EFFECT OF DIETARY CHOLESTEROL ON CHOLESTEROL AND BILE ACID METABOLISM IN RATS

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Abstract—Effect of dietary cholesterol on biliary and fecal excretion of cholesterol and bile acids was examined in rats. Cholesterol supplemented diet (2%) caused almost no change in plasma lipid levels, but produced an increase of pre β-lipoprotein and a decrease of α-lipoprotein. Dietary cholesterol increased bile secretion and biliary excretion of total bile acids but not of cholesterol. Biliary cholic and deoxycholic acids were not greatly changed but chenodeoxycholic acid was significantly increased. Dietary cholesterol also increased fecal excretion of sterols and bile acids. The excretion of coprostanol was increased about 10-fold and that of cholesterol was over 50-fold. Rat fecal bile acids consisted of lithocholic, deoxycholic, hyodeoxycholic or its 6β-isomer, α-muricholic, β-muricholic and some keto bile acids. Dietary cholesterol increased fecal excretion of mostly the secondary bile acids from chenodeoxycholic acid, and also deoxycholic acid. When a cholesterol supplemented fat-free diet was provided, no increase was found in either plasma or liver lipid levels, or in biliary excretion of bile acids, but a marked increase was produced in fecal excretion of sterols and bile acids. It is concluded that dietary cholesterol increases the formation and excretion of chenodeoxycholic acid, and also deoxycholic acid. When a cholesterol supplemented fat-free diet was provided, no increase was found in either plasma or liver lipid levels, or in biliary excretion of bile acids, but a marked increase was produced in fecal excretion of sterols and bile acids. It is concluded that dietary cholesterol increases the formation and excretion of chenodeoxycholic acid, and also that of cholic acid but to a lesser extent. The increase of bile acids, particularly chenodeoxycholic acid, is responsible for the diminution of tissue and blood cholesterol, though only a minor amount of dietary cholesterol is absorbed in rats.

Excess feeding of cholesterol causes a marked hypercholesterolemia in the rabbit, chicken or hamster while in the rat the increase is almost nil. The mouse, dog, monkey and man are also reported to be less responsive to dietary cholesterol (1, 2, 3). The reason for the species difference is not clear, but an interesting event related to the difference has been reported. The excess feeding of cholesterol markedly increases the conversion of cholesterol to bile acids in rats (4, 5), or in dogs (6), but not in rabbits (7) or in hamsters (8). These response patterns of bile acid metabolism to dietary cholesterol are consistent with changes in blood cholesterol levels in various species. Species of animals which show a good response in bile acid formation show no rise in blood cholesterol level and in cases where the response is poor, hypercholesterolemia results. In addition, Beher et al. (8, 9) have shown that cholesterol feeding decreases cholic acid pool but increases chenodeoxycholic acid pool in rats. This qualitative change may also be related to the poor response in blood

1 Some of these data was presented at the 48th Annual Meeting of the Japan Pharmacological Society (Kobe, 1975) and at the 9th International Congress on Clinical Chemistry (Toronto, 1975)
cholesterol level to dietary cholesterol in this species.

In the present experiments, the effect of dietary cholesterol on blood and liver lipid levels, biliary and fecal excretion of sterols and bile acids was examined in rats.

MATERIALS AND METHODS

Male Wistar rats weighing 200 to 300 g were maintained in an air conditioned room (25±1°C, 70% in humidity) lighted 12 hr a day (08.00 to 20.00). Either ordinary powder diet for rats (JCL-CA-1, C. J. Incorp., Tokyo) or fat-free powder diets (Oriental Kobo Kogyo Co. Ltd., Tokyo) was provided as the basal diet and cholesterol was supplemented at the rate of 2%. Rats were fed utilizing a powder diet feeding apparatus (Natsume Seisakusho Co. Ltd., Tokyo) and bottled water ad libitum.

Animals were caged individually in a specially designed cage and two-days feces were collected during the period of experiments. At the bottom of the cages were spread four or five sheets of newspapers, then a sheet of nylon filter of about 7 mm thickness (Nylon PS-150, Japan Vilene Co. Ltd., Tokyo) to absorb urine, and then a usual metal net of about 1 cm mesh making a space of about 2 cm between nylon sheet and metal net. This system was very convenient for collecting feces and avoiding a mixture with urine. Later, we found

Rat feces
dried in vacuum oven at 50°C
powdered
Extraction with abs. ethanol 
85°C, 1 hr
repeated 3 times
Saponification
1.25 N NaOH, 120°C, 6 hr
Extraction with ethylether
Ether layer
(Water layer
(Sterol fraction)
(Bile acid fraction)
GLC 1% SE-30
(GLC 1% SE-30
acidified with 2N HCl
Extraction with ethylether
Ether layer
(Water layer
(Bile acid fraction)
(Bile acid fraction)
Solvent evaporation
Extraction with pet. ether
Residue
(Pet. ether layer
(Bile acid fraction)
(GLC 3% QF-1
(Fatty acid fraction)

![Diagram of extraction and determination process](image)

**Fig. 1.** Procedures for extraction and determination of fecal sterols and bile acids.
that commercially available paper diapers are usable.

At the end of experiments, animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the bile ducts cannulated with PE-10 polyethylene tubing (14). Bile was collected exactly 30 mins from the start of cannulation, after which blood was withdrawn by heart puncture and the liver removed for lipid determination.

Plasma and liver total cholesterol levels were determined by the method reported previously (10), phospholipids by the method of Gomori (11), and triglycerides by the method of Hanahan and Olley (12) or by the acetylacetone method described by Fletcher (13). Biliary cholesterol and bile acids were determined by the method reported previously (14) and quantified by gas-liquid chromatography. Feces were dried at a reduced pressure with heat and pulverized using a small mill commercially available as a coffee mill. A portion of pulverized feces, usually 1 g, was extracted with 20 ml absolute ethanol at 85°C for 1 hr, three times (15). Extracts were filtered through a fat-free cotton pellet stuffed at the neck of a glass funnel, combined and evaporated to dryness under a reduced pressure utilizing a rotary evaporator. To the residue was added 2 to 4 ml of 1.25 N sodium hydroxide solution and the solution was hydrolyzed at 120°C for 6 hr. Sterols were extracted with ether from the reaction mixture and then bile acids after acidifying with 2 N hydrochloric acid solution, as described previously (14). Each sterol and bile acid fraction was subjected to gas-liquid chromatography (GLC) utilizing SE-30 and QF-1 column, respectively. The procedures are illustrated in Fig. 1. Serum lipoprotein pattern was analyzed by acrylamide gel electrophoresis (16) with a minor modification (17).

RESULTS

**Determination of fecal bile acids**

Determination procedures for fecal bile acids consists of three procedures; extraction, purification and quantification. We have determined rat fecal bile acids in the combination of various procedures as shown in Table 1. An aliquot of pulverized samples was subjected

| Diet        | Extraction procedure | Purification procedure | No. of Determinations | Extracted substance (mg/g feces) | Bile acids (mg) | GLC |
|-------------|----------------------|------------------------|-----------------------|----------------------------------|-----------------|-----|
| Ordinary    | Makita               | Makita                 | 3                     | 73.5                             | 12.4            | 4.1 |
| diet        | Roscoe               | Roscoe                 | 3                     | -                                | 13.5            | 3.8 |
|             | Wilson               | Makita                 | 1                     | 62.7                             | -               | 2.1 |
| Fat-free    | Makita               | Makita                 | 3                     | -                                | 2.7             |     |
| diet        | Roscoe               | Roscoe                 | 1                     | 30.8                             | 4.6             | 2.9 |
|             |                      |                        | 3                     | -                                | 4.8             | 2.9 |

Makita: Makita, M. and W. W. Wells, Anal. Biochem., 5, 523 (1963).
Roscoe: Roscoe, H. G. and M. J. Fahrenbach, Anal. Biochem., 6, 520 (1963).
Wilson: Wilson, J. D., J. Lipid Res., 2, 350 (1961).
a. Roscoe and Fahrenbach (15). b. Uchida et al. (14).
to the analysis procedures. Various combinations gave almost similar results but the quantification with titration showed higher values than with GLC. In the present experiment, the combination of extraction and purification procedures by Roscoe and Fahrenbach (15) and quantification by GLC (14) was employed, but it was later found that the purification procedure was not always necessary when determined by GLC.

**Plasma and liver lipid levels after cholesterol feeding**

Table 2 shows the effect of dietary cholesterol on plasma and liver lipid levels. Cholesterol was added to either the ordinary or fat-free diet at the rate of 2% and fed to rats *ad libitum* for a week. Plasma lipid levels were not significantly affected by dietary cholesterol in both ordinary and fat-free diet groups. Liver weight and liver cholesterol level were increased in the ordinary diet group but not in the fat-free diet group. Liver phospholipid level, as well as plasma phospholipid, was increased in the fat-free but not in the ordinary diet group. Liver triglyceride level was rather increased in the ordinary but decreased in the fat-free diet group. It is obvious from the data that when cholesterol is given without dietary fat, almost no cholesterol accumulates in the liver.

Serum lipoprotein pattern was examined in both control and cholesterol fed rats. Results shown in Table 3, indicate an increase in the ratio of pre-β-lipoprotein and a decrease in α- and β-lipoprotein.

**Biliary sterols and bile acids**

Table 4 shows the effect of dietary cholesterol on bile secretion and biliary excretion of cholesterol and bile acids. Cholesterol feeding with ordinary diet caused an increase in bile volume and biliary excretion of total bile acids but not in cholesterol excretion. In regard to changes in the components, an increase in chenodeoxycholic acid was significant while cholic acid remained unchanged. In the fat-free diet group, dietary cholesterol caused no increase in either total bile acid excretion or in the composition ratios.

**Fecal sterols and bile acids**

Table 5 shows the effect of dietary cholesterol on fecal excretion of sterols and bile acids. The mass of feces was slightly increased in both groups. Fecal excretion of sterols was significantly increased mainly in the form of cholesterol. Coprostanol was also increased but the increase was much less than that of cholesterol. The excretion of bile acids was also increased by dietary cholesterol about 2.5-fold in the ordinary diet group and about twice in the fat-free diet group. Although most of the components increased, the increase of the secondary bile acids from chenodeoxycholic acid, such as lithocholic, hyodeoxycholic, α-muricholic and β-muricholic acids, was larger than that of deoxycholic acid, suggesting that dietary cholesterol resulted in increase of the formation and excretion of chenodeoxycholic acid. These changes were similar in both groups.

**Identification of rat biliary sterols and bile acids**

Neutral fraction of rat bile showed only one large peak corresponding to cholesterol when examined by GLC. This was confirmed by gas chromatography-mass spectrometry (GC-MS). Acid fraction gave about 12 peaks. Among them lithocholic (3α), deoxycholic
Table 2. Effect of dietary cholesterol on plasma and liver lipid levels in rats

|                         | Ordinary diet |                         | Fat-free diet |                         |
|-------------------------|---------------|-------------------------|---------------|-------------------------|
|                         | Control       | Cholesterol<sup>a</sup> | Control       | Cholesterol<sup>a</sup> |
| No. of rats             | 7             | 7                       | 6             | 6                       |
| Body weight final (g)   | 243±6.7<sup>b</sup> | 237±4.3<sup>b</sup>     | 198±3.8<sup>b</sup> | 196±2.7<sup>b</sup>     |
| Body weight gain (g/week) | 32±4.6       | 26±3.6                  | 25±1.6        | 23±4.4                  |
| Plasma cholesterol (mg/100 ml) | 69±4.5 | 65±2.2                  | 50±2.9        | 56±2.7                  |
| Plasma phospholipid (mg/100 ml) | 135±7.8 | 119±6.3                 | 99±5.4        | 114±3.9<sup>p</sup>     |
| Plasma triglyceride (mg/100 ml) | 65±4.5 | 63±7.8                  | 47±5.2        | 50±6.7                  |
| Liver wt. (g/100 g BW)  | 4.1±0.09      | 4.6±0.10<sup>a</sup>    | 4.0±0.14      | 4.3±0.12                |
| Liver cholesterol (mg/g) | 2.7±0.07     | 6.9±0.27<sup>a</sup>    | 2.2±0.04      | 2.3±0.05                |
| Liver phospholipid (mg/g) | 36.5±0.45    | 35.2±0.64               | 31.2±0.9      | 33.5±0.4<sup>p</sup>    |
| Liver triglyceride (mg/g) | 7.7±0.44     | 13.5±1.43<sup>a</sup>   | 5.4±0.48      | 3.4±0.14<sup>a</sup>    |
| Diet intake (g/day/rat) | 17.6          | 17.6                    | 15.5          | 16.1                    |

<sup>a</sup> Cholesterol was supplemented at the concentration of 2% to powdered ordinary diet or fat-free diet and given to rats for one week ad libitum.
<sup>b</sup> Mean±S.E. * Statistically significant against paired control (P<0.05)

Table 3. Effect of dietary cholesterol on serum lipoprotein pattern in rats

|                 | Control       | Cholesterol<sup>a</sup> |
|-----------------|---------------|-------------------------|
| α-Lipoprotein   | 62%<sup>b</sup> | 58%<sup>b</sup>         |
| β-Lipoprotein   | 22            | 15                      |
| pre β-Lipoprotein | 16        | 27                      |

<sup>a</sup> Cholesterol was supplemented at the concentration of 2% to powdered ordinary diet and given to rats for 11 days ad libitum.
<sup>b</sup> Mean in duplicate determinations.
TABLE 4. Effect of dietary cholesterol on biliary excretion of cholesterol and bile acids in rats

|                        | Ordinary diet | Fat-free diet |
|------------------------|---------------|--------------|
|                        | Control       | Cholesterol b | Control       | Cholesterol b |
| No. of rats            | 6             | 7            | 6             | 6             |
| Body weight final (g)  | 243 ± 6.7 b   | 237 ± 4.3 b  | 198 ± 3.8 b   | 196 ± 2.7 b   |
| Bile (ml/hr/rat)       | 1.12 ± 0.069  | 1.36 ± 0.084 b | 0.83 ± 0.057  | 0.85 ± 0.043  |
| Cholesterol (mg/hr/rat)| 203 ± 20.4    | 236 ± 11.1   | 229 ± 10.7    | 203 ± 6.7     |
| Bile acids (mg/hr/rat) | 12.94 ± 1.039 | 17.22 ± 1.283 b | 5.25 ± 0.553  | 5.43 ± 0.434  |
| Lithocholic (mg/hr/rat)| 0.20 ± 0.06(2) | 0.69 ± 0.11* (4) | 0.26 ± 0.10(5) | 0.33 ± 0.14(6) |
| Deoxycholic (mg/hr/rat)| 0.32 ± 0.04(2) | 0.71 ± 0.14(4) | 0.35 ± 0.06(7) | 0.32 ± 0.06(6) |
| α-Muricholic (mg/hr/rat)| 0.09 ± 0.02(1) | 0.23 ± 0.03* (1) | 0.08 ± 0.01(2) | 0.12 ± 0.01(2) |
| Chenodeoxycholic (mg/hr/rat)| 0.81 ± 0.06(7) | 2.97 ± 0.34* (17) | 0.49 ± 0.08(9) | 0.60 ± 0.11(11) |
| Hyodeoxycholic (mg/hr/rat)| 0.23 ± 0.04(2) | 0.45 ± 0.14(3) | 0.50 ± 0.07(10) | 0.46 ± 0.14(8) |
| Cholic (mg/hr/rat)| 9.85 ± 0.79(76) | 8.94 ± 0.66(52) | 2.32 ± 0.21(44) | 2.51 ± 0.42(46) |
| Others (mg/hr/rat)| 1.44 ± 0.22(11) | 3.25 ± 0.34* (19) | 1.27 ± 0.25(24) | 1.10 ± 0.10(20) |

a, Cholesterol was supplemented at the concentration of 2% to powdered ordinary diet of fat-free diet and given to rats for one week ad libitum.
b, Mean ± S.E. * Statistically significant against paired control (P < 0.05). ( ) : Percentages in the total bile acids.
### Table 5. Effect of dietary cholesterol on fecal excretion of sterols and bile acids in rats

|                      | Ordinary diet |                      | Fat-free diet |                      |
|----------------------|---------------|----------------------|---------------|----------------------|
|                      | Control       | Cholesterol\(^{a}\) | Control       | Cholesterol\(^{a}\) |
| No. of rats          | 4             | 4                    | 6             | 6                    |
| Feces (g/day/rat)    | 5.2±0.21\(^{b}\) | 5.6±0.18\(^{b}\)    | 3.6±0.22\(^{b}\) | 4.8±0.27\(^{b}\)    |
| Total sterols (mg/day/rat) | 14.3±0.64     | 343.0±14.37\(^{c}\) | 6.3±1.45     | 349.7±27.57\(^{b}\) |
| Coprostanol (mg/day/rat) | 7.4±0.34     | 71.6±4.11\(^{*}\)  | 2.2±0.42     | 24.7±9.62\(^{*}\)  |
| Cholesterol (mg/day/rat) | 6.9±0.55     | 271.4±11.80\(^{c}\) | 4.1±1.03     | 325.0±36.13\(^{c}\) |
| Total bile acids (mg/day/rat) | 6.89±0.310    | 17.18±1.418\(^{*}\) | 2.77±0.65\(^{c}\) | 4.99±0.56\(^{c}\) |
| Lithocholic (mg/day/rat) | 0.44±0.07\(^{6}\) | 1.74±0.32\(^{*}(10)\) | 0.44±0.07\(^{16}\) | 1.05±0.04\(^{*}(21)\) |
| Deoxycholic (mg/day/rat) | 1.89±0.12\(^{27}\) | 4.06±0.39\(^{*}(24)\) | 0.20±0.07\(^{7}\) | 0.37±0.10\(^{7}\) |
| α-Muricholic (mg/day/rat) | 0.12±0.02\(^{2}\) | 0.55±0.12\(^{*}(3)\) | 0.04±0.01\(^{1}\) | 0.10±0.03\(^{2}\) |
| Hyodeoxycholic (mg/day/rat) | 2.57±0.24\(^{37}\) | 5.70±0.90\(^{*}(33)\) | 0.58±0.19\(^{21}\) | 1.03±0.40\(^{21}\) |
| β-Muricholic (mg/day/rat) | 0.51±0.10\(^{17}\) | 1.99±0.44\(^{*}(12)\) | 0.40±0.27\(^{14}\) | 0.99±0.14\(^{20}\) |
| Peak 10\(^{c}\) (mg/day/rat) | 1.17±0.20\(^{17}\) | 2.75±0.58\(^{*}(16)\) | 0.67±0.21\(^{24}\) | 0.68±0.09\(^{14}\) |
| Others (mg/day/rat)   | 0.17±0.03\(^{2}\) | 0.32±0.11\(^{12}\)  | 0.46±0.08\(^{17}\) | 0.75±0.18\(^{15}\) |

\(^{a}\) Cholesterol was supplemented at the concentration of 2% to a powdered ordinary diet or a fat-free diet and given to rats for one week \textit{ad libitum}.  
\(^{b}\) Mean±S.E.  
\(^{c}\) Mean±S.E. in 3 samples, since two rat samples each were combined.  
\(^{d}\) Unidentified, see text.  
\(^{*}\) Percentages in the total bile acids.  
\(^{*}\) Statistically significant against paired control (P<0.05).
(3α12α), α-muricholic (3α6β7α), chenodeoxycholic (3α7α), cholic (3α7α12α), and 7-oxo-lithocholic (3α7=0) acids were identified but it remained uncertain whether the peak of relative retention time (RRT) 1.35 was hyodeoxycholic (3α6α) or its 6β-isomer, 3α,6β-dihydroxycholanoic acid, since both compounds gave similar Rf, RRT, and GC-MS charts.

Fig. 2 shows typical gas-liquid chromatograms of fecal sterols of rats maintained on an ordinary diet and a fat-free diet. Coprostanol and cholesterol were identified by GC-MS. A minor peak following cholesterol probably corresponded to β'-cholestenol. In feces from rats on an ordinary diet, sterols corresponding to campesterol, β-sitosterol and their metabolites were found.

Fig. 3 shows a typical gas liquid chromatogram of fecal bile acids from a rat on an ordinary diet. Lithocholic, deoxycholic, hyodeoxycholic or its 6β-isomer, and β-muricholic acids were found. The large peak with RRT 2.15 was not identified, though it resembled 3α-

![Fig. 2. Gas liquid chromatograms of fecal sterols on 1% SE-30 column. Ordinary diet (left) and fat free diet (right).](image1.png)

![Fig. 3. Gas liquid chromatograms of fecal bile acids (trifluoroacetate-methylester) on 3% QF-1 column. Ordinary diet.](image2.png)

![Fig. 4. Gas liquid chromatogram of α-muricholic acid (trifluoroacetate-methylester) on 3% QF-1 column.](image3.png)
FIG. 4 shows a gas liquid chromatogram of \( \alpha \)-muricholic acid, giving three peaks. The largest peak corresponds to the peak of RRT 1.15 in the chart of rat biliary bile acids and fecal bile acids (Fig. 3). Fig. 5 shows gas chromato-mass spectrograms (GC-MS) of the main peak of \( \alpha \)-muricholic acid and the peak of RRT 1.15 in biliary bile acids. From the similarities in RRT values and GC-MS charts, the peak of RRT 1.15 was considered to be \( \alpha \)-muricholic acid.

Since the structure of \( \alpha \)-muricholic acid possessed three hydroxyl elements, it was expected to exhibit a large RRT value on QF-1 column as did other trihydroxycholanoic acids, for example, hyocholic, \( \beta \)-muricholic, cholic, and allocholic acids, but in fact \( \alpha \)-muricholic acid showed such a small value as 1.15 while the other acids had values of about 2.0 as shown in Table 6. Thus, it was speculated that the main peak of RRT 1.15 (Fig. 4) was a decomposed product of \( \alpha \)-muricholic acid. However, GC-MS chart of this bile acid gave the M+ peak (m/e 710), suggesting that the peak of RRT 1.15 comprised intact molecules of \( \alpha \)-muricholic acid tri-(trifluoroacetate)-methylester. It is interesting that only \( \alpha \)-muricholic acid among all trihydroxycholanoic acids gave such a small value of retention time on GLC.

**Effect of diet**

Effects of a fat-free diet feeding were also examined. Compared with an ordinary diet, a fat-free diet caused a slight decrease in plasma and liver lipid levels (Table 2), a decrease

| Compounds       | RRT  |
|-----------------|------|
| Deoxycholic     | 1.00 |
| \( \alpha \)-Muricholic | 1.15 |
| Hyocholic       | 1.87 |
| \( \beta \)-Muricholic | 1.92 |
| Cholic          | 1.98 |
| Allo-cholic     | 2.15 |

\( \alpha \), GLC on 3% QF-1 column at 230°C.
in bile volume, and biliary excretion of bile acids but not of cholesterol (Table 4). Composition ratio of cholic acid was decreased and the components of keto bile acids and unidentified peaks such as those classified as "others" in the table, increased. As shown in Table 5, the amount of feces was decreased though diet intake was not reduced (Table 2). Fecal sterols, especially coprostanol, and total bile acids were markedly decreased. Composition ratios of lithocholic and \( \beta \)-muricholic acids were increased and those of deoxycholic and hyodeoxycholic acids were decreased.

From these results, it is conceivable that a fat-free diet may cause a decrease in cholic acid formation and as a consequence, an increase in the ratio of chenodeoxycholic acid.

**DISCUSSION**

The present experiments demonstrate that dietary cholesterol causes a temporary increase in the plasma cholesterol level and a marked increase in the liver cholesterol level, biliary excretion of bile acids and fecal excretion of sterols and bile acids. In rats fed a fat-free diet, dietary cholesterol caused almost no increase in either plasma or liver cholesterol level, or biliary excretion of bile acids, but did increase fecal excretion of sterols and bile acids.

Although plasma or serum cholesterol level was not increased by dietary cholesterol (Table 2), pre \( \beta \)-lipoprotein ratio in serum was increased and \( \alpha \)-lipoprotein ratio was decreased by dietary cholesterol. This suggests a shifting of cholesterol among lipoproteins. These results are in good parallel with the results of Frnka and Reiser (18) who demonstrated that dietary cholesterol increases the synthesis and degradation of apo very low density lipoprotein (VLDL) but decreases the synthesis of apo high density lipoprotein (HDL) in rats.

It is also obvious from Table 2 that almost no cholesterol accumulated in the liver when dietary fat was excluded. This suggests that bile acids are essential for lipid absorption but the presence of neutral fat is also required.

Rat bile contains cholic, chenodeoxycholic, deoxycholic, hyodeoxycholic or its \( 6\beta \)-isomer, \( \alpha \)-muricholic, \( \beta \)-muricholic, lithocholic and some keto bile acids (14). Among them, cholic acid was a main component and occupied over 70\% in rats fed on ordinary diet and 50\% even in rats on a fat-free diet (Table 4). In contrast to this, cholesterol was a sole major sterol found in rat bile. Other sterols might be present if minute examinations were done, but such sterols were actually negligible.

In feces, lithocholic, deoxycholic, hyodeoxycholic or its \( 6\beta \)-isomer, \( \alpha \)-muricholic, \( \beta \)-muricholic and keto bile acids were the main components (Fig. 3). Cholic and chenodeoxycholic acids were negligible. Fecal sterols consisted of coprostanol, cholesterol and some phytosterols such as campesterol, \( \beta \)-sitosterol and their metabolites (Fig. 2). These phytosterols were probably derived from the diet and were not found in feces of rats on a fat-free diet. \( \Delta^2 \)-Cholestenol is reported to be found in rat feces (19, 20) and a peak showing the same retention time with that of \( \Delta^2 \)-cholestenol was found in the chart (Fig. 2), but since the peak was small and changed almost in parallel with that of cholesterol and the origin of this compound was obscure, it might be excluded from the calculation in the present ex-
The identification of hyodeoxycholic acid remained uncertain. Hyodeoxycholic acid and its 6α-isomer gave almost the same Rf and RRT values and it was difficult to determine any difference. Rat liver is reported to possess the activity of 6β-hydroxylation but not of 6α-hydroxylation. Gustafsson et al. (21) have recently demonstrated that cholesterol feeding causes an increase in the activity of 6β-hydroxylation of lithocholic acid as well as the 7α-hydroxylation of cholesterol. Therefore, if the compound is formed in rat liver, such may be 6β-hyodeoxycholic acid. However, if the compound is formed in the intestinal lumen by the action of microorganisms, some of which are known to have 6α-hydroxylation activity, the compound would be 6α-hyodeoxycholic acid. In bile fistula rats (14), the compound diminishes from the bile, supporting the postulation that the compound is a secondary bile acid.

Although bile acids found in bile and feces have yet to be fully identified, it is clear from the present results that cholesterol feeding causes an increase of chenodeoxycholic acid in bile of ordinary diet group (Table 4), and an increase of lithocholic, hyodeoxycholic or its 6β-isomer, αα- and β-muricholic acids in feces of both diet groups (Table 5). Since lithocholic, hyodeoxycholic, α-muricholic, β-muricholic or 7-oxo-lithocholic acid is considered to be derived from chenodeoxycholic acid (22), increase of these bile acids indicates the increase of chenodeoxycholic acid. These results coincide well with the reports of Wilson (4, 5) and Beher et al. (8, 9). Wilson has shown that dietary cholesterol increases fecal excretion of bile acids and Beher et al. have shown that cholesterol increases chenodeoxycholic acid, though detailed analyses of various bile acids in both bile and feces have not yet been reported.

In addition, the present results indicate that dietary cholesterol also causes an increase in fecal excretion of deoxycholic acid. Since deoxycholic acid is derived from cholic acid and not from chenodeoxycholic acid, the increase of deoxycholic acid suggests an increase of cholic acid. Beher et al. (8, 9) noted that the synthesis rate of cholic acid remained unchanged after cholesterol feeding while chenodeoxycholic acid synthesis increased. Since 7α-hydroxylation of cholesterol is increased after cholesterol feeding (21, 23), the increase of chenodeoxycholic and cholic acid excretion is considered to be a reflection of increase in their formation from cholesterol. This increase may prevent the accumulation of cholesterol in tissues and blood in rats.

The increase of chenodeoxycholic acid seems to be favorable for the decrease of cholesterol accumulation since turnover frequency for this bile acid is larger than that for cholic acid (9), and thus the increase of chenodeoxycholic acid formation may be regarded as a defence mechanism against alimentary hypercholesterolemia in this species.

Half lives of bile acids were calculated from the present data on biliary and fecal excretion of bile acid according to the formula shown below, under the assumption that the turnover frequency of bile acid was 10 times a day.

\[
\frac{1}{2} \cdot \frac{\text{Fecal bile acid (mg/day/rat)}}{\text{Biliary bile acid (mg/hr/rat)} \times 24 \times \frac{1}{10}}
\]
The half life was 2.76, 1.31, 2.76, and 1.45 days in ordinary diet controls, ordinary diet plus cholesterol, fat-free diet control, and fat-free diet plus cholesterol, respectively. These values coincide well with those reported by Beher et al. (9).

Another significant change caused by dietary cholesterol was an increase of fecal sterols (Table 5). Most of the cholesterol administered seemed to be excreted into feces, while the increase in fecal bile acids was only a few percent of the cholesterol administered. These results suggest that dietary cholesterol is scarcely absorbed in rats, at least under the conditions employed in the present experiments.

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