EFFECTS OF ETHYL-LINOLEATE ON THE ATHEROMATOUS CHANGES CAUSED BY HIGH CHOLESTEROL DIET IN THE RABBIT

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Since lowering effects of essential fatty acids and vegetable oil on the blood cholesterol level were reported by Tuttle (1), Kinsell et al. (2) and Vles et al. (3), the similar studies were further extended to unsaturated fatty acids of longer chains. The lowering effects of pure linoleate and arachidonate on the elevated blood cholesterol level were presented by Kinsell et al. (2) and Kingsbury (4). However, the recent study by Beveridge and Connell (5) has shown that the large doses of margarine which contained large amounts of unsaturated fatty acids produced a slight elevation of the blood cholesterol level in humans.

The histologically atherosclerotic or atheromatous changes of the tissues in rabbits caused by feeding with the diet containing between 1 and 3% of cholesterol for about 60 days were confirmed by many investigators including the present authors (6). In the present experiments attempts were made to observe the effects of mixing of linoleic acid ethylester in the high cholesterol diet on the atheromatous changes and to compare the difference in the effects of the mixings between the saturated and unsaturated fatty acids.

METHODS

Twenty-three young male rabbits weighing about 1.8 kg at the start of feeding were used. The control group consisted of 3 rabbits was given 88 g/day/animal of the artificial diet (defatted CR-1 supplied by the Central Laboratory for Experimental Animal, Tokyo). The defatted diet contained 1.5% low fat in which the linoleic acid content was 18.2% according to gaschromatographic analysis. Therefore, the control group received the daily dose of 218 mg of linoleic acid. The other 20 rabbits were given the defatted diet mixed with 1% of cholesterol, and were used as the high cholesterol diet groups. These groups were divided into three groups. The first group consisted of 5 rabbits was given the high cholesterol diet mixed with 10% of ethyl-linoleate (L-group). The second group consisted of 5 rabbits was given the high cholesterol diet mixed with ethyl-linoleate and stearic acid in the ratio of each 5% (S, L-group). The last group consisted of 10 rabbits was given the high cholesterol diet mixed with 10% of stearic acid (S-group). The animals were kept in the individual cage at the room temperature...
of 22±1°C for 12 weeks. The body weight and the levels of the serum cholesterol and phospholipids were individually measured. At the end of 12th week of the experiment all animals were sacrificed by cutting both common carotid arteries, and the levels of cholesterol and phospholipids in the aorta and liver were estimated. The isolated parenchymatous organs were stained with hematoxylin-eosin for histological examination.

Details of the procedure for estimation of crude lipids in the liver, phospholipids and cholesterol in the serum, liver and aorta were described elsewhere (6).

RESULTS

1. Spontaneous behaviors

No significant change in the daily food intake between the control group and the high cholesterol diet group supplemented with fatty acids was observed throughout the feeding term. Moreover, deficient symptoms of essential fatty acids such as depilation, hematuria and chyluria were also not detected. One rabbit in the S-group died at the 5th week exhibiting the pathological findings of pneumonia and the other one died at the 10th week showing the pulmonal abscess, and two animals were suffered from jaundice.

2. Body weight gain

Body weight gain of the respective four groups of the rabbits is shown in Fig. 1. Each point in the figure represents the mean body weight at the respective weeks. The increases in body weight of the L-, S-, L- and S-groups to that of the control group at the 12th week were 146%, 110% and 91%, respectively. Therefore, it was much likely that the increase in body weight was proportional to the daily doses of ethyl-linoleate.

3. Electrocardiogram

No reliable difference was detected in heart rate, level of the ST segment and height of the T-wave in the electrocardiographic recordings (standard limb lead II) at the fully sedated state between the control group and the groups received the high cholesterol diet.

4. Serum lipids

The change in the levels of the total serum cholesterol estimated every two weeks is shown in Fig. 2. The control animals maintained the levels about 40 to 70 mg/100 ml throughout the experimental term, while the levels in the high cholesterol diet groups increased markedly and pro-
progressively along with elapse of time. Levels at the 8th week were 20 to 30 folds greater than that of the control group. Until the 8th week the increase in the total serum cholesterol level was likely to correlate with daily doses of ethyl-linoleate. The total serum cholesterol levels at the 8th week in the L-, S,L- and S-groups were 1,729±290, 1,523±354, 1,219±315 mg/100 ml, respectively. Although the level in the S,L- and S-groups showed further progressive increase, the level in the L-group decreased gradually.

The levels of the serum phospholipids estimated as inorganic phosphor declined in the control group from 4.82±0.21 mg/100 ml at the beginning of feeding to 2.32

![Graph](image-url)

**FIG. 2.** The effect of ethyl-linoleate on the serum cholesterol level of the rabbits fed on the high cholesterol diet.

**TABLE 1.** The effect of ethyl-linoleate on the serum phospholipids level of the rabbits fed on the high cholesterol diet.

<table>
<thead>
<tr>
<th>Group</th>
<th>Feeding term</th>
<th>0 week (mgP/100 ml)</th>
<th>12 week (mgP/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>2.32±0.70</td>
<td></td>
</tr>
<tr>
<td>1% Chol. + 10% S.</td>
<td></td>
<td>34.74±6.11</td>
<td></td>
</tr>
<tr>
<td>1% Chol. + 5% S. + 5% L.</td>
<td>*4.82±0.21</td>
<td>33.59±5.67</td>
<td></td>
</tr>
<tr>
<td>1% Chol. + 10% L.</td>
<td></td>
<td>32.46±9.39</td>
<td></td>
</tr>
</tbody>
</table>


**TABLE 2.** The effect of ethyl-linoleate on the liver and aortic lipid contents of the rabbits fed on the high cholesterol diet.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total-cholesterol (mg/100 g)</th>
<th>Phospholipids (mg/100 g)</th>
<th>Crude-lipid (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>*479±10</td>
<td>155.3±8.4</td>
<td>2.31±0.36</td>
</tr>
<tr>
<td>1% Chol. + 10% S.</td>
<td>1336±308</td>
<td>106.7±12.1</td>
<td>7.44±1.02</td>
</tr>
<tr>
<td>1% Chol. + 5% L. + 5% S.</td>
<td>1333±176</td>
<td>132.7±5.9</td>
<td>8.20±1.41</td>
</tr>
<tr>
<td>1% Chol. + 10% L.</td>
<td>2347±154</td>
<td>127.7±6.9</td>
<td>12.15±0.88</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>115±7</td>
<td>33.1±2.8</td>
<td>---</td>
</tr>
<tr>
<td>1% Chol. + 10% S.</td>
<td>1103±289</td>
<td>43.8±3.5</td>
<td>---</td>
</tr>
<tr>
<td>1% Chol. + 5% L. + 5% S.</td>
<td>1022±470</td>
<td>53.4±8.3</td>
<td>---</td>
</tr>
<tr>
<td>1% Chol. + 10% L.</td>
<td>1003±490</td>
<td>35.7±2.8</td>
<td>---</td>
</tr>
</tbody>
</table>

±0.70 mg/100 ml at the 12th week. On the other hand, the level in the groups received the high cholesterol diet increased to about 6 folds at the 12th week (Table 1). However, there was no clear cut correlation in the increased levels between the groups supplemented with the saturated and unsaturated fatty acids in the diet.

5. Tissue lipids

1) Aorta

Feeding rabbits high cholesterol diet increased markedly the levels of aortic cholesterol and the levels at the 12th week were about 9 folds as that in the control group. But there was no significant change in the increased level between the L-, S,L- and S-groups. The levels of aortic phospholipids increased slightly in the S,L- and S-groups but not in the L-group. The results are shown in Table 2.

2) Liver

Levels of liver cholesterol and crude lipid were markedly increased by feeding on the high cholesterol diet. The elevated cholesterol levels in the L-, S,L- and S-groups were 2,347, 1,333 and 1,336 mg/100 g, respectively. The results indicated that the administration of ethyl- linoleate activated the fatty deposition including cholesterol while that of stearic acid prevented it in the liver. The high cholesterol diet lowered the levels of the liver phospholipids slightly. These decreases were most markedly observed in the S-group.

6. Pathohistological findings

The rabbits received the high cholesterol diet were characteristic in the marked proliferation of the fatty tissues especially in the retroperitoneal spaces and around the parenchymatous organs in the peritoneal space. The tissue weights are shown in Table 3.

Liver: The yellowish brown discoloration of the liver was markedly observed in the L-group. The liver was the heaviest in the L-group and its weight was about 1.7 to 2.0 folds as the control. Disarranged acinal structure with atrophic and swollen cell was the usual finding. The disorganized trabecular structure of the liver cells with less staining of the protoplasmic granules and pycnotic or disappeared nuclei were more markedly observed in the central portion of the acinus than in the

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight</th>
<th>Adrenal gland (mg)</th>
<th>Spleen (mg)</th>
<th>Kidney (g)</th>
<th>Heart (g)</th>
<th>Lung (g)</th>
<th>S, L, S, L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>290 ± 53</td>
<td>113 ± 31</td>
<td>282 ± 64</td>
<td>45 ± 13.3</td>
<td>52 ± 3.1</td>
<td>0.08 ± 0.02</td>
<td>6.62 ± 0.22</td>
</tr>
<tr>
<td>10% S, L</td>
<td>291 ± 57</td>
<td>114 ± 33</td>
<td>284 ± 67</td>
<td>45 ± 13.3</td>
<td>52 ± 3.1</td>
<td>0.09 ± 0.02</td>
<td>6.72 ± 0.22</td>
</tr>
<tr>
<td>5% S, L, S</td>
<td>290 ± 55</td>
<td>113 ± 31</td>
<td>282 ± 64</td>
<td>45 ± 13.3</td>
<td>52 ± 3.1</td>
<td>0.08 ± 0.02</td>
<td>6.62 ± 0.22</td>
</tr>
<tr>
<td>10% L</td>
<td>290 ± 53</td>
<td>113 ± 31</td>
<td>282 ± 64</td>
<td>45 ± 13.3</td>
<td>52 ± 3.1</td>
<td>0.08 ± 0.02</td>
<td>6.62 ± 0.22</td>
</tr>
</tbody>
</table>

*standard deviation, S: stearic acid, L: ethyl linoleate, **: The underlined values of tissue weights were markedly increased in comparison with the values of control.
peripheral portion. The artery in the atrophic interacinal tissues showed atheromatous proliferation of the intima. However, the liver in the S-group showed normal staining of the cells especially in the periphery of acinus (Fig. 3).

**Spleen**: The spleen of the rabbits received the high cholesterol diet was discolored light brown or white-brown and increased in weight. The increase was about 1.7 folds in the S,L-group and 3 folds in the L-group. The spleen in the L-group showed atrophic picture of the white pulp with atresic central artery showing extreme proliferation of the intima. On the other hand, the red pulp showed reduction in the capillaries, erythrocytes and splenocytes. The splenocytes containing gross and fine vacuoles were swollen and less staining. These atrophic or degenerative changes were less in the spleen of the S-group (Fig. 4).

**Adrenals**: The adrenals of the rabbits received the high cholesterol diet were discolored yellowish and increased in weight. The increase was about 2.5-3 folds as that of the control animals. The cellular structures in the fascicular and the reticular zones were disarranged, and the atrophic and swollen cells with pycnotic or less stained nucleus showed decreased staining of the protoplasmic granules. These findings were observed in every treated groups.

**Thoracic aorta**: Feeding animals the high cholesterol diet resulted in marked atheromatous proliferation of the intima and media of the thoracic aorta. Elastic fibers in
the intima were swollen and muscle fibers showed some fragmentations. These changes were more marked in the aorta of the S-group, and the aorta showed, in addition, fatty infiltration and hyalination of the muscle fiber in the media (Fig. 5-A).

**Kidney:** Although the kidney of the S-group showed increased weight about 12.5% as that of the control animals, the rabbits in the L-group produced no significant change in weight of the kidney. However, epithels in the proximal and especially distal tubules were swollen and the intratubular space was narrowed. There were reddish-brown pigmentionations in the intracellular spaces. These changes were more markedly seen in the kidney of the S-group. The tubular epithel showed marked fatty infiltration and slight hyaline degeneration. The epithels in the distal portion of the tubules showed highly sclerotic changes with pycnotic nucleus and narrowing of the glomerular space (Fig. 5-B).

**Lungs:** The weight of the lungs increased slightly in the rabbits received the high cholesterol diets. On the whole, the slightly sclerotic alveolar epithels seemed edematous. The atheromatous proliferation of the media and intima of the arteries and arterioles was not different between the L- and S-groups.

**Heart:** Feeding animals the cholesterol diet increased slightly weight of the heart. No significant histological change was detected in the heart muscle.

**Thyroid gland:** Cholesterol feeding decreased slightly weight of the thyroid gland and it was more markedly observed in the S-group. Generally, high-fat and cholesterol feeding produced a considerable adenomatous proliferation of the glandular structure and the follicle usually contained no colloid.

**Hypophysis:** The anterior hypophysis showed a significant reduction in number of the basophile cells.

**Gastro-intestinal tract:** No significant histological change was detected by feeding animals the cholesterol diet.

**Testis:** Feeding animals the cholesterol diet did not modify the weight and histological findings of the testis.
DISCUSSION

Lowering effects of essential fatty acids including linoleic acid on the blood cholesterol level in the rat and man have been accumulated in recent decades. More marked lowering of the serum cholesterol level in the rabbit by feeding on the non-treated corn oil than the cooked one has been presented by Kritchevsky et al. (7). In the present experiments, the levels of serum and liver cholesterol were more increased in the L-group and this evidence may need further experiments.

The activating effects of linoleic acid on biosynthetic processes in the liver were demonstrated in vivo by Avigan and Steinberg (8) and in vitro by Mukherjee and Alfin-Slater (9). Gordon et al. (10), on the other hand, indicated that linoleic acid served to activate the oxidation of cholesterol to cholic acids and therefore, to increase its excretion into the bile. Increased absorption of cholesterol from the intestine in the presence of unsaturated fatty acids was shown by Vahauny et al. (11). The dominant availability of unsaturated fatty acids for the esterification of cholesterol in the intestine was indicated by Alfin-Slater (12). The less absorption of saturated fatty acids such as stearic and palmitic acids in the intestine than unsaturated fatty acids was shown by Dauer et al. (13). The lowering effect of unsaturated fatty acid compared to saturated fatty acid on the serum cholesterol level reported by the Vles et al. (3) was attained by the administration of these fatty acids (no cholesterol), to the hypercholesterolemic rabbits produced by high fat and cholesterol feeding. From these points of view, even if linoleic acid activates the biosynthesis and the transport of cholesterol or the oxidative metabolic mechanism of cholesterol in the liver, more marked increase in the levels of the serum and liver cholesterol as well as in the body weight in the rabbits of L-group than in the animal of S-group may derive partly from the activated absorption of cholesterol. It was reported that there was a possibility of the existence of large concentration of cholic acids in the intestine at the time of intestinal digestion and absorption (14). This evidence presents the possibility that linoleic acid facilitates the oxidative excretion of cholesterol into the bile and accordingly, the increased level of cholic acids in the intestine activates the absorption of fat.

Feeding rabbits the low-fat diet was expected to show not only the biochemical effects of linoleic acid but also to produce the deficient symptoms of the essential fatty acids. However, no deficient symptom was observed. Therefore, the low-fat diet used in the present experiments was likely to contain unsaturated fatty acids in the amounts not smaller than the daily requirement dose, 100 mg/kg. However, the rabbits in the S-group demonstrated the icterus in two and the least level of the liver phospholipids. The findings may reflect the relative deficiency of unsaturated essential fatty acids rather than the chronic toxicity of stearic acid.

The characteristic histological findings in the rabbits received the high cholesterol diet were the marked fatty infiltration of the liver and spleen with ensuing degenerative changes, as well as the atheromatous proliferation of the aorta and arteries in the
kidney, liver and lungs. Findings in the liver and spleen were most marked in the L-group and showed the highest elevation of the level of the serum cholesterol, while findings in the aorta and arteries were found most marked in the S-group and showed least elevation of the serum cholesterol level. The liver and spleen are usually regarded to have high activity in pooling or uptaking the excess of the absorbed fat for further disposal. Alfin-Slater (12) pointed out that the cholesterol ester of linoleic acid in the organs was more labile than the same ester of saturated fatty acids. The paradoxical non-parallelism in the cholesterol levels between liver and spleen on one side and aorta and arteries on the other side may be assumed that the cholesterol levels in the former organs correlate more closely to the serum cholesterol level and the absorbed linoleate facilitates the active uptake process of cholesterol by the liver and spleen. On the other hand, the deposit of cholesterol in the aorta and arteries more markedly activated by the supplementary diet with stearic acid than by the same diet with linoleate is assumed to relate with the passive mechanism, or with the stable esterification of cholesterol with stearic acid. However, the exact mechanism was still unknown from the results in the present experiments.

In conclusion, essential fatty acids such as ethyl-linoleate have serum cholesterol lowering effect and accelerating effect on cholesterol absorption by the intestine, and it is tempting to assume that the administration of essential fatty acid supplemented with large dose of cholesterol (such as 1% cholesterol) simultaneously, promote the latter effect so that this resulted in a more marked hypercholesterolemia than that of saturated fatty acid did in it.

**SUMMARY**

Rabbits were fed low fat basal diet supplemented with 1% cholesterol and 10% fatty acids for 12 weeks. These fatty acids were: i) 10% ethyl-linoleate, (L-group), ii) 5% ethyl-linoleate+5% stearic acid (S,L-group), iii) 10% stearic acid (S-group). Further, the effects of ethyl-linoleate on the lipid metabolism were observed. The results were summarized as follows.

1. The body weight increase of rabbits in the L-group was greater than that of controls, but the growth curve in the S-group did not differ from that of controls.

2. The administration of ethyl-linoleate accelerated the increase of serum and liver cholesterol levels compared with that of stearic acid. Feeding rabbits the cholesterol diet decreased the liver phospholipids level and these decreases were most markedly observed in the S-group.

3. Feeding rabbits the cholesterol diet increased the cholesterol level of the thoracic aorta and there was no significant change in the increased levels between these three groups. The phospholipids levels of the thoracic aorta in the S- and S,L-groups increased compared with that of controls.

4. Feeding rabbits the cholesterol diet produced adenomatous proliferation of the thyroid glands, atheromatous changes of the aorta and arteries in the lungs, spleen and
kidney and lipid infiltration of the liver, spleen and adrenals. On the other hand, the coronal and meningeal arteries were not affected. These histological changes in the liver and spleen were marked in the L-group. The changes in the aorta and kidney were marked in the S-group. Histological findings in the S,L-group showed almost similar changes as that of the L-group.

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