Effects of Dietary Pantethine Levels on Contents of Fatty Acids and Thiobarbituric Acid Reactive Substances in the Liver of Rats Orally Administered Varying Amounts of Autoxidized Linoleate

Naoko HIRAMATSU,* Tadaaki KISHIDA,1 Takashi HAMANO,2 and Masato NATAKE3
1Himeji College of Hyogo, Shinzaikohoncho, Himeji 670, Japan
2Public Health Research Institute of Kobe City, Ikuta-ku, Kobe 650, Japan
3Department of Agricultural Chemistry, Faculty of Agriculture, Kobe University, Nada-ku, Kobe 657, Japan
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Summary The effects of dietary pantethine levels on the contents and compositions of fatty acids and on the levels of lipid peroxides were investigated with rat liver and its S-9 fraction under administration of 0 (non), 0.2 (low dose), and 0.35 ml (high dose) of autoxidized linoleate (AL) per 100 g body weight of the rats per day for 5 days. AL having 800 meq/kg of peroxide value (PV) and 1,700 meq/kg of carbonyl value (CV) was dosed to the rats of each group given drinking water containing 0 mg% (deficient), 6.25 mg% (adequate), and 125 mg% pantethine (excess). In the pantethine-deficient and -adequate groups, the contents of fatty acids both in the liver homogenate and in the S-9 fraction were correspondingly decreased by increasing dose levels of AL, and the decrease was remarkable especially in the pantethine-deficient group, but was not significant in the pantethine-excess group even by a high dose of AL. Particularly, in the high dose of AL, the notable decreases of oleic acid (C₁₈:1) contents in both the liver and the S-9 fraction were observed in rats of the pantethine-deficient and -adequate groups.

The thiobarbituric acid (TBA) values in the liver homogenate and the S-9 fraction were increased correspondingly by increasing dose levels of AL, and the increases were repressed in the pantethine-excess group. Key Words autoxidized linoleic acid, pantethine, fatty acid content, TBA value, rat liver, S-9 fraction

* To whom all correspondence should be addressed.
Previously, we have investigated in detail the effects of dose levels and dose periods of AL on the drug-metabolizing system in rat liver (1, 2). It has been found that both the content of cytochrome P-450 and the activity of the drug-metabolizing system were increased by a low dose of AL, and were decreased by a high dose of AL (1) or by the elongation of the dose period even in the case of a low dose of AL (2).

We have also investigated the effects of dietary pantethine levels on the drug-metabolizing system in the livers of rat given a low or a high dose of AL (3). That is, they were induced by the low dose of AL for 5 successive days and lowered by the high dose of AL for the same period, compared with respective non-AL groups in each of the three pantethine levels. In groups of the non-AL and the low dose of AL, enzyme activities of the electron transfer system in rat liver microsomes, aminopyrin-N-demethylase activity, and metabolic activation of 2-acetylaminofluorene in the S-9 fraction were significantly higher in the pantethine-deficient group than in the pantethine-adequate and -excess groups. These results suggested that the pantethine deficiency and the low dose of AL accelerated lipid peroxidation in vivo, followed by induction of the drug-metabolizing system in order to depress the enhanced in vivo lipid peroxidation. In the high dose of AL, the pantethine-excess diet seemed to effectively protect the drug-metabolizing system in comparison with the pantethine-adequate diet, though the enzyme activities in the system decreased significantly in any pantethine level groups. It was concluded from these results that pantethine might have relieved the effect of both a low and a high dose of AL on the drug-metabolizing system in rat liver.

In the present study, we planned to investigate the effect of dietary pantethine level on the contents and compositions of fatty acids and the extent of lipid peroxidation in the liver and the S-9 fraction of rats orally administered varying amounts of AL. Thus, fatty acid contents and TBA values are determined with liver homogenates and the S-9 fraction including drug-metabolizing system on the same experimental conditions as the previous report (3).

MATERIALS AND METHODS

Chemicals. Linoleic acid was of extra-pure reagent grade from Nakarai Chemicals Kyoto, and its purity as determined by gas chromatography was about 95%. Fatty acid standards (palmitic, stearic, oleic, linoleic, linolenic, arachidonic, and docosahexanoic acids) were purchased from Gasukuro Kogyo, Tokyo. Five % trifluoromethylphenyltrimethyl-ammonium hydroxide in methanol (TFM-PTAH) was from Nishio Kogyo, Tokyo. The column packing was 15% diethylene glycol succinate (DEGS) on acid-washed and dimethylchlorosilane (DMCS)-treated Chromosorb W (60–80 mesh) (Gasukuro Kogyo). All other reagents used for gas chromatography were of analytical reagent grade. Bovine serum albumin was obtained from Sigma Chemical, St. Louis, MO. Pantethine (60% solution), D-bis-(pantothenyl-β-aminoethyl) disulfide, was kindly supplied from Daiichi Seiyaku, J. Nutr. Sci. Vitaminol.
Tokyo. The other chemicals were of guaranteed reagent grade from Nakarai Chemicals.

**Animals and diet.** Male Wistar rats, 3 weeks old and each weighing about 40 g (JCL, Tokyo), were divided into 9 groups (5 rats/group), and were housed at approximately 23°C with a light and dark cycle of 12 h each. They were fed for one month on the same diet (Cleaw Japan, Tokyo) as described in the previous report (2). The composition of the diet was 24.5% vitamin-free casein, 45.5% corn starch and 1% α-starch, 10.0% granulated sugar, 6.0% corn oil, 5.0% cellulose (3.0% Avicel and 2.0% KC flock), 1.0% vitamin mixture without pantothenic acid, and 7.0% mineral mixture. The drinking water containing 0, 6.25, or 125 mg pantethine/100 ml was given to the rats, which were accordingly designated the pantethine-deficient, -adequate, and -excess groups, respectively. The diet and drinking water were provided *ad libitum*.

**Administration of AL.** After the initial feeding for one month, AL prepared in the same way as previously described (2) was orally administered to the rats of each pantethine level at a daily dose of 0, 0.2, and 0.35 ml/100 g body weight for 5 successive days. The experimental dose conditions of pantethine and AL are shown in Table 1.

**Preparation of liver homogenate and the S-9 fraction.** The liver homogenate and the S-9 fraction were prepared by a modification of the method of Yahagi (4). After decapitation, the liver was excised and thoroughly perfused with 1.15% KCl solution. The liver was finely chopped with a razor, and homogenized with 3 volumes of 1.15% KCl solution. The homogenate was centrifuged at 9,000 × g for 15 min and the postmitochondrial supernatant was used as S-9 fraction.

**Extraction of lipid and methylation.** Lipids were extracted from 1 ml of the rat liver homogenate or S-9 fraction with 3 ml of a mixture of chloroform: methanol (1:2, v/v) containing 50 ppm of butylated hydroxytoluene (BHT). The

| Group number | PaSS (mg/100 ml drinking water) | PaSS status | AL (ml/100 g B.W.) |
|--------------|--------------------------------|-------------|-------------------|
| 1            | 0                              |             | 0                 |
| 2            | 0                              | Deficient   | 0.2               |
| 3            | 0                              |             | 0.35              |
| 4            | 6.25                           |             | 0                 |
| 5            | 6.25                           | Adequate    | 0.2               |
| 6            | 6.25                           |             | 0.35              |
| 7            | 125                            |             | 0                 |
| 8            | 125                            | Excess      | 0.2               |
| 9            | 125                            |             | 0.35              |

PaSS, pantethine. The AL which showed 800 meq/kg of PV and 1,700 meq/kg of CV was prepared by autoxidizing 12.5 ml of linoleic acid at 37°C for 20 days in a 12.5 cm diameter petri dish.

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extracts were centrifuged at 3,000 rpm for 5 min. The precipitate was extracted again with 3.8 ml of a mixture of chloroform : methanol : water (1:2:0.8, v/v/v) containing 50 ppm of BHT, and was centrifuged again at 3,000 rpm for 5 min. The first and second supernatants were combined, and 2 ml of chloroform and 2 ml of water were added to the combined extract. Then, the extract stood overnight. After removal of water-soluble substances from the extracts, lipid samples were obtained after drying in a stream of nitrogen.

A 0.5 ml portion of standard solution and/or sample in benzene was pipetted into a 2 ml screw-capped test tube. To this was added 200 µl of TFM-PTAH. The tube was closed by screw-cap and left at room temperature for 15 min. In this way, the esterification of fatty acids was performed completely. An aliquot (4 µl) of this solution was analyzed by gas chromatography.

Gas chromatography. The chromatographic system consisted of a Hitachi Model 163 gas chromatograph with a flame ionization detector (FID) and a data processor (Shimadzu Model C-R3A). A glass column (200 × 0.3 cm i.d.) was packed with 15% DEGS. The column temperature was set at 180°C. Other details: the temperature of both the injector block and detector was 210°C; the flow rate of carrier gas (nitrogen) was 70 ml/min. Peaks were identified by comparison with those of standards. The content of each fatty acid in the sample was calculated by comparison with peak area of the corresponding standard fatty acids.

Measurement of TBA value. The TBA values in liver homogenate and the S-9 fraction were measured by the method of Masugi and Nakamura (5). To liver homogenate or S-9 fraction (0.25 ml) in a tube was added 0.25 ml of 0.05 M phosphate buffer (pH 7.0), 0.2 ml of 7% sodium dodecylsulfate, 2.0 ml of 0.1 N HCl, 0.3 ml of 10% phosphotungstic acid, and 1.0 ml of 0.5% TBA. The air in the tube was replaced by a stream of nitrogen gas, and the tube was capped with a glass bead. After boiling for 45 min, the reaction mixture was cooled in an ice bath and 5.0 ml of 1-butanol was added to the mixture with vigorous shaking. Then the mixture was centrifuged at 3,000 rpm for 5 min. The absorption at 532 nm was measured for the butanol extract. Using tetra-ethoxypropane as the standard, TBA value was expressed as malondialdehyde content in liver homogenate or S-9 fraction. In the liver homogenate, the TBA value was calculated per g liver weight or mg fatty acids. In the S-9 fraction, the TBA value was calculated per mg protein of mg fatty acid.

Other analytical methods. Protein was determined by the procedure of Lowry et al. (6) with bovine serum albumin as the standard.

Statistical analysis. Statistical significance of differences among values was analyzed by two-way analysis of variance (ANOVA), or by one-way ANOVA in the case of the constant level of one factor. When main effects and/or interaction were significant, least significant difference (LSD) test was performed. A probability of < 0.05 was taken as the level of significance.
RESULTS

Changes in diet and drinking water intakes and body weight of rats before and after AL administrations

The changes in diet and drinking water intakes and body weights of rats before and after the AL administration are shown in Figs. 1, 2 and 3, respectively.

During the prefeeding, no difference between the pantethine-adequate and
Fig. 2. Changes in drinking water ingestion during the whole experimental period. ×, pantethine-deficient group; ▲, pantethine-adequate group; ○, pantethine-excess group. —, non-AL; ----, low AL; ---, high AL. Each value illustrated from the first day to the 30th day is the mean of 15 rats during prefeeding periods. Each value illustrated from the 31st day to the 35th day is the mean of 5 rats during AL administration. The upper panel's pantethine-adequate and -excess groups are successively continued at point A and B of lower panels, respectively. (a) Statistical significant difference from the pantethine-adequate group. (b) Statistical significant difference from the pantethine-excess group. (c) Statistical significant difference from the high dose of AL group. *p<0.05; **p<0.01.

-excess groups was observed either in the intakes of diet and water or in the body weight of rats, but the pantethine-deficient groups was significantly different from pantethine-adequate and -excess groups with regard to the intakes of diet and water, and the body weight of rats. The pantethine-deficient rats showed not only poor growth but also other symptoms such as alopecia or scleroderma. The
pantethine-deficient rats also scattered vigorously the diet after 20 days of the prefeeding.

With the low AL administration, there were tendencies to decrease diet ingestions and to increase water intakes in the rats of any pantethine levels, but the differences were not significant in comparison with the respective non-AL group (Figs. 1 and 2). Consequently, the body weight of rats under the condition of low AL administration increased successively in a similar manner to respective non-AL in any pantethine levels (Fig. 3). With the high AL administration, the diet intake decreased markedly in any pantethine level. The water intake in the pantethine-excess group increased over the AL administration period. The water intakes in the pantethine-deficient and -adequate groups increased in the early period of the AL administration, then decreased rapidly in the latter period. Thus, after 4 or 5 days of the high AL administration, the rats in the pantethine-deficient and -adequate groups could not eat and drink (Figs. 1 and 2), whereas the rats of the pantethine-excess group could still sufficiently drink. The body weight of rats, in any pantethine levels, decreased constantly during the high AL administration for 5 successive days, as shown in Fig. 3.
Changes in liver weights after AL administration

As shown in Table 2, the effect of AL dose level on liver weight is statistically significant ($p<0.01$), and the effect of dietary pantethine level and interaction between dietary pantethine and AL dose were not significant. The liver weights were increased by the low dose of AL in comparison with non-AL, and lowered by the high dose of AL in comparison with the low dose of AL.

Changes in fatty acid composition and contents

The changes in fatty acid composition and contents in the liver homogenate and the S-9 fraction are shown in Tables 3 and 4, respectively. The effects of AL dose level ($p<0.01$) and dietary pantethine level ($p<0.01$) and the interaction between them ($p<0.01$) were statistically significant on the total fatty acid contents both in the liver homogenate and in the S-9 fraction.

In the case of non-AL, no difference in the total fatty acid contents of liver homogenates was observed between the pantethine-deficient (Group 1) and -adequate (Group 4) groups, whereas the contents of palmitic acid ($p<0.01$) and total fatty acids ($p<0.01$) in liver homogenates of the pantethine-excess group (Group 7) were significantly higher than those of the pantethine-deficient and -adequate groups (Table 3). In the S-9 fraction, the contents of total fatty acids of the pantethine-deficient groups (Groups 1–3) were significantly lower ($p<0.01$) than those of the pantethine-adequate (Groups 4–6) and -excess (Groups 7–9) groups in any AL dose level (Table 4).

Corresponding to the dose level of AL, the total fatty acid contents decreased in the rat liver homogenate of pantethine-deficient and -adequate groups and in the S-9 fraction of pantethine-deficient group. On the contrary, the decrease of fatty acids except palmitic acid and oleic acid in the liver homogenate of pantethine-excess group was not significant even by the large dose of AL. Therefore the total contents of fatty acids in the liver homogenate of the group (Group 9) maintained

| Group No. | (g/100 g body weight) | ANOVA |
|-----------|-----------------------|-------|
| 1         | 4.51±0.12             | AL    | 0.01 |
| 2         | 4.98±0.34             | PaSS  | NS   |
| 3         | 4.78±0.33             | AL×PaSS | NS   |
| 4         | 4.61±0.14             |       |      |
| 5         | 4.91±0.14             |       |      |
| 6         | 4.63±0.23             |       |      |
| 7         | 4.56±0.20             |       |      |
| 8         | 4.96±0.18             |       |      |
| 9         | 4.69±0.39             |       |      |

Groups 1–9, see Table 1. AL, autoxidized linoleate; PaSS, pantethine. Values are mean±SD for 7 rats in each group. NS, not significant.
Table 3. Composition of fatty acids in liver homogenates (%).

| Group No. | C16:0 | C18:0 | C18:1 | C18:2 | C20:4 | C22:6 | Total (mg/g liver) |
|-----------|-------|-------|-------|-------|-------|-------|-------------------|
| 1         | 9.2±0.5 | 18.1±1.2 | 13.3±0.6 | 14.7±0.7 | 33.2±0.9 | 4.5±0.6 | 16.8±0.3 |
| 2         | 9.2±0.5 | 17.3±0.9 | 12.5±0.9 | 15.0±1.0 | 34.8±0.9 | 4.2±0.4 | 15.1±0.4 |
| 3         | 11.5±1.5 | 19.5±2.4 | 8.0±1.0 | 17.7±2.2 | 32.1±5.5 | 4.2±0.5 | 10.3±1.3 |
| 4         | 9.6±0.6 | 17.2±2.0 | 14.9±0.6 | 12.7±1.2 | 34.0±0.6 | 4.6±0.4 | 16.6±0.8 |
| 5         | 10.0±0.3 | 14.4±1.4 | 12.9±1.6 | 15.2±0.9 | 35.9±0.7 | 4.5±0.3 | 15.7±0.3 |
| 6         | 10.0±0.5 | 16.6±1.9 | 8.8±0.4 | 17.8±1.3 | 35.5±1.7 | 4.2±1.1 | 13.6±0.3 |
| 7         | 11.6±0.5 | 19.3±0.7 | 14.3±0.7 | 13.0±0.6 | 31.1±1.1 | 3.8±0.4 | 18.0±0.4 |
| 8         | 11.3±0.2 | 19.0±0.5 | 12.6±0.5 | 13.6±0.3 | 32.6±0.7 | 4.1±0.2 | 17.4±0.4 |
| 9         | 10.7±0.7 | 20.8±0.5 | 12.1±0.8 | 13.4±0.5 | 31.8±0.7 | 4.1±0.3 | 16.7±0.9 |

ANOVA for content

|       | AL   | PaSS | AL × PaSS |
|-------|------|------|-----------|
| AL    | 0.01 | 0.01 | NS        |
| PaSS  | 0.01 | 0.01 | NS        |
| AL × PaSS | NS  | 0.05 | 0.05 |

ANOVA for composition

|       | AL   | PaSS | AL × PaSS |
|-------|------|------|-----------|
| AL    | 0.05 | 0.01 | NS        |
| PaSS  | 0.01 | 0.01 | NS        |
| AL × PaSS | 0.01 | 0.05 | 0.01 |

Groups 1–9, see Table 1. AL, autoxidized linoleate; PaSS, pantethine. Values are mean±SD for 5 rats in each group. NS, not significant.
Table 4. Composition of fatty acids in S-9 fractions (%).

| Group No. | C16:0 | C18:0 | C18:1 | C18:2 | C20:4 | C22:6 |
|-----------|-------|-------|-------|-------|-------|-------|
| 1         | 18.9±1.4 | 17.3±1.4 | 18.1±1.0 | 15.5±1.3 | 21.4±2.0 | 3.9±1.0 |
| 2         | 22.3±0.3 | 19.0±0.3 | 12.6±0.3 | 15.4±0.6 | 21.0±0.6 | 2.0±0.4 |
| 3         | 17.6±0.2 | 19.8±0.2 | 8.8±0.2 | 14.6±0.2 | 29.8±0.4 | 2.5±0.3 |
| 4         | 17.8±0.1 | 17.1±0.1 | 19.9±0.1 | 15.6±0.3 | 20.7±0.3 | 2.0±0.0 |
| 5         | 18.7±0.2 | 16.6±0.2 | 13.4±0.2 | 18.0±0.3 | 24.5±0.2 | 1.9±0.0 |
| 6         | 20.7±0.2 | 20.1±0.1 | 8.0±0.2 | 17.6±0.1 | 24.1±0.1 | 2.7±0.0 |
| 7         | 20.9±0.1 | 14.5±0.1 | 25.0±0.2 | 14.1±0.1 | 15.9±0.1 | 4.2±1.8 |
| 8         | 20.8±0.5 | 17.9±0.4 | 21.8±0.6 | 13.8±0.2 | 16.6±0.5 | 2.3±0.1 |

ANOVA for content:
- AL: 0.05
- AL×PASS: 0.005

ANOVA for composition:
- AL: 0.005
- AL×PASS: 0.005

Groups 1-9, see Table 1. AL, autoxidized linoleate; PsSS, pantethine. Values are mean±SD for 5 rats in each group. "Values are mean of two samples. NS, not significant."

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the same level as those in the case of non-AL in pantethine-deficient (Group 1) and -adequate (Group 4) groups. The decreases of oleic acid contents by the high dose of AL were significantly \( p < 0.01 \) more remarkable in the both pantethine-deficient (Group 3) and -adequate (Group 6) groups than in the pantethine-excess group (Group 9) in both the liver homogenate and the S-9 fraction (Tables 3 and 4).

Evidently, the fatty acid composition was effected by both AL dose level and dietary pantethine level, except docosahexanoic acid in liver homogenate, and linoleic acid and docosahexanoic acid in S-9 fraction. Especially, oleic acid content and composition lowered corresponding to the increasing level of AL dose in the pantethine-deficient (Groups 2 and 3) and -adequate (Groups 5 and 6) groups. On the contrary, the decrease rates of the other fatty acids content were less than that of oleic acid, and their compositions showed a tendency to increase corresponding to the increasing level of AL dose. Unlike the pantethine-deficient and -adequate groups, the fatty acid contents in both liver homogenate and S-9 fraction of the pantethine-excess group showed only a little decrease, and the fatty acid composition changed little (Table 4).

Changes in thiobarbituric acid value

The TBA values are expressed as malondialdehyde (MDA) nmol per wet liver weight (g) or per liver fatty acid (mg) in liver homogenate and as malondialdehyde (MDA) nmol per protein (mg) or per fatty acid (mg) in the S-9 fraction, respectively in Table 5. The TBA values expressed as amounts of MDA either per

| Group No. | Liver homogenate | S-9 fraction |
|-----------|------------------|--------------|
|           | MDA (nmol/g wet liver) | MDA (nmol/mg fatty acid) | MDA (nmol/mg protein) | MDA (nmol/mg fatty acid) |
| 1         | 144 ± 29         | 8.5 ± 1.6    | 0.77 ± 0.08            | 24.6 ± 2.5                |
| 2         | 178 ± 16         | 11.0 ± 0.9   | 1.27 ± 0.17            | 53.2 ± 5.4                |
| 3         | 201 ± 43         | 20.0 ± 6.0   | 1.15 ± 0.14            | 72.3 ± 10.4               |
| 4         | 124 ± 30         | 7.5 ± 2.1    | 0.65 ± 0.04            | 15.5 ± 1.3                |
| 5         | 189 ± 36         | 12.0 ± 2.5   | 1.76 ± 0.62            | 41.1 ± 10.3               |
| 6         | 178 ± 27         | 13.1 ± 2.1   | 1.18 ± 0.22            | 35.0 ± 4.2                |
| 7         | 155 ± 19         | 8.6 ± 1.2    | 0.97 ± 0.21            | 20.8 ± 4.3                |
| 8         | 178 ± 27         | 9.9 ± 2.9    | 1.10 ± 0.18            | 24.1 ± 5.8                |
| 9         | 191 ± 48         | 11.5 ± 3.3   | 1.31 ± 0.48            | 27.0 ± 10.5               |

ANOVA

|        | Liver homogenate | S-9 fraction |
|--------|------------------|--------------|
| AL     | 0.05             | 0.01         |
| PaSS   | NS               | NS           |
| AL × PaSS | NS               | NS           |

Groups 1–9, see Table 1. AL, autoxidized linoleate; PaSS, pantethine. Values are mean ± SD for 5 rats in each group. NS, not significant.
wet liver weight in the liver homogenate or per mg protein in the S-9 fraction showed the significant difference only on AL dose level \((p < 0.05)\). On the other hand, the TBA values expressed as MDA nmol/mg fatty acid in liver homogenates showed more clearly the significant difference on AL dose level \((p < 0.01)\). Among the values of MDA nmol/mg fatty acid, the effects of AL dose level \((p < 0.01)\) and dietary pantethine level \((p < 0.01)\) and the interaction between them \((p < 0.01)\) were statistically significant in the S-9 fractions. That is, the TBA value in S-9 fraction increased correspondingly with increasing dose level of AL, and the increase was repressed markedly corresponding to the increase of dietary pantethine level.

**DISCUSSION**

It has been reported that pantethine has a relieving effect against various injuries caused by lipid peroxidation in vivo (7–10). The absorbed pantethine, not having a reducing property by itself, might be reduced to pantetheine via the redox system in vivo, and the resultant pantetheine might have depressed lipid peroxidation in vivo by the reducing property originating from its sulphydryl group.

We have reported that the drug-metabolizing activities in the cases of both non-AL and low AL dose were significantly higher in the pantethine-deficient group than in the pantethine-adequate and -excess groups (3). It was inferred from this result that, in the pantethine-deficient non-AL group, the drug-metabolizing system was induced (11–13) in order to decompose the lipid peroxides resulting from the in vivo redox system, and thereby in vivo lipid peroxidation would be more accelerated. We have also presumed that the higher induction of drug-metabolizing system by the low AL dose might be a reasonable response of the system against the elevated accumulation of lipid peroxides in vivo (14, 15). Thus it seemed likely that the ingestion of pantethine relieves the induction of drug-metabolizing system because the pantethine derivative having antioxidative property depressed the lipid peroxidation in vivo (7–10).

In order to better understand the state of lipid peroxidation in biological systems, it is important that the loss of fatty acids is measured by a direct analysis of the tissue lipids, and also that the products resulting from peroxidation are evaluated. In the present study, we determined the changes in contents and compositions of fatty acids and TBA values in liver and the S-9 fraction obtained from rats raised under the same condition as described previously (3).

The total fatty acids contents in the liver and the S-9 fraction were decreased correspondingly with the increase of AL dose in pantethine-deficient and -adequate groups, and it was especially remarkable in the pantethine-deficient group. In the pantethine-excess group, the decrease of fatty acids contents except oleic acid was not significant in spite of the high dose of AL (Table 3). The TBA values in liver homogenate and the S-9 fraction rose with the increase of AL dose level, especially in the pantethine-deficient group, and lowered with the increase of the dietary pantethine level even in the high dose of AL (Table 5). These changes in fatty acid

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contents and TBA values showed that fatty acids in tissue and subcellular fraction were damaged by dosed AL, and pantethine relieves the damage. We also found in this connection that it is suitable to express TBA value as MDA nmol per mg fatty acid constituting lipids rather than per g liver weight or mg protein in the S-9 fraction because TBA value expresses the level of lipid peroxidation. These results supported our presumption mentioned in the previous paper (3) on changes of drug-metabolizing system produced by AL dose and dietary pantethine.

It is well known in vitro that the increase in the number of double bond of fatty acid is accompanied by the increase of reactivity in lipid peroxidation (16, 17). On the other hand, much of the work on biological effects of lipid peroxidation is based on inferential evidence (18) and has not been sufficiently clarified for mechanism of in vivo lipid peroxidation (19). Thus, the reason why oleic acid content decreased remarkably by increasing AL dose to rats of the pantethine-deficient and -adequate groups could not be explained from the hitherto-obtained knowledge of lipid peroxidation. From the viewpoint of fatty acids metabolism, we would like to speculate the reason for the remarkable decreasing of oleic acid as follows.

In the present experiments, rats lost their appetite by increasing AL dose, and starved. Fatty acids taken up from blood stream to the liver cells may be mainly either oxidized to provide energy or resynthesized into phospholipids to repair the damaged biomembrane. In this connection, oleic acid is a major component in hepatic neutral lipid, and on the contrary is a minor component in hepatic phospholipid in comparison with the other polyunsaturated fatty acids, such as linoleic acid or arachidonic acid (20). Consequently, the rat liver in the state of starvation may consume more oleic acid than the other fatty acids. Furthermore, endogenous oleic acid in rat liver is synthesized through \( \Delta^9 \)-desaturation of stearic acid by cyanide sensitive factor which is the terminal enzyme in microsomal electron transfer system (21). It has been reported by Imai et al. (22–24) that the biosynthesis of oleic acid by the \( \Delta^9 \)-desaturation of stearic acid decreased markedly in liver of starved or diabetic rats. Thus, the production of oleic acid remarkably decreases, but the consumption of oleic acid increases in the liver of starved rat. The \( \Delta^9 \)-desaturation of stearic acid in rat liver requires thioesterification of the fatty acid with CoA, and the yielded oleyl CoA is incorporated to phospholipid or triglyceride (23, 24). It has been reported that the level of CoA in the rat liver was depressed by administration of secondary autoxidation products of linoleic acid (25). Probably, the decrease of oleic acid content caused by the depression of \( \Delta^9 \)-desaturation may be more remarkable than those caused by the in vivo lipid peroxidation. Thus, the \( \Delta^9 \)-desaturation reaction in the liver of AL-administered rat which was deficient in pantothenic acid, a constituent of CoA, might be furthermore depressed because of the severe decrease of CoA. However, because the rats of pantethine-excess group were sufficiently supplied CoA from pantethine, the biosynthesis of oleic acid might be maintained in the rat liver. Moreover, the supplement of fatty acids from the adipose tissue to the liver via bloodstream might be prevented by increasing AL dose in the rats of pantethine-deficient and -adequate
groups. These seem to be the reasons why the oleic acid content decreased remarkably in the rat liver homogenate and S-9 fraction of the pantethine-deficient and -adequate groups.

In conclusion, it appears that sufficient dietary pantethine depresses in vivo lipid peroxidation and maintains normally the lipid metabolism, even under the condition of high AL administration.

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