CHRONIC ORAL TOXICITY OF 3,5,3'-TRIIODO-4'-ACETYLTYROFORMIC ACID WITH SPECIAL REFERENCE TO CHOLESTEROLYTIC EFFECTS

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Though the use of thyroid hormone in the treatment of hypercholesterolemia in the humans received wide acceptance, its practical use is limited by the high incidence of angina attack and by the influence through the augmented rate of basal metabolism. 3,5,3'-Triiodothyronine (T₃), 3,5,3'-triiodothyroacetic acid (Triac), 3,5,3'5' -tetraiodothyroacetic acid (Tetrac) and other possible metabolites of thyroxine were reported to suppress serum cholesterol levels within the range of doses which did not produce concomitant rise in basal metabolic rate (1-4). Through these experiments the evaluation of other derivatives became one of the most attractive projects not only for the clinical but also the experimental studies for attempting to separate the cholesterolytic property from the effect on the basal metabolism by changing the chemical structures. Among them, 3,5,3'-triiodothyroformic acid (T₃-F) was reported by Boyd and Oliver (3, 4) as a possible metabolite of thyroxine. Kumaoka et al. (5) demonstrated that 3,5,3'-triiodo-4'-acetyl-thyroformic acid (TBF-43) produced a moderate cholesterolytic effect without much increase of basal metabolic rate in cases of essential hypertension and myxedema when 10 to 50 mg were prescribed daily. Acute and subacute toxicities of the compound were presented by Aramaki et al. (6) and Kajihara et al. (7). They reported that subacute oral administration of TBF-43 in the daily doses above 40 mg/kg caused a retardation of growth in rats. Furthermore, the autopsy study proved an increase in the weight of the parenchymal organs, especially the heart, kidney and adrenal gland.

The present report deals with the chronic oral toxicities of TBF-43 in the rats and rabbits fed on the standard diet or high-cholesterol diet.

METHODS

Animals used in this study were albino rats of Wistar strain and albino rabbits.

Feeding of animals

1) Rats: Young male rats weighing 60 to 70 g at the beginning of the experiments were allowed to feed the standard diet (CLEA-CA-1) prepared by Zikken-dobutsu Chokenkyusho (the central laboratories of experimental animals in Japan), and water ad libitum in an air-conditioned room at temperature of 22±1°C. The composition of the
standard diet was previously described (8). The animals were kept in individual cages. The powdered diet was admitted to the diet container about 80 ml in volume, and the dispersion of the powdered food was prevented by covering with a plastic plate with several holes through which animals were allowed to pick up the food. In the experiments of the rats fed on high-cholesterol diet, the standard diet was added with 5% of hardened oil (ECONA oil, Kao-sekken Co.), 1% of cholesterol and 0.5% of taurocholic acid.

2) Rabbits: Male albino rabbits weighing about 2.0 kg at the beginning of the experiments were fed on the standard compressed diet (RC-5) of Oriental Kobo-kogyo Co., and water in an air-conditioned room at temperature of 22±1°C. The composition of the standard diet was described previously (8). The preliminary experiments showed that each rabbit given water ad libitum took up at least 80 g/day/animal of the diet. The high-cholesterol diet for the rabbits consisted of 80 g/day/animal of the standard diet added with 1% cholesterol and 10% of soya bean oil.

Administration of TBF-43

1) Rats: Since it was reported that the daily dose for the cholesterolytic use of TBF-43 is about 1 mg/kg/day (5), the daily doses of 5, 10, 30 and 100 mg/kg of TBF-43 were mixed into the powdered standard diet for 12 weeks. In another series of experiment, 1, 3 and 5 mg/kg/day of the compound were orally administered for 48 weeks. Further, the doses of 5 mg/kg/day of TBF-43 and of 5 μg/kg/day of T3 were also given to the rats fed on the high-cholesterol diet for 16 weeks.

2) Rabbits: In the rabbits fed on the high-cholesterol diet for 12 weeks the daily doses of 5, 15 and 30 mg/kg of TBF-43 were added into the diet. No animal refused to take the diet mixed with the compound.

Measurements

Details of the measurements were previously described (8). The body weight of the animals was measured once a week over a period of the experiments. In the rats, the food was supplied three times a week and the amounts of the food/week/animal were calculated from the measurement of the residual food. During and after the term of the drug administration, five to ten rats were sacrificed at random for the pathological examinations and for the measurement of the total cholesterol level in the serum and liver and of the level of lipid in the liver. In the rabbits the heart rate and serum cholesterol level were measured every other week. The determinations of total cholesterol concentration in the serum and liver was followed to ferric chloride method devised by Zak (9) and Henley (10).

Pathological examinations

The animals were sacrificed by decapitation. The lung, heart, liver, spleen, kidney, adrenal gland, thyroid gland, prostate, hypophysis and testis were separately weighed and were histologically studied by staining with hematoxylin-eosin and with lipotropic dyes. Three animals in each group were used for the microscopical examinations.
RESULTS

1. **Chronic toxicity in rats fed on the standard diet**

   1) **Toxic symptoms**

   a) **The rate of increase of body weight**

   The rate of increase of body weight of the male rats received daily 5, 10, 30 and 100 mg/kg of TBF-43 for 12 weeks are shown in Fig. 1. Each group was consisted of 10 animals. No significant deviation of growth rate from the control was noted at the 5 mg/kg/day level. The rate reduced slightly but transiently at the 10 mg/kg/day level from the 8th to 10th week, although the mechanism was not elucidated. The rate of growth was significantly (P<0.01) lower than that of the controls at the dose level of 30 mg/kg/day from the 2nd week, and the average increase was 116.0±6.03 g at the end of 12th week against 170.0±8.60 g in control. In this group one rat died at the 6th week and two rats at the 8th week. The body weight did not increase in the rats received the daily dose of 100 mg/kg of TBF-43 from the 3rd week. Five animals died at the 3rd week, two at the 4th week, two at the 6th week and one at the 7th week, even though the daily total food consumption was larger than that of the controls.

   b) **The behaviors and other symptoms**

   There were no differences in the behaviors and general symptoms between the rats which received the daily doses of 5 and 10 mg/kg of TBF-43 and the control animals. The animals at the dose of 30 mg/kg/day showed the sign of depilation, hyperemia of the conjunctiva, rhinorrhea and lacrimation after the 3rd week. The spontaneous movements were decreased. These symptoms were more markedly observed in the animals which died during the experimental period. The depilation was so marked at the 100 mg/kg/day level that the scalp, face and abdomen were covered only with short downy hair. The marked increase of the salivary secretion, conjunctivitis, hemorrhage in the paws, tachypnea and tachycardia were developed. In spite of the manifestation of ataxia before death, the animals took up the increased amounts of food.

   There was no observable abnormality in the male rats received the daily doses of 1, 3 and 5 mg/kg of TBF-43 for 48 weeks, not only of the growth rate but also of the general symptoms and behaviors.
2) Food consumption

The amount of food consumption in grams/rat/day for 12 weeks is illustrated in Fig. 2. There was no significant difference between the rats which received the daily dose of 5 mg/kg of the compound and the control rats. However, a definite increase of 1 to 2 g/rat/day was observed in the rats at the doses of 10 and 30 mg/kg/day. Much larger amounts of food were taken up by the rats which received the daily dose of 100 mg/kg of TBF-43. Therefore, it was necessary to adjust the mixing rate of TBF-43 in the food at least once a week in order to maintain the predetermined oral doses exactly.

However, the average daily food consumption in the rats by the oral administration of 1, 3 and 5 mg/kg/day of TBF-43 was not significantly different during 48-week experimental period from that in the control rats fed on the standard diet alone.

2. Experiments in rats fed on high-cholesterol diet

1) Body weight and food consumption

The high-cholesterol diet containing 5% of hardened oil, 1% of cholesterol and 0.5% of taurocholic acid was fed to 70 young male rats for 4 weeks. Ten rats were sacrificed at random for the determination of cholesterol concentrations in the serum and liver. The remaining 60 animals were subdivided into three groups, each consisted of 20 rats. One group was allowed to receive further the high-cholesterol diet, and was used as a control. Another two groups received the same diet supplemented with 5 mg/kg/day of TBF-43 and 5 μg/kg/day of 3,5,3'-triiodothyronine (T₃), respectively. At the end of the 4th, 8th and 12th weeks of the drug administration five rats from the control and treated groups were sampled at random for sacrifice. The addition of cholesterol and taurocholic acid to the diet was stopped at the end of the 16th week of the cholesterol feeding, and the remaining five rats in each group were fed on the standard diet added with 5% of hardened oil alone for another 4 weeks. Even during these regimes, the same doses of TBF-43 and T₃ was administered further in the treated groups.

The growth and food consumption of the control and treated animals are presented in Table 1. The rise of body weight in the cholesterol-fed rats which received the daily dose of 5 μg/kg of T₃ was slightly lesser after the 5th week of the drug administration than those of the control rats fed on the high-cholesterol diet alone. However, there was no difference in the body weight gain between in the rats which were fed on cholesterol diet with the daily dose of 5 mg/kg of TBF-43 and in the control animals.
Average daily food consumption for rats received TBF-43 or T₃ was not significantly different from that of the control rats. The similar conclusion was presented by the determination of the amounts of food/100 g body weight/day. In addition, the animals which received the high-cholesterol diet supplemented with TBF-43 or T₃ did not show any abnormality of the general symptoms.

2) Total cholesterol level in serum

As was reported previously (8), the feeding of the rats on the high-cholesterol diet supplemented with taurocholic acid increased the level of total cholesterol in the serum. The peak effect was usually observed at the 8th week of the cholesterol-feeding. Thereafter, the level of cholesterol declined spontaneously despite the further cholesterol-feeding.
The administration of 5 mg/kg/day of TBF-43 or 5 μg/kg/day of T3 to the cholesterol-fed rats resulted in lower serum cholesterol values than that observed in the cholesterol-fed controls, as shown in left column of Table 2. There was no significant difference between both effects of TBF-43 and T3. Though the depressive effect of TBF-43 on the elevation of cholesterol level in the serum at the 4th week of the administration was less than that of T3, TBF-43 accelerated the spontaneous decline of cholesterol level more markedly than T3. However, the cholesterol level in the serum of the rats fed on the high-cholesterol diet for 16 weeks was still higher than that of the rats before the cholesterol-feeding in spite of simultaneous administration of TBF-43 or T3. The withdrawal of cholesterol and taurocholic acid from the high-cholesterol diet at the end of 16th week and further feeding of the animals on the diet containing hardened oil alone resulted in the progressive reduction of the cholesterol level in every groups of animals.

3) Total cholesterol and lipid levels in liver

It was previously reported that the feeding of the rats on the high-cholesterol diet increased the level of total cholesterol in the liver to 38.80 mg/g wet tissue weight, which was about 8 times higher than that before the cholesterol-feeding, at the 4th week (8). The level, however, decreased spontaneously and progressively despite the further feeding of the animals on the same diet.

As shown in middle column of Table 2, the oral administration of 5 mg/kg/day of TBF-43 did not modify the spontaneous decline of the cholesterol level in the liver. On the other hand, the cholesterol level failed to decrease when a dose of 5 μg/kg/day of T3 was supplemented the high-cholesterol diet. The effect of T3 on the spontaneous decline differed sharply from that of TBF-43 during the term of the cholesterol-feeding. The withdrawal of cholesterol and
taurocholic acid from the high-cholesterol diet at the end of 16th week and further feeding of the rats on the standard diet added with hardened oil alone for 4 weeks decreased the total cholesterol level in every group of animals rapidly.

The feeding of the rats on the high-cholesterol diet for 4 weeks also increased the lipid level in the liver to 76.0 mg/g wet tissue weight which was about 2.5 times higher than that before the cholesterol-feeding. However, the concentration of lipid did not decline in parallel with the decrease in the concentration of total cholesterol. The daily administration of T₃ accelerated the increase of the lipid level in the liver markedly, while TBF-43 did not. The removal of cholesterol and taurocholic acid from the diet at the 17th week and further feeding of the rats on the diet mixed with hardened oil alone for 4 weeks decreased the lipid level in the liver of both treated and untreated animals.

3. Experiments in rabbits fed on high-cholesterol diet

1) Body weight and heart rate

The high-cholesterol diet containing 10% of soya bean oil and 1% of cholesterol was fed to 20 male rabbits for 4 weeks. Thereafter, the animals were subdivided into four groups, each consisted of five rabbits. One group was allowed to receive further the high-cholesterol diet as control. Another three groups received the same diet supplemented with 5, 15 and 30 mg/kg/day of TBF-43, respectively. The time course of the average body weight in these four groups of the rabbits is illustrated in Fig. 3. It

![Graph](image)

**Fig. 3.** The effect of TBF-43 on the body weight of rabbits fed on high-cholesterol diet.

Each point in Figs. 3 and 4 represents the mean value of five animals.

C : Starting the cholesterol-feeding,
T : Starting the daily administration of TBF-43.
was demonstrated that the administration of TBF-43 not only depressed the body weight gains but also declined the weight in proportion to the doses. The death of the animals received 30 mg/kg/day of TBF-43 was observed in two at the 6th week, and one at the 8th week. One rabbit at the dose level of 15 mg/kg/day died at the 8th week. The dead animals showed signs of profuse icterus and marked emaciation. The decreasing effect of TBF-43 on the body weight and the death rate of the animals are likely to correspond with the daily dose levels of the compound. The rabbit at the dose of 30 mg/kg/day was revealed a decrease in spontaneous movement and a tendency of depilation after the 4th week of the administration. The latter symptom was also observed in some animals which received 15 mg/kg/day of TBF-43.

The average heart rate of five rabbits fed on the high-cholesterol diet for 14 weeks was in the range of 235 to 275/min, and these values were not significantly different from those of the animals fed on the standard diet alone. The heart rate of the cholesterol-fed rabbits which received the daily dose of 5 mg/kg of TBF-43 was within the control range (235 to 290/min). On the other hand, the administration of 15 and 30 mg/kg/day of the compound caused a marked increase in the heart rate from the 4th week, and the average heart rate was in the range of 320 to 345/min. These results are shown in Fig. 4.

2) Total cholesterol level in serum
The level of total cholesterol in the serum of the rabbits was markedly and progressively increased along with the length of the cholesterol-feeding, as was reported previously (8). No spontaneous decline of the cholesterol level was observed in the rabbits.

The daily oral administration of 5, 15 and 30 mg/kg of TBF-43 failed to prevent the increase of the total cholesterol level in the serum, as shown in Table 3. Moreover, the large doses (15 and 30 mg/kg/day) of the compound accelerated the increase in the cholesterol level. The most prominent acceleration of the increase in the cholesterol level was observed on the rabbits received the daily dose of 30 mg/kg of TBF-43 for 6 weeks.

3) Total cholesterol and lipid levels in liver
The total cholesterol and lipid concentration in the liver of the rabbits fed on the high-cholesterol diet for 16 weeks were 64.41 and 113.0 mg/g wet tissue weight, respect
These values were markedly larger than the corresponding value in the rabbits fed on the standard diet alone.

When the rabbits fed on the high-cholesterol diet were simultaneously administered the daily doses of 5, 15 and 30 mg/kg/day of TBF-43 from the 5th week to the 16th week, the levels of total cholesterol in the liver at the 16th week were 59.81, 43.39 and 37.19 mg/g, respectively. These results showed that the supplement of the compound induced a slight protecting effect against the deposit of cholesterol in the liver. On the other hand, the concentrations of lipid in the liver of the rabbits received 5, 15 and 30 mg/kg/day of TBF-43 were 119.0, 134.3 and 146.0 mg/g, respectively. Therefore, the administration of the compound accelerated slightly the deposit of lipid in the liver. The results are shown in Table 4.

### Table 3. The effect of TBF-43 on the serum cholesterol level of rabbits fed on high-cholesterol diet.

<table>
<thead>
<tr>
<th>Administ. of TBF-43 or T3 (weeks)</th>
<th>Cholesterol-feeding (weeks)</th>
<th>No. of rabbits in each group</th>
<th>Serum total cholesterol (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20 (Total)</td>
<td></td>
<td>44.1</td>
</tr>
<tr>
<td>2</td>
<td>6 (40)</td>
<td></td>
<td>259.2</td>
</tr>
<tr>
<td>4</td>
<td>5 (30)</td>
<td></td>
<td>440.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control</th>
<th>TBF-43 (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>15</td>
</tr>
</tbody>
</table>

886.3 621.5 625.8 619.7 708.8 541.2 849.0 1054.2 860.2 1040.1 1261.8 1661.8 965.6 1045.7 1311.1 1478.5(3)* 1220.6 1264.4 1425.5(4)* 1276.8(2)* 1282.9 1205.7 1614.1(4)* 1432.4(2)*

*: Number of animals, See the text.

### Table 4. The effect of TBF-43 on the liver cholesterol and lipid levels of rabbits fed on high-cholesterol diet for 16 weeks.

<table>
<thead>
<tr>
<th>Doses of TBF-43 (mg/kg/day)</th>
<th>No. of rabbits</th>
<th>Liver total cholesterol (mg/g)</th>
<th>Liver lipid (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>64.41</td>
<td>119.0</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>59.81</td>
<td>119.0</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>43.39 (44.65) *</td>
<td>134.3 (113.2) *</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
<td>37.19 (42.55) *</td>
<td>146.0 (118.6) *</td>
</tr>
</tbody>
</table>

*: The mean value of five rabbits including the animals which died during the experimental period.

4. **Autopsy and histopathology**

1) **Rats received 5, 10, 30 and 100 mg/kg/day of TBF-43 for 12 weeks**

Autopsy were performed on all the rats which died during the course of the study. At the end of 12th week, the surviving control and treated rats were sacrificed. Autopsies and microscopic examinations of the representative tissue specimens were performed. Table 5 summarized the mean wet weight of the various tissues such as heart, lung, liver, spleen, kidney, adrenal gland, thyroid gland, hypophysis and testis in the control
and treated animals in reference to the tissue weight per body weight (100 g).

The tissue weight per body weight of lung and left kidney in the rats which received 5 mg/kg/day of TBF-43 were significantly (P<0.01) larger than those of the controls. In only one among three rats at the dose of 5 mg/kg/day, the thyroid gland showed slight atrophic changes of the follicular epithelia. Other tissues in the animals received 5 mg/kg/day of TBF-43 failed to show any remarkable alterations which might be attributed to the thyrotrophic activity of the compound.

In the rats received the daily dose of 10 mg/kg of TBF-43, the heart, spleen and kidney showed a significant increase in weight at P<0.01. The central portion of lobules in the liver and the white pulp of the spleen showed slight atrophic pictures in two out of three animals. The thyroid gland in all the animals was revealed signs of flattening of the follicular epithelia and atrophy of the follicles which were not uniform in degree. In one rat, the content of colloid substances in the follicles of thyroid gland was decreased, and the number of mature sperma cells in the testis was slightly reduced.

In the rats received the daily dose of 30 mg/kg of TBF-43, the significant increase in the tissue weight was observed in the heart, spleen and kidney, while the significant decrease was noted in the thyroid gland, hypophysis and right testis at P<0.01. Since the animals showed a marked retardation of the body weight gains during 12 weeks of the drug administration, the increase in tissue weight of the heart, lung, spleen, kidney and testis was more clearly demonstrated by expressing the tissue weight per body weight (Table 5). Compared with the control animals the treated rats showed a marked reduction of the fatty tissues in the vicinity of parenchymal

<table>
<thead>
<tr>
<th>Table 5: The mean wet weight of tissues of rats received TBF-43 for 12 weeks.</th>
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<tbody>
<tr>
<td>Doses of TBF-43 (mg/kg/day)</td>
</tr>
<tr>
<td>------------------------------</td>
</tr>
<tr>
<td>0 mg/kg/day</td>
</tr>
<tr>
<td>10 mg/kg/day</td>
</tr>
<tr>
<td>30 mg/kg/day</td>
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</table>

The values in ( ) represent the mean of tissue weight per body weight (100 g). Significantly increased from the control (P<0.01). Significantly decreased from the control (P<0.01).
organs. In the microscopic examinations, the rats at the 30 mg/kg/day level showed atrophic pictures of the peribronchial lymph glands, the central portion of the hepatic lobules and the white pulp of the spleen. In two among three rats, there were slight hyaline and hypertrophic degenerations in the capillary epithelia of the glomerulus and in the epithelia of the proximal tubules of the kidney. The hyaline casts were also detected in the tubular canals of the renal cortex in one animal. The marked flattening of the follicular epithelia in the thyroid gland was attributable to the progressive atrophy. In one rat, there were considerable infiltrations of plasma cells and lymphocytes in the interstitial tissues of the thyroid gland.

The marked increase of the tissues weight per body weight in the rats received the daily dose of 100 mg/kg of TBF-43 was partly derived from the extreme deprivation of the body weight. The increase in weight of the liver, heart, kidney and adrenal gland of the dead animals from the 4th to 7th week were 2.5, 2.8, 3.4 and 3.9 times of the control weight by expressing the tissue weight per body weight, respectively. The macroscopical observation proved the marked reduction of the fatty tissues. In the rats at the dose of 100 mg/kg/day for 4 to 7 weeks, the moderate congestion of the lung were accompanied with marked atrophic changes of the peribronchial lymph glands. The congestion and central atrophy of the hepatic lobules were also observed. In the spleen the disappearance of the central structure and outer band of the white pulp was a strong indication for the atrophic changes. The scattered lesions of the degeneration of the neutrophilic cells were detected in the spleen of one animal. The capillary epithelia of the renal glomerulus showed hypertrophic changes and deposit of hyaline substances accompanied with compensatory dilatation of the adjacent tubular canals. These pictures were an indication for the regenerative process of glomerular structures. The degenerative changes in the epithelia of the proximal and distal tubules were also observed. The adrenal gland showed an atrophic picture revealing the reduction of the lipoid contents and the disappearance of the zonular structures. The thyroid gland showed a marked atrophy exhibiting the extreme reduction of the number of follicles, the marked flattening of the epithelial cells, the reduction of the colloid substances and the proliferation of the interstitial tissues. The number of mature sperm cell in the testis was also reduced.

2) Rats received 1, 3 and 5 mg/kg/day of TBF-43 for 48 weeks

At the ends of the 24th, 36th and 48th weeks of the oral administration of 1, 3 and 5 mg/kg/day of TBF-43, the control and treated rats were sacrificed for autopsies. Table 6 summarized the mean wet weight of the tissues such as heart, lung, liver, spleen, kidney, adrenal gland, thyroid gland, prostate and testis. The weight of the adrenal gland of the rats which received 5 mg/kg/day of TBF-43 for 36 weeks were significantly (P<0.05) larger than that of the respective control. There was slight but insignificant increase in the weight of lung in the rats at the dose of 5 mg/kg/day for 24, 36 and 48 weeks as compared to the corresponding controls.

Histological examinations of the tissues in the rats received the daily dose of 1
mg/kg of TBF-43 failed to show any remarkable alterations which might be attributed to the toxicity of the compound. In one rat at the 3 mg/kg/day level, height of the follicular epithelium of thyroid gland was increased and many small vacuoles were detected in the follicles. Two out of three rats which received the daily dose of 5 mg/kg of TBF-43 showed a slight atrophy of the central portion of hepatic lobules accompanied with the manifestation of the pycnotic cells. A slight atrophy of the white pulp in the spleen and a decrease of the mature sperma cells in the testis were observed in only one animal at the 5 mg/kg/day level. The latter picture was considered to indicate a depression of the spermatogenic activity. In two rats of the same group, the thyroid gland showed slight atrophy and degeneration of the follicular epithelia. Furthermore, the colloid substances in the follicles decreased markedly.

From the results described above, it is concluded that the daily administration of TBF-43 above the dose of 5 mg/kg results in the slight atrophic and degenerative changes of the thyroid gland and liver in the rats.

3) Effects of TBF-43 and T₃ on the rats fed on high-cholesterol diet

The rats fed on the high-cholesterol diet were orally administered the daily dose of 5 mg/kg of TBF-43 or 5 μg/kg of T₃, for 12 weeks. Thereafter, the administration of the same dose of TBF-43 or T₃ was continued in the animals fed on the high-fat, cholesterol-free diet for another 4 weeks. At the ends of 4th, 8th, 12th and 16th weeks of the drug...
administration, five rats from the control and treated groups were sampled and killed for autopsies and pathological examinations of representative tissues. Though some increases of the weight of heart, lung, liver and adrenal gland were observed at the 4th and 8th weeks of the administration of TBF-43, these increases were insignificant. The administration of T_3 resulted in slight but insignificant increases in the weight of lung at the 4th and 8th weeks as compared to the corresponding tissue of the control animals fed on the high-cholesterol diet. The withdrawal of cholesterol and taurocholic acid from the diet during the 17th to 20th week caused a marked decrease of the weight of liver in both untreated and treated animals. In all rats fed on the high-cholesterol diet for 16 weeks, the macroscopical examinations showed marked proliferative increases of the subcutaneous and retroperitoneal fatty tissues, the yellow-brown coloring of the liver and the light yellow-violet discoloration of the adrenal gland. Any detectable difference of change was not observed macroscopically in the organs between the untreated rats and the rats treated with TBF-43 or T_3.

The microscopical examination of the parenchymatous tissues in the rats fed on the high-cholesterol diet for 4 weeks showed following results. In the liver, there were atrophic cells with many small vacuoles and degenerative nuclei in the intermediary portion of the lobular structures. The zonular structures of the adrenal gland were not distinct and many vacuoles were detected in the cells of the gland. In the thyroid gland the height of the follicular epithelia were increased. In some animals, colloid substances in the follicles were reduced and some vacuoles were detected. The number of mature sperma cells in the testis was slightly reduced. Almost similar pathological changes of the tissues were observed in the rats fed on the high-cholesterol diet for 8 weeks.

The oral administration of 5 mg/kg/day of TBF-43 for 4 weeks caused slight improvement of the pathological changes induced by the cholesterol-feeding. Especially, the picture of fatty degeneration in the liver was ameliorated. No abnormality was observed in the thyroid gland. However, the increased manifestation of the vacuoles and disorganization of the zonular structures in the adrenal gland were still detected. On the other hand, the administration of 5 mg/kg/day of T_3 for 4 weeks failed to improve the pathological abnormalities caused by the cholesterol-feeding. The fatty degeneration of the hepatic cell and the atrophy of follicular epithelia in the thyroid gland were observed. Moreover, the vacuoles in the adrenal gland were increased in number and the disorganization of the zonular structures was also observed.

The pathological signs of the tissues, especially of the liver, improved spontaneously despite the continued feeding of the rats on cholesterol and taurocholic acid. The fatty degeneration of the liver in the rats fed on the high-cholesterol diet for 12 weeks was reduced in degree. However, the atrophic and degenerative changes of the follicular epithelia were observed in some portion of the thyroid gland. The oral administration of 5 mg/kg/day of TBF-43 for 12 weeks did not modify the spontaneous improvement of the pathological signs of the tissues during the term of the cholestrol-feeding. On the other hand, the fatty degeneration of the liver did not improved spontaneously, and
yet many large vacuoles, atrophic cells and pyknotic cells were observed in the medial portion of the hepatic lobules when a dose of 5 μg/kg/day of T3 was supplemented in the high-cholesterol diet. Atrophic and degenerative changes of the follicular epithelia in the thyroid gland of the rats received T3 were more marked than those of the untreated rats or treated rats with TBF-43.

The withdrawal of cholesterol and taurocholic acid from the diet during the 17th to 20th week resulted in a marked improvement of the pathological changes produced by the cholesterol-feeding. The vacuoles in the hepatic lobules disappeared completely and only the pyknotic cells were found being scattered. Though rare degenerative signs of the follicular epithelia in the thyroid gland were still detected, most parts of the thyroid structures showed normal picture. The oral administration of TBF-43 accelerated these improving effects slightly. Though there were some pyknotic cells in the intermediary portion of the hepatic lobules and very slight atrophy of the follicular epithelia of thyroid gland in the rats received TBF-43, the progressive recovery of the tissue changes were confirmed. The administration of T3 did not worsen the improvement of histological changes in the liver, which was produced by the interruption of the feeding on cholesterol and taurocholic acid. Some vacuoles and pyknotic cells in the hepatic lobules were slightly observed. The thyroid gland in the rats received T3, however, showed considerably marked atrophic pictures of the follicular epithelia. Slight reduction of number of the sperma cells in the testis was also observed.

4) Effects of TBF-43 on the rabbits fed on high-cholesterol diet

There were marked proliferations of the subcutaneous and retroperitoneal fatty tissues in the rabbits fed on the high-cholesterol diet for 16 weeks. The liver showed a yellow-brown discoloration and the adrenal gland showed a light yellow discoloration. In the cholesterol-fed rabbits which received the daily dose of 5 mg/kg of TBF-43 for 12 weeks, the subcutaneous and retroperitoneal fatty tissues were reduced. Furthermore, the fatty tissues almost dis-

<table>
<thead>
<tr>
<th>Doses of TBF-43 (mg/kg/day)</th>
<th>Heart</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>Right Kidney</th>
<th>Left Kidney</th>
<th>Right Adrenal gland</th>
<th>Left Adrenal gland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>mg</td>
<td>mg</td>
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<td>2.21</td>
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The values in ( ) represent the mean tissue weight per body weight (1 kg).
appeared in the animals received the daily dose of 15 and 30 mg/kg of the compound. Even in these animals the discoloration of the liver and adrenal gland was observed. Table 7 summarized the mean wet weight of the tissues such as heart, lung, liver, kidney, adrenal gland, thyroid gland and testis. Though slight decrease in weight was observed in the lung, liver, adrenal gland and thyroid gland of the rabbits received 5 mg/kg/day of TBF-43, these increases were insignificant. In the rabbits received the daily dose of 15 mg/kg of the compound, the weight of the liver, thyroid gland and testis showed some decrease, while the weight of the lung, spleen, kidney and adrenal gland increased slightly despite the reduction of the body weight. Marked decrease in the weight was observed in the liver, thyroid gland and testis of the rabbits which received the daily dose of 30 mg/kg of TBF-43. Since the administration of TBF-43 reduced the body weight, the tissue weight per body weight was generally increased, particularly in the heart, lung, kidney and adrenal gland.

Deposit of fats in the various tissues was markedly increased in the rabbits fed on the high-cholesterol diet for 16 weeks. The heart tissue showed signs of some congestion, and in one animal there were hyaline degeneration of the muscle and proliferation of the connective tissues. In all cholesterol fed rabbits, the large and intermediate arteries in the lung showed a moderate degree of hypertrophy of the intima and media in which deposit of lipid substances was demonstrated by the staining with the lipotropic dye. The similar atheromatous hypertrophy was detected in the ascending and descending aortae. The diffuse fatty infiltration was observed in the central and intermediary portions of the hepatic lobules in all animals and the fatty infiltration extended to the peripheral portion of the lobules in two out of three rabbits. The white pulp of the spleen in one rabbit exhibited an atrophic picture and a disappearance of the central structure. There were many vacuoles in the adrenal gland of all animals, especially many fatty granules in large size was scattered in the glomerular zone of one animal and in the fascicular zone of another. In the thyroid gland the size of the follicles was uneven, but otherwise no abnormality was detected. These changes in the various tissues of the rabbits fed on the high-cholesterol diet contrasted sharply to the less marked pathological changes found in the rats on the cholesterol-feeding for corresponding term.

The brain arteries were not affected by the cholesterol-feeding alone, but the administration of 5 mg/kg/day of TBF-43 caused a slight or moderate hypertrophy of the endarteries in the meninges and parenchymas and a periarterial edema (dilatation of the Virchow-Robin's space) in the cholesterol-fed rabbits. Many vacuoles and slight atrophic pictures of the heart muscle fibers were observed in two out of three rabbits received 5 mg/kg/day of TBF-43. In the lung of all animals there was marked hypertrophy of the media of the middle-sized arteries and proliferation of the elastic fibers between the media and intima. In one animal the similar atheromatous change of the intima was observed in the large arteries of the lung. Furthermore, the marked atheromatous hypertrophy was detected in the endothelium of the aorta of animals.
In one out of three animals, extreme hypertrophy of the endothelium resulted in a stenosis of the aortic space. The diffuse lobular fatty infiltration in the liver of the rabbits received 5 mg/kg/day of TBF-43 was more marked than that of the untreated animals. The fatty infiltration was observed even in the peripheral part of the hepatic lobules. In the adrenal gland the gross vacuoles were detected in the glomerular zone, inner layer of the fascicular zone and small vacuoles in the reticular zone. The border between the glomerular and fascicular zones was vague in one animal. The size of the follicles of thyroid gland was not uniform and the shape of the follicular epithelia were polymorphous. Moreover, the flattening of the epithelia and the uneven coloration of the intrafollicular colloid were indicative for the atrophy of the thyroid structures. The reduction of number of the sperma cells was observed in the testis.

In general, the pathological changes of the tissues mentioned above were more marked in the rabbits which received the daily dose of 15 mg/kg of TBF-43. There was hyaline hypertrophy of the intima of the arteries and arterioles in the brain parenchyma. The hyaline hypertrophy of the intima and the obstructive changes in the coronary arteries resulted in the proliferation of the interstitial tissues and the fatty degeneration of the heart muscle. One animal showed many regions of the bionecrosis indicative for the manifestation of the heart infarct. In the lung, the atheromatous hypertrophy of the intima and media caused the arterial and arteriolar stenosis. The similar hypertrophy was also observed in the large arteries of the lung. The hypertrophy was also observed in the large arteries of the lung. The hypertrophic changes of the aorta was almost in the same degree as in the animals received 5 mg/kg/day of TBF-43. The diffuse fatty infiltration of the hepatic lobules was always observed. In two out of three rabbits, there were atrophic changes of white pulp in the spleen accompanied with the infiltration of neutrophilic cells. Moreover, many foam cells take up lipoid substances were observed in red pulp of the spleen. In the adrenal gland the glomerular zone showed signs of hypertrophy and the vacuolization was observed in the reticular zone. The border between the glomerular and fascicular zones was not distinct. The follicular epithelia of the thyroid gland showed polymorphism and the size of the follicles was smaller than that in the untreated animals. The follicular colloid was stained lightly and the vacuoles were detected in the follicles. In one animal the proliferation of the interstitial and lymphatic tissues of thyroid gland was observed.

The more marked pathological changes were detected in rabbits received the daily dose of 30 mg/kg of TBF-43. The edema and hypertrophy of the intima of the brain arteries were more marked than those in the animals at the 15 mg/kg/day level. Especially, in the rabbit died at the 6th week of the drug administration the obstruction of the arteries and arterioles due to the hypertrophy of the intima was observed in the brain and heart. The pathological pictures in the lung, aorta and liver of the rabbits received 30 mg/kg/day of the compound were almost the same as those in the animals at the 15 mg/kg/day level. The glomerular zone of the adrenal gland showed
a marked hypertrophy and the extent of the zone was 2 to 3 times of that in the untreated animals. On the other hand, the fascicular zone was narrow in width and contained many gross vacuoles. The pathological changes of the thyroid gland and testis were similar to those in the glands of the rabbits which received 15 mg/kg/day of TBF-43.

**DISCUSSION**

Though the cholesterolytic effect of thyroid hormone in the experimental animals and humans has generally been accepted, the mode of action remains to be settled. In an attempt to separate the cholesterolytic effect from the basal metabolic one, various iodothyroalkyl acids were extensively subjected to experimental and clinical trials. 3,5,3'-Triiodo-4'-acetyl-thyroformic acid (TBF-43) was reported to exhibit a considerably strong cholesterolytic effect with little increase in the basal metabolic rate in the hypercholesterolemic patients (5). The acute and subacute toxicity of the compound in the mice and rats showed that the margin of safety for the cholesterolytic effect was relatively narrow (6, 7).

The chronic toxicity of TBF-43 in the rats and rabbits fed on the standard diet or the high-cholesterol diet was described in the present report. The daily administration of the compound above the dose of 30 mg/kg caused a significant depression on the body weight gains in the rats fed on the standard diet. Moreover, the animals treated with TBF-43 took up larger amount of food compared with controls. The increased uptake of the food was always observed in the rats received the daily dose above 10 mg/kg. The rats which received the daily dose of 30 mg/kg or more showed a sign of depilation and inflammatory change of the mucous membranes. In the rats fed on the high-cholesterol diet the daily administration of 5 mg/kg of TBF-43 did not affect the body weight gains and the food consumption. On the other hand, the same procedure of 5 μg/kg of 3,5,3'-triiodothyronine (T₃) depressed the body weight gains slightly. It was reported that the clinical cholesterolytic doses of TBF-43 and T₃ were 1 mg/kg/day and 1 μg/kg/day, respectively (4, 5). Therefore, the results obtained in the present experiments showed that the depressive effect of TBF-43 on the body weight gain in the rats is weaker than that of T₃.

The feeding of the rats on the high-cholesterol diet for 8 weeks elevated considerably the cholesterol level in the serum and liver, thereafter the increased level declined spontaneously in spite of the continuation of the cholesterol-feeding. The daily administration of 5 mg/kg of TBF-43 or of 5 μg/kg of T₃ depressed slightly the elevation of the cholesterol level in the serum induced by the cholesterol-feeding. The spontaneous decline of the serum cholesterol level was also accelerated by both compounds. The more pronounced decrease of the cholesterol level was obtained by the withdrawal of cholesterol and taurocholic acid from the diet. In the liver, the daily administration of TBF-43 did not modify the spontaneous decline of the level during the term of cholesterol-feeding. On the other hand, the daily administration of T₃ caused a marked...
increase in the accumulation of cholesterol in the liver and failed to show the spontaneous decline of the liver cholesterol level during the extended feeding of the animals on the high-cholesterol diet. These results indicate that the cholesterolytic effect of T₃ in the liver is relatively weaker than that of TBF-43.

In the rabbits fed on the high-cholesterol diet, the daily administration of TBF-43 depressed the body weight gains and the larger doses of the compound produced a marked emaciation. The rabbits which received the daily dose above 15 mg/kg showed an increase in the heart rate. Moreover, some rabbits at the doses of 15 and 30 mg/kg/day exhibited a marked degree of emaciation and icterus, and died. These toxic symptoms are likely to derive from the elevation of the basal metabolic rate and the atrophic and degenerative changes of the parenchymatous organs and the arteries. These symptoms were observed more markedly in the rabbits than in the rats. It was previously reported (8) that the cholesterol level of the serum and liver in the rabbits increased progressively along with the length of the term of the cholesterol-feeding and did not decrease unless the deprivation of cholesterol from the diet. In the rabbits the daily administration of TBF-43 failed to prevent the increase in the cholesterol level of serum. On the contrary, the larger doses of the compound accelerated it. However, the accumulation of cholesterol in the liver was slightly inhibited by the administration of TBF-43.

Autopsies of the rats received the daily doses of 5 and 10 mg/kg of TBF-43 for 12 weeks showed slight increase in the weight of heart, lung and kidney. In the microscopic examinations, slight atrophic pictures were detected in the follicular epithelia of the thyroid gland, the central portion of the hepatic lobules and the white pulp of the spleen. However, the daily doses above 30 mg/kg of the compound produced a marked diminution of the fatty tissues in spite of a significant increase in weight per body weight of the parenchymatous organs. The increase of the relative value of tissue weight per body weight is due to a marked retardation of the body weight gains. Furthermore, congestive changes of some parenchymatous organs and atrophic pictures of the lymph gland, liver, spleen, kidney, adrenal gland, thyroid gland and testis were the common findings.

The proliferative increase of the fatty tissues in the rats fed on the high-cholesterol diet was not markedly affected by the daily administration of TBF-43 or T₃. The fatty infiltration of the hepatic lobules and the atrophic changes of the adrenal and thyroid glands observed in the rats fed on the high-cholesterol diet were slightly improved by the daily administration of 5 mg/kg of TBF-43 for 4 weeks. On the other hand, the daily administration of 5 μg/kg of T₃ for 4 weeks failed to improve these changes. The fatty infiltration and atrophic pictures in the organs were gradually and spontaneously ameliorated despite the continuation of the cholesterol-feeding. The daily administration of TBF-43 more than 8 weeks did not significantly affect the spontaneous improvement of the tissue changes, while the same procedure of T₃ worsened the atrophic changes occasionally. The progressive improvement of the pathological changes caused
by the withdrawal of cholesterol from the diet was markedly activated by the daily administration of TBF-43 but not that of T₃.

There were marked proliferations of the fatty tissues in the rabbits fed on the high-cholesterol diet. The daily administration of 15 mg/kg of TBF-43 produced a decrease in weight of the liver, thyroid gland and testis and a slight increase in weight of the lung, spleen, kidney and adrenal gland. In accord with the progressive elevation of the cholesterol level in the serum and liver, atheromatous changes of the large and intermediate arteries and atrophic-degenerative pictures of the heart, liver, spleen, adrenal gland, thyroid gland were observed by the microscopic examinations. The intima and media of the arteries and aortae showed hypertrophic changes mainly consisted of lipotropic substances. In the rabbits, the daily administration of large dose of TBF-43 accelerated the atheromatous changes of the arteries and the degenerative pictures of the parenchymatous and endocrine organs, though the proliferations of the subcutaneous and retroperitoneal fatty tissues were markedly depressed or even completely diminished. These effects of the compound were proportional in intensity to the dose administered. Therefore, it is concluded that the effects of TBF-43 are much different between rats and rabbits. The leading role of rabbits as experimental animal for the research of atherosclerosis was emphasized by Mann (11) and Davidson (12). The results obtained in the present report show clearly that the rats can excessively metabolize the administered cholesterol in some different pathway from the rabbits in order to maintain the normal cholesterol level in the blood and liver. It is assumed that regulating mechanism in the rats is reflected in the spontaneous decline of the cholesterol level which developed gradually after the manifestation of the peak of cholesterol level.

It is considered that the rabbits lack the regulating mechanism and show a progressive elevation of cholesterol level in the serum and liver along with length of the cholesterol-feeding. The administration of TBF-43 resulted in the accelerating manifestation of the atheromatous hypertrophy of the intima and media of arteries and of the atrophic-degenerative changes of the parenchymatous organs in the rabbits. These effects of TBF-43 are assumed to relate with the lack of regulating mechanism against the elevated cholesterol level in the rabbits. The comparative study of TBF-43 and T₃ on the experimental arteriosclerosis in rabbits will be described by Osumi (13) elsewhere.

SUMMARY

The chronic oral toxicity of 3,5,3'-triiodo-4'-acetyl-thyroformic acid (TBF-43) in the rats and rabbits fed on standard diet and high-cholesterol diet was described in the present report.

In the rats fed on the standard diet, the daily administration of TBF-43 above the dose of 10 mg/kg resulted in toxic pictures of some parenchymatous organs. The cholesterololytic effects of the compound differed markedly between rats and rabbits. In the rats, TBF-43 showed preventing effects on the elevation of the cholesterol level in
serum and liver and the improving effects on the atrophic-degenerative changes of the parenchymatous organs produced by the cholesterol-feeding. In the rabbits, however, the compound did not show the improving effects on the cholesterol level in serum and on the pathological changes of the arteries and parenchymatous organs caused by the cholesterol-feeding. These results are assumed to relate with the differential metabolic pathway of cholesterol between both species.

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