SPECIES DIFFERENCES OF SERUM AND LIVER CHOLESTEROL LEVELS BETWEEN RABBITS AND RATS FED ON HIGH-CHOLESTEROL DIET

SHUJI TAKAORI AND KIRO SHIMAMOTO
Department of Pharmacology, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto

Received for publication August 2, 1963

The experimental atheromatosis in various animals fed on high-fat, high-cholesterol diet has widely been used for the elucidation of the pathogenesis of clinical arteriosclerosis and for the laboratory evaluation of lipolytic agents. Page et al. (1) and Myasnikov (2) have shown that rabbits are highly susceptible to the exogenously administered cholesterol, while rats are relatively resistant to the effect of cholesterol feedings. For the sake of producing the increased level of serum cholesterol and the aortic atheromatosis in rats, the feeding of the animal on high-cholesterol diet mixed with cholic acid or taurocholic acid has been recommended (1, 3, 4). In comparative studies on the effects of lipolytic agents in rabbits, rats and chicken, Loustalot et al. (5) have emphasized that it is impossible to generalize a conclusion from experimental results of lipolytic agents obtained in one species.

In the course of a series of studies on the hypolipemic effects of thyroxine derivatives in rats and rabbits fed on high-cholesterol diet, the authors have noticed marked differences of serum and liver cholesterol levels between both species. These differences are likely to give an important clue to know the cholesterol metabolism and also the mode of action of lipolytic agents in both species.

METHODS

Male albino rabbits and male rats of Wistar strain were used. The feeding of the animals on high-cholesterol diet mixed with fatty oil was started in young rats weighing 60 to 70 g and in young rabbits weighing about 2 kg. The animal was kept individually in animal cages at a room temperature of 22±1°C. The rats were allowed to feed the standard powdered diet, CLEA-CA-1 prepared by Zikken-dobutsu Chuo-kenkyusho (The central laboratories of experimental animals in Japan) and water ad libitum. The composition of the diet was as follows: crude proteins 25.5%, crude fats 4.0%, crude fibers 4.0%, crude ashes 7.0% (Ca 1.8%, P 1.2%, K 0.5%, Na 0.3%) and crude carbohydrates 51.5%. One kilogram of the diet contained 12,000 I.U. of vitamin A, 2,400 I.U. of vitamin D, 20 mg of vitamin E, 7 mg of vitamin B₁, 10 mg of vitamin B₂, 4 mg of vitamin B₆, 80 mg of nicotinic acid, 0.2 mg of folic acid, 1,400 mg of choline, 30 mg of...
pantothenic acid and 0.02 mg of vitamin B₁₂. The rats which had gained their body weight by feeding of the standard diet for five days were divided into two groups. One group of the animals was allowed to be fed on the standard diet, as a control. Another group of the rats was fed on the standard diet mixed with cholesterol in 1%, hardened oil in 5% (ECONA oil, Kao-sekken Co.) and taurocholic acid in 0.5%. Since it has been shown that the addition of cholesterol alone to the food does not elevate the cholesterol levels in the serum of rats (4), further addition of taurocholic acid was necessary in the present experiments. The powdered diet was admitted to a diet container (Gan-ken type) about 80 ml in volume and dispersion of the food was prevented by covering with plastic plate with several holes, through which animals were allowed to eat the food. Twenty-four hours after the diet was given, the residual amounts of the food were measured and the eaten amounts of the food, hardened oil and cholesterol were calculated daily for each animal. The body weight of the animal was measured once a week.

The rabbits which had been confirmed to gain their body weight by feeding of the standard compressed diet, RC-5 (Oriental Kobo-kogyo Co.) and water ad libitum for four weeks were divided into two groups. The composition of the diet was as follows: water 7.0%, crude proteins 21.5%, crude fats 4.8% crude ashes 6.7%, crude fibers 11.5% and soluble non-nitrogen substances 48.5%. The preliminary experiments showed that the rabbits given water ad libitum took up at least 80 g/day/animal of the diet. One group of rabbits was given 80 g/day/animal of the the diet added 1% of cholesterol and 10% of soya bean oil. Another group which was fed on same amounts of the standard diet alone was used as a control. The calculation of the amounts of cholesterol and soya bean oil/day/animal and the measurement of the body weight were the same as those in the rats.

Five rats from the control group at the end of 4th week of the feeding and five to ten rats from the test group at each end of the 4th, 8th, 12th and 16th week were sampled at random and sacrificed by decapitation for the determination of cholesterol and lipid concentrations in the serum and liver. The level of total cholesterol in the serum prepared by the centrifugation of the blood at 3,000 rpm for 15 minutes was determined following ferric chloride method devised by Zak (6) and Henley (7). Cholesterol in 1 g of fresh liver was extracted with the mixture of equal amount of alcohol and ether in a boiling water bath and was determined by the same ferric chloride method. Ten grams of fresh liver collected from two to three rats in the same group was homogenized in 30 g of anhydrous sodium sulfate. Lipid in the homogenate of the liver was extracted with ten volumes of ether and was weighed as a residual obtained by the evaporation of ether. The addition of cholesterol and taurocholic acid to the standard diet was cut off at the end of 16th week and the rats were fed on the standard diet added 5% of hardened oil alone (high-fat, cholesterol-free diet) for another series of 4 weeks. After 4 weeks on these regimes, the animal were sacrificed for the determination of total cholesterol in the serum and liver and lipid in the liver.
During the feeding of the rabbits on the standard diet mixed with cholesterol and soya bean oil, the body weight, the heart rate and the total cholesterol level in the serum prepared from the blood taken from the cut marginal ear vein were measured. The heart rate was calculated from the electrocardiogram at 30 minutes after the fixation of the neck. The determination of cholesterol concentration in the serum and cholesterol and lipid concentrations in the liver were performed by the same procedures as in the rats.

The histo-pathological studies of the animals which had received the high-cholesterol diet will be described elsewhere (8).

RESULTS

1. Body Weight and General Effects

1) Rats

Fig. 1 shows the average increase of the body weight in the control and test groups of rats. There was no significant difference of the body weight gain between both groups the 7th week after the commencement of the experiments when the rats had the body weight about 250 g. Thereafter, the increase in the body weight was more marked in the test group of rats received the diet containing cholesterol and hardened oil (282 g in average increase during 16 weeks of the feeding) than in the control group (245 g in average increase during 16 weeks). The enhanced increase in the body weight is considered to derive from the uptake of cholesterol and hardened oil. The average amounts of food/day/animal showed no significant difference between both groups of rats, though the average amounts showed a gradual increasing along with that of the body weight. The removal of cholesterol and taurocholic acid from the diet at the 17th week and the further feeding of the rats on the diet mixed with hardened oil alone failed to
affect the average amounts of food/day/animal, while the same procedure inhibited slightly the body weight gain.

2) Rabbits

During four weeks of the feeding on the standard diet the rabbits showed a slight increase in the body weight from the average weight of 2.00 to 2.12 kg. The feeding of the rabbits on the standard diet added cholesterol and soya bean oil facilitated the increase in the body weight and the average body weights at the end of 2nd, 4th, 8th and 16th week were 2.35, 2.42, 2.56 and 2.78 kg, respectively. The average heart rate before, and 2, 4, 8 and 16 weeks after the commencement of the cholesterol feeding showed no significant change and was in the range of 235 to 275/min.

II. Level of Total Cholesterol in Serum

1) Rats

The level of total cholesterol in the serum taken from the rats which had been fed on the standard diet for 4 weeks andweighed about 200 g was 67.7±4.28 (standard error) mg/100 ml. Further feeding of the rats on the standard diet added cholesterol, hardened oil and taurocholic acid elevated markedly the level of total cholesterol in the serum. The level was 176.8±12.20 mg/100 ml at the 4th week and 247.6±23.02 mg/100 ml, 3.7 times of the control level, at the 8th week, as shown in Table 1 and Fig. 2. Thereafter, the level of total cholesterol in the serum declined gradually and progressively despite the further feeding of the animals on the diet containing cholesterol, hardened oil and taurocholic acid. The level of total cholesterol in the serum of rats at the 16th week of the cholesterol feeding was 148.5±10.72 mg/100 ml. The abolition of the supplement of cholesterol and taurocholic acid to the diet and the further feeding of the animals on the diet containing hardened oil alone facilitated slightly the decline of the cholesterol level in the serum. The level of total cholesterol at the 4th week after the removal of the cholesterol feeding was 107.7±4.93 mg/100 ml, only 1.6 times higher than the level before the cholesterol feeding.

Table 1. The levels of serum total cholesterol, liver total cholesterol and liver lipid of the male rats fed on high-fat, high-cholesterol diet.

<table>
<thead>
<tr>
<th>Duration (weeks)</th>
<th>Number of rats</th>
<th>Serum Total cholesterol (mg/100 ml)</th>
<th>Liver Total cholesterol (mg/g)</th>
<th>Liver Lipid (mg/g)</th>
<th>Liver Total cholesterol Lipid x100 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>67.7±4.28**</td>
<td>4.82±0.35**</td>
<td>30.5</td>
<td>15.8</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>176.8±12.20</td>
<td>38.76±3.05</td>
<td>76.2</td>
<td>50.9</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>247.6±23.02</td>
<td>36.71±3.04</td>
<td>102.1</td>
<td>36.0</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>126.0±8.94</td>
<td>29.80±4.15</td>
<td>114.8</td>
<td>26.0</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>148.5±10.72</td>
<td>21.25±4.14</td>
<td>100.2</td>
<td>21.2</td>
</tr>
<tr>
<td>20*</td>
<td>5</td>
<td>107.7±4.93</td>
<td>6.32±0.49</td>
<td>52.0</td>
<td>12.2</td>
</tr>
</tbody>
</table>

*: The rats fed on the high-fat, cholesterol-free diet after the 17th week.
**: Standard error.
Fig. 2. The levels of serum total cholesterol, liver total cholesterol and liver lipid of the male rats fed on high-fat, high-cholesterol diet.
C: Control rats.
*: The rats fed on high-fat, cholesterol-free diet after the 17th week.

2) Rabbits

The concentration of total cholesterol in the serum was 44.1±4.83 (standard error) mg/100 ml at the end of 4 weeks of the feeding on the standard diet. The feeding of the rabbits on the standard diet mixed with cholesterol and soya bean oil increased the level of total cholesterol to 259.2 mg/100 ml at the 2nd week, 440.7 mg/100 ml at the 4th week and 708.8 mg/100 ml, which was 16.1 times higher than that before the cholesterol feeding, at the 8th week. The further prolongation of the cholesterol feeding period was followed with the more pronounced elevation of cholesterol level in the

Table 2. The levels of serum total cholesterol, liver total cholesterol and liver lipid of the male rabbits fed on high-fat, high-cholesterol diet.

<table>
<thead>
<tr>
<th>Duration (weeks)</th>
<th>Serum Total cholesterol (mg/100 ml)</th>
<th>×</th>
<th>Liver Total cholesterol (mg/g)</th>
<th>Lipid (mg/g)</th>
<th>Total cholesterol Lipid ×100(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>44.1±4.83*</td>
<td></td>
<td>3.72±0.19*</td>
<td>33.2</td>
<td>11.2</td>
</tr>
<tr>
<td>2</td>
<td>259.2</td>
<td>5.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>440.7</td>
<td>10.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>886.3</td>
<td>20.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>708.8</td>
<td>16.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>860.2</td>
<td>19.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>965.6</td>
<td>21.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1220.6</td>
<td>27.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1282.9</td>
<td>29.1</td>
<td>64.41</td>
<td>113.0</td>
<td>57.0</td>
</tr>
</tbody>
</table>

*: Standard error.
serum, 1282.9 mg/100 ml at the 16th week. The stoppage of the cholesterol feeding at the end of 16th week resulted in a gradual and progressive fall of the cholesterol level in the serum. These results are shown in Table 2.

III. Levels of Total Cholesterol and Lipid in Liver

1) Rats

The levels of total cholesterol and lipid in the liver of rats fed on the standard diet for 4 weeks and weighed about 200 g were 4.82±0.35 mg/g wet tissue weight and 30.5 mg/g wet tissue weight, respectively. As shown in Table 1 and Fig. 2, the feeding of the animals on the diet containing cholesterol, hardened oil and taurocholic acid elevated both levels in the liver. The concentration of the liver cholesterol at the 4th week of the cholesterol feeding increased to 38.76 mg/g, about 8 times of the control level. Though the concentration of the liver lipid also increased in response to the cholesterol feeding, the increase of lipid was not so marked as that of total cholesterol. Accordingly, the percentage of total cholesterol to lipid in the liver increased and the half of lipid in the liver at the 4th week was occupied by total cholesterol.

The level of total cholesterol in the liver decreased spontaneously and progressively despite the continued feeding of the rats on cholesterol, hardened oil and taurocholic acid. The average total cholesterol concentration in the liver of rats at the 16th week was 21.25 mg/g. On the other hand, the concentration of lipid in the liver did not decline in parallel with the decrease of the level of total cholesterol and the former showed 2.5 to 3.7 times of the control level. Therefore, the percentage of total cholesterol to lipid in the liver increased and the half of lipid in the liver at the 4th week was occupied by total cholesterol.

The removal of cholesterol and taurocholic acid from the diet at the end of 16th week and further feeding of the rats on the high-fat, cholesterol-free diet for another series of 4 weeks decreased the levels of total cholesterol and lipid in the liver to one-third (6.32 ±0.49 mg/g) and to half (52.0 mg/g) of those at the 16th week. The percentage of total cholesterol to lipid in the liver decreased to 12.2% which was rather smaller than that before the cholesterol feeding.

2) Rabbits

Total cholesterol and lipid concentrations in the liver of rabbits which had been fed on the standard diet for four weeks were 3.72±0.19 mg/g and 33.2 mg/g, respectively (Table 2). The levels, however, increased to 17.4 times (64.41 mg/g) in the total cholesterol concentration and 3.4 times (113.0 mg/g) in the lipid concentration at the 16th week of the feeding on cholesterol and soya bean oil. Therefore, the percentage of total cholesterol to lipid in the liver increased from 11.2% to 57.0%.

IV. Pathological Findings

1) Rats

The control rats fed on the standard diet were sacrificed at the end of 12th and 24th weeks after the feeding. The animals which had received the standard diet mixed
with cholesterol, hardened oil and taurocholic acid for 4, 8, 12 and 16 weeks and the animals which had been fed on the high-fat, cholesterol-free diet for another series of 4 weeks after the high-cholesterol feeding for 16 weeks were sacrificed; and the various organs were weighed and were macroscopically observed.

The weight of the various organs in these animals are shown in Table 3. The yellow-brown coloring of the liver and the light yellow-violet discoloration of the adrenal glands were characteristic of the rats fed on the high-cholesterol diet. However, the removal of cholesterol and taurocholic acid from the diet at the end of 16th week and the further feeding of the animals on the high-fat, cholesterol-free diet for 4 weeks restored the discoloration of the liver and adrenal glands of almost normal. The weight of the liver in rats received the high-cholesterol diet (13.85 g at the 12th week) was significantly (P<0.01) larger than that of the control animals (9.67 g at the 12th week). Though some increases of the weight of the kidney and adrenal glands were also observed in the rats fed on the high-cholesterol diet, the weight of the heart, spleen, thyroid gland, prostate and testis showed no significant change. The increased weight of the liver in the rats fed on the high-cholesterol diet was reduced by the removal of cholesterol and taurocholic acid from the diet.

2) Rabbits

The rabbits received the standard diet mixed with cholesterol and soya bean oil were sacrificed at the 16th week for macroscopical examinations of the various organs. The yellow-brown discoloration of the liver and the light yellow discoloration of the adrenal glands were noticed in all animals. Moreover, there were marked increase in the subcutaneous and retroperitoneal fatty tissues as well as the proliferation of fatty tissues around the parenchymatous organs.

### Table 3. Influence of the high-fat, high-cholesterol diet on the mean wet weight of tissues of the male rat.

<table>
<thead>
<tr>
<th>Duration (weeks)</th>
<th>Number of rats</th>
<th>Body weight (kg)</th>
<th>Heart weight (mg)</th>
<th>Lungs weight (mg)</th>
<th>Liver weight (mg)</th>
<th>Spleen weight (mg)</th>
<th>Kidney weight (mg)</th>
<th>Right Adrenal weight (mg)</th>
<th>Left Adrenal weight (mg)</th>
<th>Testis weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>5</td>
<td>354.0 ± 8</td>
<td>117.5 ± 8</td>
<td>206.8 ± 8</td>
<td>598 ± 8</td>
<td>913 ± 8</td>
<td>1140 ± 8</td>
<td>945 ± 8</td>
<td>883 ± 8</td>
<td>903 ± 8</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>395.8 ± 10</td>
<td>1306 ± 10</td>
<td>2206 ± 10</td>
<td>9638 ± 10</td>
<td>432 ± 9</td>
<td>1020 ± 9</td>
<td>997 ± 9</td>
<td>396 ± 9</td>
<td>1146 ± 12</td>
</tr>
</tbody>
</table>

- **Diet**: Standard diet
- **Duration**: 12 weeks

<table>
<thead>
<tr>
<th>Duration (weeks)</th>
<th>Number of rats</th>
<th>Body weight (kg)</th>
<th>Heart weight (mg)</th>
<th>Lungs weight (mg)</th>
<th>Liver weight (mg)</th>
<th>Spleen weight (mg)</th>
<th>Kidney weight (mg)</th>
<th>Right Adrenal weight (mg)</th>
<th>Left Adrenal weight (mg)</th>
<th>Testis weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>5</td>
<td>317.6 ± 14</td>
<td>1106 ± 14</td>
<td>1248 ± 14</td>
<td>13850 ± 14</td>
<td>631 ± 14</td>
<td>1003 ± 14</td>
<td>1003 ± 14</td>
<td>1003 ± 14</td>
<td>1150 ± 14</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>201.9 ± 8</td>
<td>875 ± 8</td>
<td>926 ± 8</td>
<td>10938 ± 8</td>
<td>495 ± 8</td>
<td>1003 ± 8</td>
<td>883 ± 8</td>
<td>883 ± 8</td>
<td>903 ± 8</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>325.2 ± 14</td>
<td>1249 ± 14</td>
<td>1420 ± 14</td>
<td>12798 ± 14</td>
<td>618 ± 14</td>
<td>1116 ± 14</td>
<td>1116 ± 14</td>
<td>1116 ± 14</td>
<td>1188 ± 14</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>360.2 ± 14</td>
<td>1244 ± 14</td>
<td>1692 ± 14</td>
<td>1990 ± 14</td>
<td>596 ± 14</td>
<td>1230 ± 14</td>
<td>1230 ± 14</td>
<td>1230 ± 14</td>
<td>1220 ± 14</td>
</tr>
</tbody>
</table>

- **Diet**: High-fat, cholesterol-free diet
- **Duration**: 16 weeks

The increased weight of the liver in the rats fed on the high-cholesterol diet was reduced by the removal of cholesterol and taurocholic acid from the diet.
DISCUSSION

For the chemical assay of concentrations of total cholesterol in the serum and tissues of animals, the Liebermann-Buchard’s color reaction has been used by Bloor et al. (9), Schönheimer and Sperry (10) and Sperry and Webb (11). Afterward, the ferric chloride method has been devised by Zak (6) and Henley (7); and it has been proved by Kobayashi (12) and Yoshikawa et al. (13) that this method is relatively specific for the determination of the total cholesterol concentration with the simpler technic. Thus, the level of total cholesterol in the serum and the liver of the rats and rabbits fed on the standard diet mixed with cholesterol and fatty oil for 4 to 16 weeks were estimated by the method of Zak and Henley.

The levels of total cholesterol in the control rat fed on the standard diet were 67.7 ±4.28 mg/100 ml in serum and 4.82±0.35 mg/g in liver. The levels of total cholesterol in the control rabbit fed on the standard diet were 44.1±4.83 mg/100 ml in serum and 3.72±0.19 mg/g in liver. The level of lipid in the liver was 30.5 mg/g in the control rat and 33.2 mg/g in the control rabbit. Boyd and Oliver (14) have shown that the levels of total cholesterol in the serum of normal rabbit and rat are 40 and 50 mg/100 ml, respectively. Kritchevsky et al. (15) have demonstrated that the level of total cholesterol in the serum of normal rabbit is 55 mg/100 ml and the level in the liver is 1.4 mg/g. It has been reported by Alfin-Slater (16) that the total cholesterol concentration in the liver of normal rabbit is 2.3 mg/g. Although some variations of the levels of cholesterol may derive from the factors such as the species differences, the differential compositions of the standard diet and the differences of the assay procedures, the levels of cholesterol in the serum and liver of both species observed in the present experiments agree approximately well with those presented by the above-mentioned authors. There were slight and insignificant individual variations of the level of cholesterol, but the levels of cholesterol were not elevated by the feeding of the standard diet for at least four weeks inspite of the considerable increase of the body weight.

The feeding of the rabbits on the standard diet mixed with 1% of cholesterol and 10% of soya bean oil elevated the serum cholesterol in parallel with the length of the feeding period. The level of total cholesterol in the serum at the 4th week was about 10.0 times (440.7 mg/100 ml) of that in the serum of the control rabbit, and the level increased to 16.1 times (708.8 mg/100 ml) at the 8th week, and 29.1 times (1282.9 mg/100 ml) at the 16th week. The levels of total cholesterol and lipid in the liver of the rabbits fed on the standard diet mixed with cholesterol and soya bean oil for 16 weeks were 64.41 and 113.0 mg/g, respectively. The percentage of total cholesterol to lipid in the liver increased from 11.2% in the control animal to 57.0% at the 16th week of the cholesterol feeding. Wang et al. (17) have shown that the oral administration of cholesterol in the dose of 1.0 g/day/animal in the rabbit rises the level of plasma cholesterol from 49 mg/100 ml (the control level) to 641 mg/100 ml at the 4th week, 914 mg/100 ml at the 8th week, 1179 mg/100 ml at the 16th week and reaches to 1213 mg/100 ml at the
20th week. The similar results have been obtained by Kritchevsky et al. (15, 18). They have observed that the feeding of rabbits on the standard diet mixed with 2% of cholesterol and 6% of corn oil for 8 weeks induces a marked increase of cholesterol concentration in the serum (1122-2334 mg/100 ml) and the liver (24.6-75.7 mg/g).

In our studies the similar experiments were extended on the rats fed on the standard diet mixed with 1% of cholesterol, 5% of hardened oil and 0.5% of taurocholic acid, anticipating that there will be a progressive increase in the cholesterol concentration in the serum and liver of rats in similar way to that of rabbits. The elevation of cholesterol level in the serum of the rats proved markedly less than that in the serum of the rabbits. After 4 weeks of the cholesterol feeding, the average total serum cholesterol of the rats rose to 2.6 times of normal (176.8 mg/100 ml); an additional moderate elevation (3.7 times of the control, 247.6 mg/100 ml) was seen after 8 weeks. Thereafter, the level of the serum total cholesterol declined spontaneously and gradually despite the further feeding of the rats on the same diet, and the serum cholesterol concentration at the 16th week was only 2.2 times (148.5 mg/100 ml) of the control level. The spontaneous decline of the level of the serum total cholesterol in the rats showed a sharp contrast to the progressive elevation in the rabbits. These results indicate that the absorption and metabolism of cholesterol or fat differ strikingly in the rat from that in the rabbits. After the cholesterol feeding for 16 weeks, further feeding of the rats on the high-fat, cholesterol-free diet for 4 weeks reduced the level of the serum cholesterol to 1.6 times (107.7 mg/100 ml) of the control level.

The level of total cholesterol in the liver of the rats rose to 8 times (38.76 mg/g) of the control by the feeding of the animals on the high-cholesterol diet for 4 weeks. The further feeding of the rats on the same diet reduced the liver cholesterol concentration gradually, and the level at the 16th week was 4.5 times (21.25 mg/g) of the control. The patterns of the liver total cholesterol in the rat showed not only less elevation but also spontaneous decline in contrast to the marked and progressive elevation in the rabbit. On the other hand, the increased level of lipid in the liver of rats at the 16th week of the cholesterol feeding (3.2 times of the control level, 100.2 mg/g) was almost the same as that in the rabbits. Accordingly, the ratio of total cholesterol to lipid in the liver of rats increased to 50.9% at the 4th week of the feeding and decreased to 21.2% at the 16th week. The increased levels of total cholesterol and lipid in the liver declined progressively to 1.5 times of the respective control levels by the further feeding of the animals on the cholesterol-free diet for another series of 4 weeks.

Ruegamer and Silverman (19) have studied the effect of some lipolytic agents on the plasma and tissue cholesterol and lipid concentrations in the rats fed on a high-fat diet containing 1% of cholesterol, 29% of lard and 0.5% of cholic acid for 3 weeks. They have reported that the levels of plasma cholesterol, liver cholesterol and liver lipid increase to 222 mg/100 ml, 47.28 mg/g and 197.7 mg/g, respectively. The animals have been then placed on the unsupplemented high-fat diet mixed with 29% of lard
alone. After 3 weeks on these regimes, all levels of plasma and liver cholesterol and liver lipid have been lowered to 123 mg/100 ml, 21.47 mg/g and 162.4 mg/g, respectively. The levels in their experiments are considerably higher than obtained in our studies. The differences might be derived from higher amounts of lard (29%) in the diet of their experiments. Alfin-Slater (16) has shown that the feeding of the rats on the diet mixed with cholesterol and cotton seed oil for 12 weeks elevates the levels of plasma cholesterol, liver cholesterol and liver lipid to 91 mg/100 ml, 32.5 mg/g and 147.2 mg/g, respectively. The less elevation of the plasma cholesterol level in his experiments than that observed in our experiments may be derived from the absence of taurocholic acid, which activates the intestinal absorption of cholesterol from the diet (3, 4). On the other hand, the levels of the liver cholesterol and lipid of the rats in his experiments were higher than those shown in the present experiments. These results suggest that the patterns of cholesterol and lipid in the liver of rats are not a simple reflection of the patterns of cholesterol in the blood. There may be other mechanisms which regulate and limit the levels of cholesterol and lipid in the liver of rats. The mechanism may serve for the limitation of absorption, the degradation or the excretion of the excess cholesterol. The limited elevation of the level of serum and liver cholesterol and the spontaneous decline of the levels despite the continuation of the feeding of the rats on high-cholesterol diet may support this speculation.

**SUMMARY**

The changes of body weight, behaviors, levels of total cholesterol in the serum and liver and of lipid in the liver and pathological findings in the autopsy were studied in the rabbits and rats which were fed on the standard diet mixed with cholesterol and fatty oil for long term of days. The results obtained were summarized as follows:

1. The body weight gains of the animals fed on the high-fat, high-cholesterol diet were more marked than those in the control animals.

2. The peak of the serum and liver cholesterol concentrations in the rats fed on the standard diet mixed with cholesterol, hardened oil and taurocholic acid was obtained at the 4th to 8th week of the feeding. Thereafter, the levels of cholesterol decline spontaneously and progressively despite the continuation of the cholesterol feeding. On the other hand, the level of liver lipid showed a gradual increase. Accordingly, the percentage of total cholesterol to lipid in the liver of rats increased during the cumulative period of the liver cholesterol and decreased during the decline of the liver cholesterol.

3. At the end of 16th week after the commencement of cholesterol feeding, the rats were placed on unsupplemented high-fat diet mixed with hardened oil alone. After 4 weeks on these regimes, the levels of serum and liver cholesterol and liver lipid reduced to about 1.5 times of the corresponding control levels.

4. The levels of total cholesterol in the serum and liver of the rabbits were increased progressively along with the length of the feeding of the animal on the standard diet mixed with cholesterol and soya bean oil. No spontaneous decline of the cholesterol
levels was observed. The percentage of total cholesterol to lipid in the liver of the rabbits also increased at the 16th week of the cholesterol feeding.

5. The autopsy proved certain signs of fatty degeneration in the liver and adrenal glands accompanied with the weight increase of the former organ in both species fed on the high-fat, high-cholesterol diet. Marked deposition of fats in the subcutaneous, retroperitoneal and periparenchymatous tissues was also observed.

REFERENCES
1) PAGE, I. H. AND BROWN, H. B. : Circulation 6, 681 (1952)
2) MYASNIKOV, A. L. : Ibid. 17, 99 (1958)
3) PHIL, A. : Acta physiol. scand. 34, 206 (1955)
7) HENLEY, A. A. : Analyst 82, 286 (1957)
8) TAKAORI, S., OSUMI, Y. AND SHIMAMOTO, K. : This Journal, to be published
10) SCHÖNHEIMER, R. AND SPERRY, W. M. : Ibid. 106, 745 (1934)
13) YOSHIKAWA, H. et al. : Igaku-no-ayumi 33, 375 (1960) (Japanese)