic acid.

[e] In some samples lithocholic was detected, but accounted for less than 2% of the total. 12-Keto-lithocholic was not detected.

with reduced rates of fecal steroid excretion (8, 9, 36–38). This hypercholesterolemia has been shown to be due to the casein component of the semipurified diets (4–7). The present experiments strongly suggest that the casein is also responsible for the reduced rates of fecal steroid excretion. Substitution of soy protein isolate for casein resulted in increased rates of cholesterol turnover, cholesterol oxidation, and fecal steroid excretion. The soy protein diets also reduced cholesterol absorption. The results obtained with the soy protein diets were similar to those observed with commercial feed.

Kinetic parameters derived from the die-away curves (Table 3) showed that diets containing soy protein isolate produced a twofold increase in cholesterol turnover rate and a significantly smaller mass of pool A and estimated size of pool B, compared to the casein diets. Possible reasons for the difference in cholesterol turnover rate between rabbits fed casein or soy protein diets were explored by measuring rates of oxidation of cholesterol and rates of excretion of neutral steroids and bile acids. Oxidation of cholesterol, calculated from the output of $^{14}CO_2$ in expired air following administration of [26-$^{14}$C]cholesterol, oc-

TABLE 6. Comparison of methods for determination of cholesterol turnover and excretion[a]

| | Bile Acid Excretion | | Neutral Steroid Excretion | | Total Sterol Excretion | | Cholesterol Turnover[h] |
|---|---|---|---|---|---|---|---|
| | GLC Method (feces + urine) (1) | $^{14}CO_2$ Method[b] (2) | GLC Method[c,d] (3) | Isotopic Sterol Balance[d,e] (4) | GLC Method[f] (5) | Combined Sterol Balance[g] (6) | Kinetic Analysis (7) |
| | | | | *mg/day* | | | |
| *Casein* | | | | | | | |
| Low fat | 9 ± 1 | 13 ± 1 | 20 ± 3 | 14 ± 2 | 29 ± 2 | 23 ± 3 | 38 ± 4 |
| High fat | 21 ± 4 | 19 ± 4 | 29 ± 3 | 29 ± 3 | 49 ± 6 | 51 ± 6 | 49 ± 5 |
| *Soy Protein* | | | | | | | |
| Low fat | 39 ± 3 | 35 ± 2 | 53 ± 5 | 39 ± 3 | 90 ± 3 | 75 ± 4 | 86 ± 4 |
| High fat | 45 ± 2 | 41 ± 2 | 41 ± 4 | 30 ± 2 | 87 ± 3 | 75 ± 5 | 80 ± 5 |
| *Commercial* | 51 ± 1 | 54 ± 5 | 34 ± 2 | 22 ± 2 | 85 ± 2 | 74 ± 2 | 84 ± 9 |

[a] Results expressed as mean ± S.E. Fecal steroids (columns 1, 3, 5) were determined by GLC for four of six rabbits (per dietary group) used in the cholesterol turnover study. Values listed in columns 2, 4, 6, 7 determined by radioactive methods, are means for the same four rabbits (per dietary group) used for fecal steroid analysis by GLC.

[b] Calculated as expired $^{14}CO_2$ (dpm) divided by the specific activity of plasma cholesterol (21).

[c] Values for the high fat diets have been corrected for unabsorbed dietary cholesterol.

[d] Values have been corrected for recovery of dietary β-sitosterol.

[e] Calculated as dpm in fecal neutral steroid fraction divided by specific activity of plasma cholesterol, measured 2 days prior to the midpoint of the fecal collection period.

[f] Sum of columns 1 and 3.

[g] Sum of columns 1 and 4.

[h] The metabolic turnover of cholesterol ($R_A$) determined by kinetic analysis following i.v. injection of [26-$^{14}$C]cholesterol.

curred at a faster rate on the soy protein diets than on the casein diets, but not so fast as in rabbits on commercial feed (Table 3). Since bile acids are the major products of oxidation of cholesterol in mammals (39) in steady state conditions, the rate of oxidation of cholesterol should approximate bile acid excretion. As pointed out by Kim, Hamilton, and Carroll (21), the method used for estimating rates of cholesterol oxidation involves a number of assumptions and uncertainties. Therefore, bile acids were analyzed directly by GLC in order to confirm the cholesterol oxidation measurements (Table 6). Both methods gave remarkably similar results that led us to conclude that in the rabbit the measurement of cholesterol oxidation by the $^{14}CO_2$ method approximates the true rate of bile acid excretion. Also, our values for steroid excretion and cholesterol turnover rates agreed well with those reported by other investigators for rabbits fed casein, high fat, semipurified diets (8, 9) or commercial feed (8, 15, 28, 30, 38, 40).

Rabbits fed the commercial or soy protein diets excreted about 10% of total bile acids in the urine. Dietary protein had little effect on the absolute amounts excreted. However, the proportion increased to 25% in rabbits on casein diets, because of the decreased excretion of fecal bile acids. This large proportion of urinary steroid excretion in the rabbit fed commercial diet or semipurified diets has been reported previously and is thought to be related to the high concentration of bile acids in rabbit plasma (8, 30).

In the present experiments, the type of dietary protein has a significant effect on the bacterial conversion of cholesterol to coprostanol but has no apparent effect on the relative proportions of the fecal bile acids (Table 5). In rabbits fed the semipurified diets, deoxycholic, lithocholic and 12-ketolithocholic were the major bile acids detected. These were present in approximately equal proportions (Fig. 3A, Table 5). Other investigators have reported these to be the major bile acids excreted by rabbits on semipurified diets (8, 41, 42). They also found small amounts of $3\beta,12\alpha$-dihydroxycholanoic and 3,12-diketo-cholanoic acids. These may have been present in very small quantities, but were not detected under our experimental conditions. The existence of non-bile acid peaks with retention times close to identified bile acids may have obscured their detection (Fig. 3A). These unknown peaks, which were found only in the feces of rabbits fed the soy protein diets, were not bile acids as confirmed by GLC-mass spectrometry. In measuring total bile acids as their ketonic derivatives (Fig. 3B) the $3\beta,12\alpha$-dihydroxy- and 3,12-diketocholanoates, if present, would be measured along with

deoxycholic and 12-ketolithocholic as the 3,12-diketo derivatives. Consistent with previous reports (8, 42), we found no chenodeoxycholic or cholic acid in rabbit feces.

The major neutral steroids found in rabbit feces were cholesterol and coprostanol (Table 5), in agreement with previous studies (9). Rabbits fed the casein diets excreted mainly cholesterol, whereas rabbits fed the soy protein diets or commercial feed excreted coprostanol as the major neutral steroid. These results suggest that the soy protein diets and commercial diet favor the bacterial conversion of cholesterol to coprostanol. It seems probable that the intestinal bacterial population is altered by diet, as suggested by Smith (43).

Total fecal neutral steroid excretion, as measured by GLC, was significantly greater in rabbits fed the soy protein diets, compared to those fed the casein diets (Table 4). It is questionable whether the increased conversion of cholesterol to coprostanol in rabbits fed the soy protein diets or commercial feed is important with respect to their higher rate of cholesterol metabolism. Dietschy and Wilson (44) pointed out that since this bacterial reaction is limited for the most part to the terminal gastrointestinal tract, it is of marginal importance in the regulation of cholesterol metabolism.

Values for the cumulative fecal recovery of $[^3H]\beta$-siterosterol, determined in the cholesterol absorption experiments, were essentially the same as those for the fecal recovery of dietary $\beta$-sitosterol, analyzed by GLC (Table 4). This close agreement suggests that the results obtained are probably an accurate measure of intestinal degradation of neutral steroids. The recovery of $\beta$-sitosterol in rabbits fed commercial diet was significantly higher ($P < 0.01$) than for those fed the semipurified diets. Intestinal degradation of neutral steroids has been demonstrated for man (20) and for rats (29), but such losses have not been repoted previously for rabbits on semipurified diets. It would appear that the bacterial degradation of neutral steroids was independent of the bacterial conversion of cholesterol to coprostanol. Substantial degradation of $\beta$-sitosterol was observed in rabbits fed either of the semipurified diets (Table 4) despite the marked differences in coprostanol excretion (Table 5). The results obtained for cholesterol absorption and $\beta$-sitosterol recovery in rabbits on commercial feed are similar to those reported previously (28, 30).

Endogenous neutral steroid excretion was measured by isotopic sterol balance (35) and the results obtained for rabbits on commercial diet were similar to those reported by Ross and Zilversmit (28) and Klauda and Zilversmit (40). However, isotopic sterol balance

gave lower results than the GLC method, for both the commercial and soy protein diets (Table 6). The isotopic sterol balance method may give low results because it does not measure cholesterol synthesized by the intestinal tract and transferred directly into the lumen without becoming equilibrated with the plasma cholesterol pool (27). This non-exchangeable cholesterol would be measured by the GLC method. The close agreement between the two methods for rabbits fed the casein diets may be related to their increased ability to absorb cholesterol (Table 4). The increased absorption may be due to a greater bile acid pool size in rabbits fed a casein, semipurified diet relative to those on commercial feed (8). Dietschy and Wilson (44) reported that an increase in bile acid pool size decreases intestinal synthesis of cholesterol and increases cholesterol absorption.

In the comparison of different methods of estimating total steroid excretion (Table 6), the results from the GLC method (column 5) are higher than those from the combined sterol balance method (column 6) for rabbits fed commercial and soy protein diets. This is due to differences in estimated amounts of fecal neutral steroid, as discussed above. Values obtained by kinetic analysis (column 7) were remarkably similar to those obtained by the GLC method (column 5). The one discrepancy, the case of the casein-low fat diet, might be due to extrahepatic tissue deposition of cholesterol, which would be accounted for by the kinetic analysis but not by the GLC method.

As pointed out by Grundy and Ahrens (35), the turnover of endogenous cholesterol in steady state conditions equals the sum of synthesized cholesterol and absorbed dietary cholesterol. From our results with cholesterol-free diets, it can therefore be concluded that rabbits on the low fat, soy protein or commercial diets synthesize about twice as much cholesterol as those on the low fat, casein diet. A similar difference exists for the high fat diets after corrections are made for cholesterol absorbed from the diet.

Our studies have shown that, for rabbits in a steady state, cholesterol oxidation is equivalent to bile acid excretion under various dietary conditions. Cholesterol turnover, measured isotopically, was also found to be equivalent to fecal steroid excretion determined directly by GLC. The use of [26-$^{14}$C]cholesterol for simultaneous determination of cholesterol oxidation and cholesterol turnover may prove useful for study of other factors affecting cholesterol metabolism.

The results reported in this paper show clearly that replacement of casein by isolated soy protein not only prevents the hypercholesterolemia, but also increases the rates of turnover and oxidation of cholesterol and the amount of fecal steroid excreted, and decreases

cholesterol absorption. It seems probable that these mechanisms are the means by which low plasma cholesterol levels are maintained in rabbits fed the soy protein diets. Our earlier experiments suggested that the differences in plasma cholesterol may be due in part to the amino acid composition of the dietary proteins (4, 5). However, further studies will be required to elucidate the mechanisms by which dietary protein influences the turnover and metabolism of cholesterol.

The increased excretion of fecal steroids, resulting from replacement of casein by soy protein isolate in a semipurified diet, may be due to sequestration of the steroids by indigestible material in the intestine. It is doubtful that this effect is due to the small amount of crude fiber (0.5% w/w) associated with the isolated soy protein.[3] Addition of a number of different fiber sources to the casein semipurified diet (40% w/w) did not prevent the hypercholesterolemic response (45).

The hypercholesterolemia produced in rabbits by feeding a cholesterol-free, casein, semipurified diet (2, 6) can also be prevented by including high levels of corn oil in the diet (15% w/w), which initially suggested an essential fatty acid deficiency (3). This proved unlikely since the extraction of the fat from commercial feed and addition to a casein semipurified diet made little difference to the effects of these diets on either serum cholesterol or atherogenesis (46). Also, Ross, Minick, and Zilversmit (47) demonstrated that rabbits fed a low fat casein diet similar to the one used in this study, developed hypercholesterolemia and atherosclerosis, but analysis of red blood cell phospholipids did not reveal any evidence of essential fatty acid deficiency. In other experiments we have shown that increasing the levels of corn oil from 1% (w/w) to 5% (w/w) of the casein diet did not prevent the hypercholesterolemia (48).

Our studies show a marked effect of dietary protein on plasma cholesterol levels in rabbits, a species susceptible to hypercholesterolemia and atherosclerosis, and the findings may not be relevant to human nutrition. There is evidence to suggest that dietary protein may also influence human serum cholesterol levels (7, 49). A number of different dietary trials (50–52), indicate that replacement of animal protein in the diet by vegetable protein leads to a decrease in serum cholesterol. Other workers, however, failed to demonstrate an effect of dietary protein on the level of serum cholesterol (53). The effects observed in some of the above experiments may have been due to variations in dietary constituents other than protein.

Other studies from our laboratory (54) were designed to study the effect on plasma cholesterol levels

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[3] Technical bulletin, Central Soya, Chicago, IL.

of replacing animal protein in the diet by isolated soy protein, without changing the fat or sterol content. These studies in normal subjects showed that the levels of plasma cholesterol were slightly, but significantly, lower on the soy protein diet in comparison to the diet containing animal protein. Sirtori et al. (55, 56) reported a much greater decrease in plasma cholesterol levels of Type II hypercholesterolemic patients as a result of replacing animal protein in the diet by soy protein. Thus, further studies on the mechanism by which dietary protein affects plasma cholesterol levels and the exact nature of the dietary components involved may be of practical as well as theoretical interest.▊

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