Effects of Feeding a Protein-Free Diet on the Mitochondrial Respiration and on Phospholipid Fatty Acids of Mitochondrial and Microsomal Membranes in the Rat Liver

Eiji MIYAZAWA¹ and Masao KAMETAKA²

¹Department of Culture, Seijo Junior College, Setagaya-ku, Tokyo 157, Japan
²Department of Food Science and Nutrition, Kyoritsu Women’s University, Chiyoda-ku, Tokyo 101, Japan

(Received December 26, 1989)

Summary In our previous report, we have described morphological changes in hepatocytes, e.g., enlargement of mitochondria and a change in the lamellar formation of rough endoplasmic reticulum, produced by short-term feeding of a protein-free diet to rats. In order to examine whether or not these morphological changes in the subcellular organella are accompanied by any functional and compositional changes, we measured the P/O ratio and respiratory rate of mitochondria, and the phospholipid fatty acids of the mitochondrial and microsomal membranes in the liver of rats fed a protein-free diet for a short period. Feeding rats the protein-free diet for 4 days or 27-28 days had no effect on the rate of hepatic mitochondrial oxygen consumption (State 3 respiration). The diet significantly decreased the P/O ratio on the 4th day, but did not affect it on the 27-28th days. The decreased P/O ratio observed on the 4th day returned to the control level after overnight refeeding of a 20% casein diet. Main compositional changes induced by feeding rats the protein-free diet for 2 days were significant decreases in the phospholipid/protein ratios of the total liver and mitochondrial inner membrane, a tendency of an increase in the ratio of phosphatidylcholine (PC) to total phospholipids in the mitochondrial outer membrane, a significant decrease and a tendency of decrease in the arachidonate/linoleate ratio in the phosphatidylethanolamine (PE) and PC, respectively, in the mitochondrial outer membrane. Some of these results were discussed in relation to the morphological changes in mitochondria and rough endoplasmic reticulum produced by short-term feeding of the protein-free diet which we previously reported.

Key Words protein-free diet, mitochondrial respiration, mitochondrial membrane, microsomal membrane, phospholipids, fatty acids, rat liver
Our morphometrical study showed previously that short-term (2 or 7 days) feeding of a protein-free diet induced various morphological changes in the hepatocytes of rats such as an enlargement of individual mitochondria with a concomitant decrease in the number of mitochondria per unit volume of hepatocytes (especially on the 2nd day of the protein-free diet) and a change in the lamellar formation of RER (rough endoplasmic reticulum), i.e., a decrease in the number of flattened cisternae per parallel array of RER and an increase in the formation of RER composed of a single flattened cisterna which encircled the mitochondria (1).

It has been reported that enlargement of individual mitochondria of hepatocytes was often accompanied by alterations in their function in rats administered substances such as cortisone (2), alloxan (3), essential fatty acid-deficient diets (4–7), or a low protein diet for a long period (8). Furthermore, experimental conditions which induced morphological changes in mitochondria and endoplasmic reticulum have often provoked alterations in the composition of their membrane lipids as well, such as in rats fed an essential fatty acid- (4, 6, 11, 12), choline- (9, 10), or protein-deficient diet for a long period (8, 13–20).

Considering the findings presented above, it can be expected that short-term feeding of a protein-free diet, which was found to induce morphological changes of the mitochondria and RER in the hepatocytes of rats, induces functional and/or compositional changes as well.

Therefore, in the present study we examined the P/O ratio and respiratory rate of mitochondria, and the phospholipid fatty acids of the mitochondrial and microsomal membranes in the liver of rats fed a protein-free diet for a short period. Since the phospholipid compositions of the mitochondrial inner and outer membranes are different from each other, they were examined separately.

**EXPERIMENTAL**

*Animals and diets.* Male Wistar rats weighing approximately 180 g were used. The rats were housed in individual cages with wire bottoms in a room at a temperature of 22±2°C, relative humidity of 60±5%, and a 6 a.m. to 6 p.m. light cycle. Diets (a protein-free diet and a 20% casein diet) and water were given ad libitum. The composition of diets was as follows (in weight %): casein, 0 or 20; soybean oil, 5.0; mineral mixture (Harper), 5.0; vitamin mixture (Harper), 0.85; choline chloride, 0.15; and α-cornstarch to 100 (21). In experiment I (Exp. I) 24 rats were divided into six groups (4 animals each). Rats were fed either a 20% casein diet or a protein-free diet for 4 or 27–28 days throughout, or fed initially a protein-free diet for 4 or 27–28 days and refed the 20% casein diet overnight, i.e., from 17:00–18:00 to 10:00 the next day. Among these groups, the group refed after protein-depletion for 27–28 days was not used since there was no difference between the results of the groups fed the 20% casein diet for 27–28 days and the protein-free diet for 27–28 days. In experiment II (Exp. II) 24 rats were divided into two groups (12 animals each), and fed either the 20% casein diet or the
protein-free diet for 2 days. At 10:00 to 11:00, the rats were sacrificed by decapitation, and the livers were removed quickly and cooled in ice in both experiments.

**Preparation of mitochondria (Exp. I).** Mitochondria were prepared according to the modified method of Harada (8). The preparation medium contained 0.21 M mannitol, 0.07 M sucrose, 0.1 mM EDTA, and 0.01 M Tris-HCl buffer (pH 7.4). The livers were minced as finely as possible, placed in six volumes of ice-cold preparation medium, and homogenized at 0°C with a loosely-fitted Teflon homogenizer to obtain intact mitochondria. The glass vessels were moved slowly up and down three times. The mitochondria obtained were used within 2 h after homogenization.

**Assay of mitochondrial respiration (Exp. I).** Respiration was measured polarographically by the method of Hagihara (22), using an oxygen electrode (Yanagimoto Mfg. Inc., Japan) and succinate as the substrate. Initial oxygen concentration in the reaction mixture was assumed to be 223 μmol/ml (23). The P/O ratio was calculated by dividing the number of ADP molecules consumed by the number of oxygen atoms consumed during ADP-stimulated (State 3) respiration.

**Preparation of mitochondria and microsomes (Exp. II).** Mitochondrial and microsomal fractions were prepared by the modified method of Colbeau et al. (24). About 36 ml of 10% (w/v) liver homogenate in 0.27 M sucrose solution buffered by 2 mM Tris-HCl, pH 7.6, was centrifuged at 600 × g for 10 min and the supernatant obtained was further centrifuged at 8,500 × g for 10 min. From the precipitate (I) and supernatant (II), mitochondrial and microsomal fractions, respectively, were obtained after the following treatment. The precipitate (I) was resuspended in ca. 18 ml of 0.27 M sucrose solution buffered by 2 mM Tris-HCl, pH 7.6, centrifuged at 600 × g for 10 min; the supernatant obtained (Ia) was centrifuged at 8,500 × g for 10 min and then the precipitate obtained was washed two times with ca. 10 ml of 0.27 M sucrose solution buffered by 2 mM Tris-HCl, pH 7.6. To the supernatant (II) 1 mM MgCl₂ at the final concentration was added. The mixture was centrifuged at 19,500 × g for 10 min; the supernatant obtained was further centrifuged at 78,000 × g for 60 min and the supernatant was discarded.

**Separation of inner and outer membranes of mitochondria (Exp. II).** From the mitochondrial fraction obtained, mitochondrial outer and inner membrane fractions were separated on a 3-layer gradient according to the method of Sottocasa et al. (25). Three mitochondrial subfractions, i.e., the supernatant subfraction, light subfraction, and heavy subfraction were obtained from the top layer, the interface between the 0.76 and 1.32 M sucrose layers, and the bottom of the centrifuge tube, respectively. The purity of each mitochondrial subfraction was assessed by the marker enzymes. The activities of monoamine oxidase, succinate cytochrome c reductase, and glutamic dehydrogenase were determined by the methods of Schnaitman and Greenawalt (26), Tisdale (27), and Beaufay et al. (28), respectively. As shown in Table 1, the heavy subfraction was practically free from contamination by the mitochondrial outer membrane and the light subfraction was only slightly contaminated by the mitochondrial inner membrane. Therefore, the
Table 1. Activities of marker enzymes for mitochondrial subfractions.

| Mitochondrial subfraction | Monoamine oxidase$^1$ | Succinate cytochrome c reductase$^2$ | Glutamic dehydrogenase$^3$ |
|---------------------------|----------------------|-------------------------------------|---------------------------|
| Light subfraction$^a$     | 23.58                | 62.8                                | 29                        |
| Heavy subfraction$^b$     | 1.06                 | 287.0                               | 158                       |
| Supernatant subfraction$^c$| 11.19                | 6.9                                 | 86                        |

$^{a,b,c}$ Subfractions composed of outer membrane, inner membrane plus matrix contents, and part of the matrix contents plus contents of the inter-membrane space, respectively. $^{1,2,3}$ Marker enzymes for mitochondrial outer and inner membrane, and matrix contents, respectively.

light and heavy subfractions were used as the mitochondrial outer and inner membrane fractions, respectively.

**Extraction and separation of lipids, and fatty acid analysis (Exp. II).** Extraction of lipids from the mitochondrial inner and outer and microsomal membrane fractions, and separation of phospholipids from the extracted lipids were performed according to the method of Dawson et al. (29). Phospholipids were separated by thin-layer chromatography on silica gel G in a solvent system of chloroform–methanol–water (65:25:4, v/v) (30) and each phospholipid was eluted from the silica gel by the method of Arvidson (31). After methylation of fatty acids by the method of Stoffel et al. (32), the fatty acid composition of each phospholipid extract was determined by gas-liquid chromatography (Shimadzu 6C-6A, Japan) with a flame ionization detector on a column (2 m × 2 mm) packed with 15% diethylene-glycol succinate on 60 to 80 mesh Chromosorb W at 222°C. N$_2$ was used as carrier gas, and its flow rate was 30 ml per min. The fatty acids were identified by their retention times relative to those of standard methyl ester mixtures (Wako Chemical Inc., Japan). Phosphorus in phospholipids was measured by the method of Chen et al. (33).

**Protein assay.** Protein was determined by the method of Lowry et al. (34).

**Statistical analysis.** Results are given as mean ± SEM. Statistical analysis was performed using Student’s t-test. Differences were considered to be statistically significant when $p < 0.05$.

**RESULTS AND DISCUSSION**

**Respiration of the hepatic mitochondria (Exp. I)**

Effects of feeding a protein-free diet on respiration of the hepatic mitochondria are shown in Table 2. The oxygen consumption rate (State 3 respiration) was unaffected by the protein-free diet either over a short period (4 days) or over a longer period (27–28 days). Harada (8) reported previously that in the livers of

*J. Nutr. Sci. Vitaminol.*
Table 2. Effect of feeding a protein-free diet on the respiration of hepatic mitochondria.

| Rats (period of diets)                                      | Oxygen consumption¹ | P/O ratio²   |
|-----------------------------------------------------------|---------------------|-------------|
| Control (for 4 days)                                      | 156±4ᵃ             | 1.43±0.03ᵇ  |
| Protein-depleted (for 4 days)                             | 156±11ᵇ            | 1.27±0.04ᵉ  |
| Refed overnight after protein-depletion (for 4 days)      | 163±6ᵃ             | 1.42±0.01ᵇ  |
| Control (for 27–28 days)                                  | 122±6ᵃ             | 1.54±0.03ᵈ  |
| Protein-depleted (for 27–28 days)                         | 126±9ᵇ             | 1.52±0.03ᵇᵈ |

Rats were fed the 20% casein diet (Control rats) or fed the protein-free diet (Protein-depleted rats) for 4 or 27–28 days. One group of rats was refed the 20% casein diet overnight after protein-depletion for 4 days (Refed rats). Results are given as mean±SEM of 4 rats. Within each column, values not sharing a common superscript are significantly different (p<0.05). ¹O₂ m mol/mg protein/min during ADP-stimulated (State 3) respiration. ²ADP molecules consumed/oxygen atoms consumed during ADP-stimulated respiration.

Rats fed a 4% casein diet for 40 to 50 days, swelling of isolated mitochondria occurred and simultaneously the oxygen consumption rates (State 3 respiration) of the mitochondria was significantly increased. The different dietary protein level and feeding period might have caused the different results between Harada's experiment and ours.

Table 2 also shows that feeding the protein-free diet for 4 days to rats decreased the P/O ratio of the hepatic mitochondria whereas longer feeding of the diet had no effect and that overnight refeeding of the 20% casein diet to rats restored the decreased P/O ratio to the control level. We reported previously (1) that the feeding of a protein-free diet for 2 days produced an increase in the volume of individual mitochondria in all sub-lobular areas (i.e., central, midzonal, and peripheral areas) of the liver of rats whereas feeding of the diet for 7 days did not produce such a change in the midzonal area (the other areas were not examined). As should be expected from these previously reported results, if the increase in the volume of individual mitochondria is a transient phenomenon observed only in the very early phase of acute protein deficiency such as when a protein-free diet is fed to rats for a few days, then no increase in the volume of the hepatic mitochondria of rats fed the protein-free diet for 27–28 days in the present experiment might be expected.

Thus, as the decrease in the P/O ratio of the mitochondria might be closely related to their enlargement, it seems tempting to speculate that the decrease in the P/O ratio of mitochondria causes enlargement of the mitochondria. Examining cortisone-administrated rats, Kimberg et al. (2) and Kimberg and Loeb (3) noted that enlargement of hepatic mitochondria might occur due to fusion of mitochondria, which in turn might be caused by a decrease in the P/O ratio. Wilson and

Vol. 36, No. 3, 1990
Leduc (4) assumed that enlargement of hepatic mitochondria found in essential fatty acid-deficient mice was brought about by a deficiency of ATP owing to the uncoupling of oxidative phosphorylation. Rafael et al. (36), however, observed no change in the P/O ratio in essential fatty acid-deficient rats but rather observed increased oxygen consumption in either State 3 or State 4 respiration. They suggested that increased fragility of essential fatty acid-deficient mitochondria to isolation procedures was the most plausible explanation of the decrease in the P/O ratio. Therefore, it cannot be denied that the decreased P/O ratio of the mitochondria found in the rats fed a protein-free diet for 4 days in the present experiment was an artifact brought about by fragility of the mitochondria because of their enlargement, i.e., that the decreased P/O ratio of mitochondria was not a cause but rather a result of mitochondrial enlargement.

Phospholipid/protein ratio of the liver fractions (Exp. II)

Effects of feeding the protein-free diet for two days on the phospholipid/protein ratios of the total liver and on those of the hepatic mitochondrial inner and outer and microsomal membrane fractions are shown in Table 3. A significant decrease, a tendency to decrease (p < 0.1), and no changes were observed in the phospholipid/protein ratios of the total liver and mitochondrial inner membrane fraction, in that of the whole mitochondrial fraction, and in those of the mitochondrial outer membrane and microsomal membrane fractions, respectively. Fleck et al. (37) observed a decrease in this ratio of the total liver in rats fed the protein-free diet for a few days. Gerson (19) reported that the phospholipid/protein ratios of the hepatic mitochondrial outer membrane and microsomal membrane fractions were decreased to 75.0 and 38.2% of the control values, respectively, when rats were fed a protein-free diet for 10 weeks. Harada and Mogi (16) observed an increase and no change in the phospholipid/protein ratios of the whole mitochondrial and microsomal fractions, respectively, in the livers of rats fed a 4 or 7% casein diet for about 6 months compared with rats fed a 25% casein diet for the same period.

Table 3. Effects of feeding a protein-free diet on the phospholipid/protein ratios of the total liver and on those of the hepatic mitochondrial inner and outer and microsomal membrane fractions.

| Rats              | Total liver | Mitochondrial inner membrane | Mitochondrial outer membrane | Whole mitochondria | Microsomal membrane |
|-------------------|-------------|------------------------------|------------------------------|--------------------|---------------------|
|                   | (μg phospholipids/mg protein) |                  |                               |                    |                     |
| Control           | 231±4       | 232±4                        | 623±26                       | 286±5              | 464±15              |
| Protein-depleted  | 196±10°     | 209±5°                       | 644±18                       | 239±18             | 451±14              |

Rats were fed the 20% casein diet (Control rats) or the protein-free diet (Protein-depleted rats) for 2 days. Results are given as mean±SEM of 3 samples, each of which was composed of 4 rats. a,b p<0.05 and p<0.02 compared with control values, respectively.

J. Nutr. Sci. Vitaminol.
Although the data are limited and fragmentary, our present results and those presented above (16, 19, 37) indicate that feeding a protein-free diet decreases the phospholipid/protein ratios of some subcellular membrane fractions of the liver. Presumably a protein-free but not a low-protein diet would reduce synthesis of phospholipid more severely than that of protein.

**Phospholipid composition of liver membrane fractions (Exp. II)**

Effects of feeding the protein-free diet on the relative contents of the major phospholipids of hepatic mitochondrial inner and outer and microsomal membrane fractions are shown in Table 4. In the mitochondrial inner membrane fraction, the relative contents of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and cardiolipin (CL) and in the microsomal membrane fraction, those of PC and PE were not changed by feeding the protein-free diet. In the mitochondrial outer membrane fraction, however, the relative content of CL was decreased and that of PC tended to be increased \((p < 0.1)\). CL is said to be located exclusively in the mitochondrial inner membrane (24). Therefore, if a purer mitochondrial outer membrane fraction had been obtained, *i.e.*, the mitochondrial outer membrane fraction had been less contaminated with the inner membrane which showed no change in the relative contents of PC and PE, the extent of the change in the relative content of the PC found in the mitochondrial outer membrane fraction would have been greater. Gerson (19) showed no change in the relative contents of PC and PE in the mitochondrial outer membrane fraction but showed a significant decrease and increase in PC and PE, respectively, in the microsomal fraction in the longer-term (10 weeks) experiment on a protein-free diet mentioned above. Rogers (18) found a significant decrease and increase in the PC and PE, respectively, both in the whole mitochondrial and microsomal fractions in rats fed a 4% casein diet for 6 weeks. In the later report, however, Rogers (20) showed a significant decrease in the PC/PE ratio only in the microsomal fraction and not in the whole mitochondrial fraction in rats fed a 4% casein diet for 6.5 months. Harada and Sugita (38) found no change in the relative contents of phospholipids in the whole mitochondrial fraction in rats fed a 4% casein diet for 40 to 60 days.

We reported previously (1) that besides enlargement of the mitochondria, an increase in the proximity between the mitochondria and RER occurred in the liver of rats fed a protein-free diet for 2 or 7 days. Stacked parallel lamellar formation of RER decreased, RER was dispersed more in the cell, and each mitochondrion was generally surrounded by a single cisterna of RER (1). Consequently, the ratio of the mitochondrial outer membrane in close proximity to the RER membrane increased to about 1.8- to 2.0-fold (1). This would modify the rate of phospholipid exchange between the mitochondria and RER (39) and thus affect the phospholipid composition of the mitochondria. This might be one of the reasons for the increase in the value of PC/PE ratio of the mitochondrial outer membrane toward that of microsomal membrane observed in the present experiment.
Table 4. Effect of feeding a protein-free diet on the relative contents of the major phospholipids of hepatic mitochondrial inner and outer and microsomal membrane fractions.

| Rats          | Mitochondrial inner membrane | Mitochondrial outer membrane | Microsomal membrane |
|--------------|-----------------------------|------------------------------|---------------------|
|              | Control | Protein-depleted | Control | Protein-depleted | Control | Protein-depleted |
| (Phospholipid) |         |                  |         |                  |         |                  |
| PC           | 45.3±3.2 | 43.2±4.4         | 48.3±2.4 | 54.5±0.9         | 64.6±4.0 | 64.8±3.1         |
| PE           | 30.2±2.7 | 27.7±4.0         | 32.2±2.6 | 27.8±1.1         | 19.7±2.5 | 19.0±1.8         |
| CL           | 15.0±2.1 | 16.5±2.2         | 8.2±0.5  | 5.9±0.2*         |         |                  |
| PC/PE        | 1.52±0.17 | 1.66±0.34      | 1.53±0.19 | 1.97±0.11      | 3.44±0.67 | 3.49±0.46       |

Rats were fed the 20% casein diet (Control rats) or the protein-free diet (Protein-depleted rats) for 2 days. Results are given as mean±SEM of 3 samples, each of which was composed of 4 rats. *p<0.01 compared with control values. PC, PE, and CL: phosphatidylcholine, phosphatidylethanolamine, and cardiolipin, respectively.

Table 5. Effect of feeding a protein-free diet on the phospholipid fatty acids of the hepatic mitochondrial inner and outer and microsomal membrane fractions.

| Rats          | Mitochondrial inner membrane | Mitochondrial outer membrane | Microsomal membrane |
|--------------|-----------------------------|------------------------------|---------------------|
|              | Control | Protein-depleted | Control | Protein-depleted | Control | Protein-depleted |
| Fatty acid   |         |                  |         |                  |         |                  |
| 16:0         | 23.0±1.6 | 21.8±1.3         | 21.3±1.4 | 23.9±1.8         | 22.5±0.6 | 24.1±0.5         |
| 16:1         | 1.5±0.1  | 1.6±0.3          | 1.5±0.3  | 1.4±0.3          | 1.5±0.0  | 1.6±0.1          |
| 18:0         | 19.5±0.7 | 18.9±0.3         | 21.4±0.9 | 24.6±2.3         | 21.6±0.8 | 21.1±0.9         |
| 18:1         | 9.1±0.2  | 10.0±0.6         | 9.0±0.3  | 9.3±0.2          | 8.6±0.3  | 9.6±0.5          |
| 18:2         | 15.6±1.8 | 16.0±0.7         | 13.5±0.4 | 15.0±0.7         | 15.1±2.0 | 17.0±0.3         |
Rats were fed the 20% casein diet (Control rats) or the protein-free diet (Protein-depleted rats) for 2 days. Results are given as mean±SEM of 3 samples, each of which was composed of 4 rats.  

|                | 20:4 | 22:6 | 20:4/18:2 |
|----------------|------|------|-----------|
| Control rats   | 25.8±0.2 | 5.5±0.2 | 1.69±0.19 |
| Protein-depleted rats | 26.1±0.8 | 5.8±0.9 | 1.64±0.11 |
|                 | 26.5±1.6 | 6.8±0.5 | 1.97±0.14 |
|                 | 21.7±3.0 | 4.0±0.6 | 1.44±0.15 |
|                 | 24.5±1.9 | 6.1±0.2 | 1.72±0.36 |
|                 | 21.9±0.6 | 5.0±0.5 | 1.27±0.02 |

Number of carbon atoms: number of double bonds. 

1 Not determined. 

2 Number of carbon atoms: number of double bonds. 

a,b p<0.025 and p<0.05 compared with control values, respectively. PC, PE, and CL: phosphatidylcholine, phosphatidylethanolamine, and cardiolipin, respectively.
Fatty acid composition of phospholipids from the liver membrane fractions (Exp. II)

The effects of feeding the protein-free diet on the fatty acid composition of phospholipids from the hepatic mitochondrial inner and outer and microsomal membrane fractions are shown in Table 5. No significant change in the percent of fatty acids was observed in PC, PE, and CL of the mitochondrial inner membrane fraction. A significant decrease in docosahexaenoate and a tendency of decrease in the arachidonate/linoleate ratio in PC (p<0.1), and a significant decrease in palmitate and the arachidonate/linoleate ratio and a significant increase in linoleate in PE were found in the mitochondrial outer membrane fraction. In the microsomal membrane fraction a significant decrease in palmitate and a significant increase in stearate were observed in PE but no significant change was observed in PC.

Our result that the arachidonate/linoleate ratio was decreased for PE or tended to be decreased for PC in the mitochondrial outer membrane fraction is consistent with the previous findings (19,20). Gerson (19) observed a decrease in the arachidonate/linoleate ratio in the total phospholipids of the mitochondrial outer membrane fraction in rats fed a protein-free diet for 10 weeks. Rogers (20) found a decrease in this ratio for PC and PE in rats fed a 4% casein diet for 6.5 months, although his analysis was not on the mitochondrial outer membrane fraction but on the whole mitochondrial fraction. In addition to mitochondrial phospholipids, Gerson (19) found a decrease in this ratio in the microsomal total phospholipids, and Rogers (20) also reported a decrease in this ratio in microsomal PC and PE. A decrease in the mean value of this ratio of microsomal PC was observed in the present experiment, although the difference was not significant. It has been observed that protein deficiency decreases the activity of Δ⁶-desaturase (40) which is said to be the rate-limiting enzyme for conversion of linoleate to arachidonate (41). The results of the present experiment were consistent with the view that a decrease in the activity of this enzyme occurred in the liver of rats maintained in a very early phase of acute protein deficiency.

A correlation between the changes in the P/O ratio and the compositions of the mitochondrial inner membrane (Exp. I and II)

Short-term feeding of a protein-free diet decreased the P/O ratio without affecting oxygen consumption rate (Table 2) and also decreased the phospholipid/protein ratio in the mitochondrial inner membrane (Table 3) without affecting the phospholipid and the fatty acid compositions of the mitochondrial inner membrane (Tables 4 and 5). We cannot correlate definitely these two changes at present. However, a 10% decrease in the phospholipid/protein ratio found in this study would be enough to change the membrane fluidity and this would in turn change the coupling state of the oxidative phosphorylation in mitochondria.
REFERENCES

1) Miyazawa, E., and Kametaka, M. (1988): A quantitative description of alterations in the ultrastructure of acutely protein-depleted rat liver parenchymal cells. *Nutr. Rep. Int.*, 37, 523–536.

2) Kimberg, D. V., Loud, A. V., and Wiener, J. (1968): Cortisone-induced alterations in mitochondrial function and structure. *J. Cell Biol.*, 37, 63–79.

3) Hall, J. C., Sordahl, L. A., and Stefko, P. F. L. (1960): The effects of insulin on oxidative phosphorylation in normal and diabetic mitochondria. *J. Biol. Chem.*, 235, 1536–1539.

4) Wilson, J. W., and Leduc, E. H. (1963): Mitochondrial changes in the liver of essential fatty acid-deficient mice. *J. Cell Biol.*, 16, 281–296.

5) Levin, E., Johnson, R. M., and Albert, S. (1957): Mitochondrial changes associated with essential fatty acid deficiency in rats. *J. Biol. Chem.*, 228, 15–21.

6) Klein, P. D., and Johnson, R. M. (1954): Phosphorus metabolism in unsaturated fatty acid-deficient rats. *J. Biol. Chem.*, 211, 103–110.

7) Tulipule, P. G., and Williams, J. N. (1955): Study of the role of essential fatty acids in liver metabolism. *J. Biol. Chem.*, 217, 229–234.

8) Harada, N. (1967): Morphological and respiratory changes in rat liver mitochondria resulting from a low casein diet. *J. Nutr.*, 93, 263–272.

9) Bruni, C., and Hegsted, D. M. (1970): Effects of choline-deficient diets on the rat hepatocytes. Electron microscopic observations. *Am. J. Pathol.*, 61, 413–436.

10) Chen, S.-H., Estes, L. W., and Lombardi, B. (1972): Lecithin depletion in hepatic microsomal membranes of rats fed on a choline-deficient diet. *Exp. Mol. Pathol.*, 17, 176–186.

11) Hayashida, T., and Portman, O. W. (1960): Swelling of liver mitochondria from rats fed diets deficient in essential fatty acids. *Proc. Soc. Exp. Biol. Med.*, 103, 656–659.

12) Hayashida, T., and Portman, O. W. (1963): Changes in succinic dehydrogenase activity and fatty acid composition of rat liver mitochondria in essential fatty acid deficiency. *J. Nutr.*, 81, 103–109.

13) Porta, E. A., and Hartroft, W. S. (1970): Protein deficiency and liver injury. *Am. J. Clin. Nutr.*, 23, 447–461.

14) Koch, O. R., Porta, E. A., and Hartroft, W. S. (1968): A new experimental approach in the study of chronic alcoholism. III. Role of alcohol versus sucrose or fat-derived calories in hepatic damage. *Lab. Invest.*, 18, 379–386.

15) Svoboda, D., and Higginson, J. (1964): Ultrastructural changes produced by protein and related deficiencies in the rat liver. *Am. J. Pathol.*, 45 353–379.

16) Harada, N., and Mogi, S. (1966): The influence of low protein diet on the composition of rat liver cell fractions. *Agric. Biol. Chem.*, 30, 274–277.

17) Harada, N., Kurahashi, M., and Haga, M. (1969): The influence of low casein diet on the fatty acid composition of rat liver mitochondrial phosphatides. *Agric. Biol. Chem.*, 33, 168–175.

18) Rogers, C. G. (1971): Lipid composition and metabolism in liver mitochondria and microsomes of rats fed a low protein diet. *J. Nutr.*, 101, 1547–1554.

19) Gerson, T. (1974): A comparison of the effects of dietary protein and lipid deprivation.
on lipid composition of liver membranes in rats. *J. Nutr.*, 104, 701–709.

20) Rogers, C. G. (1972): Fatty acids of phosphatidylcholine and phosphatidylethanolamine in liver mitochondria and microsomes of rats fed a low protein diet. *Nutr. Rep. Int.*, 5, 381–390.

21) Noguchi, T., Miyazawa, E., and Kametaka, M. (1974): Protease and protease inhibitor activity in rat skeletal muscle during growth, protein deficiency and fasting. *Agric. Biol. Chem.*, 38, 253–257.

22) Hagihara, B. (1961): Techniques for the application of polarography to mitochondrial respiration. *Biochim. Biophys. Acta*, 46, 134–138.

23) Oda, T., and Seki, S. (1969): Mitochondria, (i) Structure and function, *in* Research Technique for Subcellular Organella I (in Japanese), ed. by Asakura, A., and Ohnishi, K., Yoshioka-shoten, Inc., Tokyo, pp. 89–280.

24) Colbeau, A., Nachbaur, J., and Vignais, P. M. (1971): Enzymic characterization and lipid composition of rat liver subcellular membranes. *Biochim. Biophys. Acta*, 249, 462–492.

25) Sottocasa, G. L., Kuylenstierna, B., Ernst, L., and Bergstrand, A. (1967): Separation and some enzymatic properties of the inner and outer membranes of rat liver mitochondria. *Methods Enzymol.*, 10, 448–463.

26) Schnaitman, C., and Greenawalt, J. W. (1968): Enzymatic properties of the inner and outer membranes of rat liver mitochondria. *J. Cell Biol.*, 38, 158–175.

27) Tisdale, H. D. (1967): Preparation and properties of succinic-cytochrome c reductase (Complex II-III). *Methods Enzymol.*, 10, 213–215.

28) Beaufay, H., Bendall, D. S., Baudhin, P., and De Duve, C. (1959): Tissue fractionation studies. 12. Intercellular distribution of dehydrogenases, alkaline deoxyribonuclease and iron in rat-liver tissue. *Biochem. J.*, 73, 623–628.

29) Dawson, R. M. C., Hemington, N., and Lindsay, D. B. (1960): The phospholipids of the erythrocyte 'ghosts' of various species. *Biochem. J.*, 77, 226–230.

30) Itasaka, O., Hori, T., and Sugita, M. (1969): Biochemistry of shellfish lipids. XI. Incorporation of ³²P-orthophosphate into ceramide ciliatine (2-aminoethylphosphonic acid) of the fresh-water mussel, *Hyriopsis schlegelii*. *Biochim. Biophys. Acta*, 176, 783–788.

31) Arvidson, G. A. E. (1968): Structural and metabolic heterogeneity of rat liver glycerophosphatides. *Eur. J. Biochem.*, 4, 478–486.

32) Stoffel, W., Chu, F., and Ahrens, E. H. (1959): Analysis of long-chain fatty acids by gas-liquid chromatography. Micromethod for preparation of methyl esters. *Anal. Chem.*, 31, 307–308.

33) Chen, P. S., Toribara, T. Y., and Warner, H. (1956): Microdetermination of phosphorus. *Anal. Chem.*, 28, 1756–1758.

34) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193, 265–275.

35) Kimberg, D. V., and Loeb, J. N. (1972): Effects of cortisone administration on rat liver mitochondria. *J. Cell Biol.*, 55, 635–643.

36) Rafael, J., Patzelt, J., Schafer, H., and Elmadfa, I. (1984): The effect of essential fatty acid deficiency on basal respiration and function of liver mitochondria in rats. *J. Nutr.*, 114, 255–262.

37) Fleck, A., Wunner, W. H., Henderson, A. R., Ballantyne, F. C., and Tilstone, W. J. *J. Nutr. Sci. Vitaminol.*
(1971): Response of the liver to protein feeding. Proc. Nutr. Soc., 30, 42–46.

38) Harada, N., and Sugita, T. (1968): The influence of low casein diet on the in vivo incorporation of $^{32}$P-orthophosphate into individual phosphatides of rat liver mitochondria. Agric. Biol. Chem., 32, 340–344.

39) Wirtz, K. W. A., and Zilversmit, D. B. (1968): Exchange of phospholipids between liver mitochondria and microsomes in vitro. J. Biol. Chem., 243, 3596–3602.

40) Narce, M., Poisson, J.-P., Belleville, J., and Chanussot, B. (1988): Time-course effects of protein malnutrition on hepatic fatty acids $\Delta^4$- and $\Delta^5$-desaturation in the growing rat. Br. J. Nutr., 60, 389–402.

41) Brenner, R. R. (1981): Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog. Lipid Res., 60, 389–402.