Depletion of Hepatic Coenzyme A Derivatives Is One of the Markers of the Toxicity of Orally Administered Secondary Autoxidation Products of Linoleic Acid in Rat

Kazuki Kanazawa,1 Hitoshi Ashida,2 Masashi Mizuno,2 and Masato Natake1

1Department of Agricultural Chemistry and 2Division of Utilization of Biological Resources, Kobe University, Nada-ku, Kobe, Hyogo 657, Japan (Received August 1, 1988)

Summary When 400 mg/rat/day of secondary autoxidation products of linoleic acid was orally administered 3 times to rats, they died at 30–40 h after the third dose. To search the markers of the toxicity of secondary products in vivo, the rats were killed at 24 h after the third dose, and conditions of their digestive tracts and liver were analyzed. In the stomach, macroscopically, inflation, retention of undigested food, and edema were seen. Slight congestions were detected in the small intestines. It was considered that these injuries led to reduction in food consumption and then depression of the growth, but did not lead to the death of the animals. The lipid peroxide levels in the liver and the activities of its detoxifying enzymes were increased as compared to those in the control groups. The hepatic lipid contents and unsaturated fatty acid compositions were also not changed. The endogenous lipid peroxidation, therefore, did not give the rats a severe stress. The activities of hepatic acetyl-CoA carboxylase and carnitine palmitoyltransferase were 20 and 35% lower than those of control, respectively. The levels of CoASH, acetyl-CoA, and long-chain acyl-CoA were 1/9, 1/2, and 1/4 of those in control, respectively. Thus, one of the markers of the toxicity of secondary products was the depletion of hepatic CoA derivatives. In rat, bio-energy was reduced by the decrease in the intestinal absorption of nutrients, and the depletion of hepatic CoA derivatives also failed to supply energy with ß-oxidation.

Key Words autoxidation products, lipid peroxide, marker of toxicity, depletion of CoA, hepatotoxicity, lipid metabolism, toxicity by oral intake, injury of alimentary canal (rat)
Oral intake of autoxidation products of unsaturated fatty acids causes damage to an animal body (1). It is important to know the markers of the toxicity of autoxidation products in vivo. When 700 mg/rat of secondary autoxidation products of linoleic acid was orally administered to rats, their hepatic functions were significantly injured (2). We have studied the hepatotoxocities of secondary products of linoleic acid (3–9). When the dose of 700 mg/rat/day was continued, the body weight of the animal was remarkably reduced and later the animal died (2), while the consecutive dose of linoleic acid hydroperoxides did not lead to death of the animal (10).

Three effects as the lethal toxicity of secondary products in rat body are assumed. (a) Secondary products may injure the animal digestive tracts, make an absorption of nutrients difficult, and then lead to the death by starvation. (b) We previously reported on the toxicities of the single dose of secondary products (3, 5). Secondary products increased the content of hepatic lipid peroxides, which were easily detoxified later. However, the consecutive dose of secondary products makes the detoxication difficult and a biomembrane may be severely injured. (c) We also reported that secondary products were easily incorporated into liver and attacked biologically important components (4, 6–9). The consecutive dose of secondary products may drastically destroy the components and disturb the hepatic metabolism.

In this study, rats were administered secondary products at a dose of 400 mg/rat/day. The first dose did not affect the growth of the rats, but after the second dose the growth and food consumption were markedly depressed. In the preliminary experiment, when 48 rats received the 3 consecutive doses of secondary products, all animals died at 30–40 h after the third dose. To search the markers of the toxicity, the rats were killed at 24 h after the third dose and we attempted to determine the lethal cause. The present study focussed on the above-mentioned effect (c) and thus on a search of the markers in the hepatic lipid metabolism.

**MATERIALS AND METHODS**

**Secondary autoxidation products of linoleic acid.** Linoleic acid was purchased from Tokyo Kasei Kogyo Co., Ltd., and autoxidized at 37°C for 7 days. The secondary product fraction was obtained from the autoxidized linoleic acid by silica gel column and thin-layer chromatographies, and was analyzed by gas chromatography-mass spectrometry and Sephadex LH-20 gel filtration-chromatography (11). The secondary product fraction consisted of 36% mixture of polymers, 26% epoxyhydroperoxides or endoperoxides (identified on gas chromatography (12)), 4.8% 9-oxononanoic acid, 3.7% hexanal, 2.5% nonanedioic acid, 2.4% short-chain carboxylic acids, 0.75% 8-oxooctanoic acid, 0.34% 12-oxidodecadienoic acid, and other smaller unidentified compounds.

**Animals and diet.** Male Wistar rats, 5 weeks old and each weighing about 110 g (KY, SPF: Shizuoka Laboratory Animal Center), were housed at 23°C with a

12 K. KANAZAWA et al.
light and dark cycle of 12 h each, and fed on a fresh diet for 1 week. The diet was prepared daily and its peroxide value was maintained at less than 0.5 meq/kg. The detailed composition of the diets has been described previously (5). Briefly, it consisted of 30% sucrose, 25% casein, 24% corn starch, 15% soybean oil, 4% McCollum’s salt mixture, 1% cellulose powder, and 1% vitamin mixture. The rats were divided at random into 5 groups of 8 rats each and their foods were withheld for 4 h before the treatments. The first group was untreated rats. The second and third groups were intragastrically given 400 mg/rat/day of linoleic acid and secondary products for 3 days, respectively, using a tuberculin syringe equipped with a stomach tube. The other two groups were given 400 mg/rat/day of saline solution for 3 days and one of them was fed on a limited amount of diet to use it as a pair-feeding group with the secondary product group. After the administrations, the food and water were supplied ad libitum for these groups of rats except for the pair-feeding group. The animals were sacrificed at 72 h after the start of experiments and their organs and tissues were analyzed macroscopically.

Collection of blood and preparation of a liver homogenate. Blood was collected by cardiac puncture. The rat liver was perfused for 10 s with a saline solution using a cannula and then treated immediately according to the freezed-clamped method of Williamson et al. (13). The liver was frozen with lead blocks precooled in liquid nitrogen. The frozen liver was pulverized in a mortar with the frequent addition of liquid nitrogen. The liver powder was subjected to the following analyses within 24 h. A part of the powder was homogenized with 10 volumes of a 1.15% KCl solution. The protein concentration in the homogenate was estimated by the method of Lowry et al. (14).

Measurement of serum tocopherol level and hepatic lipid peroxide content. The tocopherol level was determined in serum by the method of Taylor et al. (15). To measure the hepatic lipid peroxide content, thiobarbituric acid (16) and hemoglobin-methylene blue (17) tests were carried out using the liver powder homogenate. Both tests may be indirect ones for the determination of lipid peroxides. However, many data on endogenous lipid peroxidation have been obtained with both methods; thus, they were also used here.

Determination of detoxifying enzyme activities and glutathione levels. The liver homogenate was subjected to the analyses of enzyme activities and metabolite levels. The activity of glutathione peroxidase was determined by the method of Little et al. (18) using pure linoleic acid hydroperoxides as its substrate (19). The activities of glutathione reductase and catalase were measured by the methods of Zanetti (20) and Bergmeyer et al. (21), respectively. The levels of reduced form of glutathione (GSH) and its oxidized form (GSSG) were quantified by fluorometry (22).

Hepatic lipid content and fatty acid composition. Lipids were extracted from the liver powder homogenate with 3 volumes of a methanol–chloroform (2:1) mixture using a Potter-type homogenizer. The chloroform layer was retained and washed with water. The chloroform was evaporated in vacuo and then the dry
matter was weighed to determine the lipid content in the liver. Lipids were dissolved again in 1 ml of chloroform and then applied on a silica gel column (Wakogel C-100, 1 × 17 cm). The neutral lipid, glycolipid, and phospholipid fractions were eluted with chloroform, acetone, and methanol, respectively, as shown previously (5). The neutral lipid and phospholipid fractions were dried up, weighed, and then subjected to fatty acid composition analysis. Lipids were hydrolyzed and then methyl-esterified with 0.5 N sodium methylate in 30% benzoic methanol (23). The methyl esters were extracted with one volume of hexane 3 times and then subjected to the gas chromatography (Shimadzu Gas Chromatograph, Model GC-6AM, with a 2 m column of EGSS-X (2%)) at a constant temperature of 180°C.

**Lipogenic and lipolytic enzyme activities.** The liver homogenate was centrifuged at 700 × g for 10 min and the resultant supernatant was subjected to centrifugation at 12,000 × g for 20 min. The pellet was used as the mitochondrial fraction and the supernatant was centrifuged again at 105,000 × g for 1 h. The resultant supernatant was referred to as the cytosol fraction. Carnitine palmitoyl-transferase activity in the mitochondrial fraction was assayed according to Markwell et al. (24). Acetyl-CoA carboxylase activity was measured by the 14C-labeled sodium bicarbonate fixation method using the cytosol fraction (25).

**Levels of CoA derivatives.** One gram of the liver powder was submitted to acid-extraction with 4 ml of cold 9% perchloric acid. The levels of CoASH and acetyl-CoA present in the extract were determined enzymatically by the methods of Michal and Bergmeyer (26), and Decker (26), respectively. Acid-insoluble long-chain acyl-CoA present in the precipitate was converted to CoASH (26) and then quantified.

**Statistic analysis.** When F-test for homogeneity of variance showed that variances were heterogeneous, Student’s t-test was employed to determine the statistical significance. The variability of the data is presented as means ± SE and a 0.05 probability level was chosen.

**RESULTS**

**Injury in the digestive tracts**

When 400 mg/rat/day of secondary products were orally administered for 3 days, the rats consumed half the amount of food as compared to the amounts in untreated rats after the first dose, and after the second dose they almost did not approach the foods. Subsequently, the digestive tracts of these rats were macroscopically analyzed, and compared to those of the four other groups as controls: untreated rats, rats triply dosed with linoleic acid (400 mg/rat/day), rats triply dosed with saline solution (400 mg/rat/day), and pair-feeding rats. In order to have the body weights of pair-feeding rats coincide with those of the secondary product group, the diet amount of pair-feeding rats was reduced to half at the first day, and later their foods were withheld (water was not). When the foods of the pair-feeding group were further withheld for an additional few days in a preliminary experiment,
DEPLETION OF COENZYME A

Fig. 1. Photographs of gastrointestinal tracts of linoleic acid-triply dosed rat (LA) and secondary product-triply dosed rat (SP). The arrows show the slightly congested parts.

the rats remained alive.

Abnormalities were detected in the digestive tracts of the secondary product group, but not in those of these four control groups. Figure 1 shows stomach, duodenum, and a part of the jejunum of a secondary product rat together with those of a linoleic acid rat. In the stomachs of the secondary product group, inflation near cardia (diameter was ca. 1 cm), retention of undigested food, and edema were seen. Slight congestions were detected on their small intestines. Macroscopically, hemorrhage and necrosis were not detected on the other organs and tissues in all groups of rats. It was considered that the reduction in food consumption of the secondary product group was caused by the injury of their digestive tracts.

The activities of intestinal enzymes, sucrase, maltase and alkaline phosphatase, were also measured with the method as previously described in detail (27). Sucrase and maltase are believed to distribute to the outer membrane of the microvilli of the epithelial cell and alkaline phosphatase to the inner membrane of the cell (28). It is generally considered that their activities can reflect the representative integrity and function of the intestinal brush border membrane (29). These enzyme activities in the rats with single doses of saline and linoleic acid were shown in a previous paper (27), and the similar activities were also obtained in the four control groups. On the contrary, in the secondary product group, the activities of sucrase and maltase were $0.203 \pm 0.048$ and $0.408 \pm 0.028$ nmol/min/mg protein, respectively, and were half that of the activities in the four control groups. The activity of
Table 1. Final body and liver weights.

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Untreated</th>
<th>Linoleic acid</th>
<th>Saline</th>
<th>Pair-feeding</th>
<th>Secondary products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>161 ± 2</td>
<td>161 ± 2</td>
<td>159 ± 2</td>
<td>124 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>122 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>10.0 ± 0.5</td>
<td>10.3 ± 0.9</td>
<td>9.8 ± 0.6</td>
<td>5.8 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.2 ± 0.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver weight/BODY weight (%)</td>
<td>6.21 ± 0.36</td>
<td>6.48 ± 0.55</td>
<td>6.18 ± 0.38</td>
<td>4.66 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.92 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significant difference from the saline group. <sup>b</sup>Significant difference from the pair-feeding group.

alkaline phosphatase was 17.5 ± 1.0 nmol/min/mg protein and was only slightly low. Thus, the triple dose of secondary products injured the intestinal membrane, but the extent of injury was not so strong as to lead to a leak of the inner membranous enzyme, alkaline phosphatase.

Effects on the rat growth

The effects of triple dose of secondary products on rat body and liver weights were observed, as compared to those of the four control groups (Table 1). The final body and liver weights in untreated, linoleic acid, and saline groups were about 160 and 10 g, respectively. As compared to them, those in the pair-feeding and secondary product groups were significantly low. The extent of decrease in the liver weights of pair-feeding rats was greater than that in their body weights, and the relative liver weight was 4.7%. In the secondary product group, the relative liver weight was 5.9% and was similar to those in untreated, linoleic acid, and saline groups. Thus, it was considered that the liver of the secondary product group was hypertrophied, and that the liver condition was different from that in the pair-feeding group.

Lipid peroxide content in the liver

In the following experiments, the changes in the metabolic functions of liver by the triple dose of secondary products were examined. The changes in the hepatic functions should be analyzed with two factors—the reduction in food consumption and the hepatotoxicity of secondary products. Therefore, the saline and pair-feeding groups were taken up as the control groups.

The hepatic lipid peroxidation decreases the serum tocopherol level. In the secondary product group, the tocopherol level was half that in the control groups (Table 2). The amounts of hemoglobin-methylene blue and thiobarbituric acid-reactive substances were also elevated to 1.5-fold. On the other hand, it is believed that the endogenous peroxides were reduced by catalase and glutathione peroxidase.

Table 2. Levels of serum tocopherol and hepatic lipid peroxides.

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Pair-feeding</th>
<th>Secondary products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocopherol</td>
<td>Content in serum (µg/ml)</td>
<td>15.8 ± 0.6</td>
<td>14.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Content in liver (nmol/mg protein)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin-methylene blue-reactive substances (as linoleic acid hydroperoxide)</td>
<td>0.521 ± 0.035</td>
<td>0.590 ± 0.039</td>
<td>0.751 ± 0.038&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thiobarbituric acid-reactive substances (as malonaldehyde)</td>
<td>2.19 ± 0.17</td>
<td>2.81 ± 0.50</td>
<td>3.76 ± 0.73&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significant difference from the saline group. <sup>b</sup>Significant difference from the pair-feeding group.

Table 3. Activity of hepatic detoxifying system.

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Pair-feeding</th>
<th>Secondary products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>326 ± 12</td>
<td>330 ± 12</td>
<td>375 ± 9&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>90.2 ± 4.5</td>
<td>98.0 ± 7.8</td>
<td>103.1 ± 4.3&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>47.9 ± 1.1</td>
<td>50.2 ± 2.7</td>
<td>70.2 ± 2.0&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Contents (µg/mg protein)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td>15.1 ± 0.7</td>
<td>15.3 ± 0.6</td>
<td>12.4 ± 0.6&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSSG</td>
<td>4.1 ± 0.3</td>
<td>3.8 ± 0.2</td>
<td>4.4 ± 0.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significant difference from the saline group. <sup>b</sup>Significant difference from the pair-feeding group.

in the liver. Glutathione peroxidase uses GSH as a reductant and forms GSSG. Coupled with this reaction, glutathione reductase recycles GSSG to GSH. Table 3 shows the changes in the activities of these enzymes and levels of GSH and GSSG. In the secondary product group, catalase activity was slightly increased as compared to those in controls. The activities of glutathione peroxidase and reductase were significantly increased to 3-fold and 1.5-fold, respectively. The decrease in GSH level, however, was only 20% and no accumulation of GSSG was detected. This indicates that this enzyme system was satisfactorily detoxifying the endogenous lipid peroxides in the liver of the secondary product group.

Hepatic lipid contents and unsaturated fatty acid compositions

The hepatic total lipid content in the secondary product group as well as in the
pair-feeding group was decreased by 45% as compared to that in the saline group (Fig. 2). The total lipids were separated into neutral lipids and phospholipids and their amounts were measured. The neutral lipid contents in both the secondary product group and its pair-feeding group were 65% lower than that in the saline group, and the phospholipid contents remained unchanged. Table 4 shows the compositions of unsaturated fatty acids in the neutral lipids and phospholipids.
DEPLETION OF COENZYME A

Table 5. Activities of lipogenic and lipolytic enzymes.

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Saline</th>
<th>Pair-feeding</th>
<th>Secondary products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enzyme activity (nmol/min/mg protein)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetyl-CoA carboxylase</td>
<td>2.64 ± 0.30</td>
<td>1.73 ± 0.07^a</td>
<td>1.36 ± 0.10^a,b</td>
</tr>
<tr>
<td>Carnitine palmitoyltransferase</td>
<td>28.3 ± 1.4</td>
<td>70.2 ± 12.9^a</td>
<td>44.7 ± 2.5^a,b</td>
</tr>
</tbody>
</table>

^aSignificant difference from the saline group. ^bSignificant difference from the pair-feeding group.

Table 6. Levels of CoASH, acetyl-CoA, and long-chain acyl-CoA in the liver.

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Saline</th>
<th>Pair-feeding</th>
<th>Secondary products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contents (nmol/whole liver)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CoASH</td>
<td>800 ± 66</td>
<td>1,079 ± 78^a</td>
<td>115 ± 10^a,b</td>
</tr>
<tr>
<td>Acetyl-CoA</td>
<td>393 ± 37</td>
<td>198 ± 24^a</td>
<td>117 ± 15^a,b</td>
</tr>
<tr>
<td>Long-chain acyl-CoA</td>
<td>379 ± 61</td>
<td>331 ± 21</td>
<td>80 ± 15^a,b</td>
</tr>
</tbody>
</table>

^aSignificant difference from the saline group. ^bSignificant difference from the pair-feeding group.

Arachidonic and docosahexaenoic acids in the neutral lipids were increased in both the pair-feeding and the secondary product groups as compared to those in saline group, and no difference between the secondary product group and its pair-feeding group was detected. In the phospholipids, only arachidonic acid of the secondary product group was slightly decreased (20%). Thus, the lipid contents in the secondary product group showed the same change as those in its pair-feeding group and the changes of fatty acid composition in the secondary product group were negligible.

Lipogenesis and lipolysis in the liver

To search the markers of the toxicity of secondary products in the liver, the hepatic lipid metabolism was examined. Table 5 shows the activity of one of the key enzymes in lipogenesis, acetyl-CoA carboxylase, and the activity of one of the key enzymes in lipolysis, carnitine palmitoyltransferase. The former enzyme activity was decreased and the latter was increased in the secondary product group and its pair-feeding group, as compared to those in the saline group. When these activities were compared between the groups of secondary products and its pair-feeding, the activities of acetyl-CoA carboxylase and carnitine palmitoyltransferase in the...
secondary product group were, respectively, 20 and 35\% lower than those in its pair-feeding group. Then, the hepatic levels of substrates of lipogenesis (CoASH and acetyl-CoA) and lipolysis (long-chain acyl-CoA) were measured (Table 6). In the pair-feeding group, the level of CoASH was 35\% higher than that in saline group, the level of acetyl-CoA was 50\% lower, and the level of long-chain acyl-CoA remained unchanged. On the contrary, in the secondary product group, the levels of these CoA derivatives were remarkably low. The levels of CoASH, acetyl-CoA, and long-chain acyl-CoA were reduced to 15, 30, and 20\%, respectively, as compared to those in the saline group. Thus, the changes in the levels of CoA derivatives in the secondary product group were different from those in its pair-feeding group. The triple dose of secondary products obviously depleted the CoA derivatives in the rat liver.

DISCUSSION

This study demonstrated that one of the markers of the toxicity of secondary products in vivo is the hepatic CoA derivatives and the depletion of them may be a lethal cause in the rat. The triple dose of secondary products (400 mg/rat/day) leads to the death of the rats. The triple dose injured the stomach and congested the small intestines (Fig. 1). These damages cause the reduction in absorption of nutrients and then result in stress such as starvation. The starvation suppresses the rat growth (Table 1); however, it is not a cause of death because the pair-feeding rats remained alive for a few more days.

On the other hand, the liver of the secondary product-triply dosed rats was hypertrophy as compared to that of its pair-feeding rats. We previously reported the increase in content of hepatic lipid peroxides and their detoxifying enzyme activities after the single dose of secondary products (5), and described the changes in the hepatic lipid metabolism as compared to the doses of linoleic acid and its hydroperoxides (9). In the present study, these changes were measured in rats at the point of death by the triple dose of secondary products. The triple dose of secondary products elevated the contents of hepatic lipid peroxides (Table 2) and increased the activity of its detoxifying system (Table 3). However, the extent of these changes was similar to that by the single dose (5). In the case of a single dose, such level of peroxide almost recovered to the normal level 96 h after the dose. Drastic endogenous lipid peroxidation allowed prediction of loss of membrane lipids. The triple dose of secondary product, however, did not induce the severe changes in both hepatic lipid contents and fatty acid compositions when compared to the case of its pair-feeding group (Fig. 2 and Table 4). Therefore, the detoxifying system satisfactorily worked in the liver of the triply dosed rats, and such level of the endogenous lipid peroxides was not so toxic to the animals. This indicates that the effect of endogenous lipid peroxidation is different from the toxicity of secondary products in vivo.

The content of hepatic total lipids in the pair-feeding group was decreased. It is
suggested that the animals used the depot lipids for supply of biological energy through $\beta$-oxidation, because the rats were starving. The increase of lipolytic activity and the decrease of lipogenic activity in the pair-feeding group also support this suggestion (Table 5). The triple dose of secondary product gave similar results except for the extent of changes in the lipid metabolic activity. Therefore, in the secondary product-triply dosed group, a part of the changes in lipid metabolism activity was due to the starvation.

The decreases of the CoA derivatives in the secondary product triply dosed group were remarkable, compared to those in its pair-feeding group (Table 6). The depletion of CoA derivatives further facilitated a decrease in the activity of the lipid metabolism. Inhibiting the absorption of nutrients in the secondary product group, the decrease in the supply of energy with lipolysis leads to severe reduction of biological energy. Therefore, it seems likely that one of the lethal toxicities of the secondary products is the depletion of CoA derivatives in the rat liver. In the pair-feeding group, the increase in the CoASH level and the decrease in the acetyl-CoA level were detected, as compared to the saline group. It was considered that the synthesis of acetyl-CoA from CoASH was reduced by the decrease in glycolysis which was caused by the disrupt supply of glucose from the diet.

The incorporated secondary products into liver may directly destruct the CoA derivatives or may disturb its supplemental system. We think that the toxic components contained in the orally administered autoxidation products are aldehydes, but not hydroperoxides, because aldehydes are easily incorporated into the animal liver unchanged in forms, but hydroperoxides are not (3-9). The incorporated aldehydes may specifically react with SH compounds such as CoASH as described by Esterbauer et al. (30). The single dose of secondary products reduced the CoASH and acetyl-CoA levels by 80, 35%, respectively, and the long-chain acyl-CoA level was unchanged, as reported previously (9). This result suggests that CoASH is firstly reduced in the rat liver. The depletion of CoASH inevitably results in the decrease of the acetyl-CoA and long-chain acyl-CoA levels.

Secondary autoxidation products may have other toxic effects in the rat body. A recent study showed that the dietary lipid hydroperoxides had severe effects on lymphoid tissues (31). We will examine the changes in levels of adenine nucleotides and activities of the related metabolic pathways in the liver, because ATP is another carrier of the biological energy and its amount is closely related to the levels of CoA derivatives in the liver.

REFERENCES


DEPLETION OF COENZYME A


Vol. 35, No. 1, 1989