Liver Microsomal Mixed-Function Oxidases in Response to Dietary Whole Egg Protein Levels in Rats

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(Received September 20, 1991)

Summary Relation between the activity of liver microsomal mixed-function oxidase system and dietary protein level was investigated in rats using purified whole egg protein, i.e. free from limiting amino acids. The animals were given either a diet containing 0, 5, 10, 20 or 40% of protein (experiment 1) or a diet containing 5, 10, 15 or 20% of protein (experiment 2) for 16 days. In experiment 2, half of the rats of each group were intraperitoneally injected sodium phenobarbital (PB) to induce the mixed-function oxidase system. The cytochrome P-450 content plateaued even at 5% level of dietary protein in experiment 1 and in the PB-untreated groups of experiment 2. However, it showed the highest value at 15% protein level in the PB-treated groups of experiment 2, indicating a shift of the response peak to a higher protein level due to an increase in protein requirement. Cytochrome P-450 reflected most specifically the dietary protein levels when the enzyme system was induced by PB. The 15% protein level, equivalent to 14.1 protein calories %, is a little higher than the optimal dietary level of whole egg protein ever obtained by usual nutritional indices.

Key Words dietary protein level, whole egg protein, mixed-function oxidase, cytochrome P-450, phenobarbital

The cytochrome P-450-dependent liver microsomal mixed-function oxidase (MFO) system plays an important role in the biotransformation of a wide variety of compounds including drugs, environmental chemicals, natural food toxicants, carcinogens and even endogenous substrates such as steroids (1,2). The activity of the MFO system is very dependent on the nutritional status (1-3). It is generally accepted that quantity and quality of dietary protein influence the activity of the MFO system (2,4). In particular, the cytochrome P-450 which plays a pivotal role in the MFO system is very sensitive to protein quantity and quality of the diet (2, 4, 5). It is well established in general that the activity of liver microsomal MFO system increases as dietary protein levels are increased from deficient or low to
normal levels (2, 4, 6–16). By contrast, dietary protein excess is reported to increase (11) or decrease (13) the activity of liver microsomal MFO system. However, inconsistent and differential effects of dietary protein excess on some of the MFOs have also been observed at the same time (17). As for the protein quality, on the other hand, dietary proteins of poor quality such as gluten generally decrease the activity of liver microsomal MFO system (2, 4, 18).

From these studies, a considerable amount of information is available on the effects of quantity and quality of dietary protein on the activity of liver microsomal MFO system. However, the proteins used in these studies had limiting amino acids and, besides, the dietary protein levels were mostly limited to a small range. Therefore, a dose response of the activity of the MFO system to dietary protein levels has not been clarified yet. Furthermore, the induction of the MFO system by xenobiotics such as phenobarbital (PB) has not always been introduced to the experimental conditions mentioned above.

We carried out the present study in rats to clarify the dose response of the liver microsomal MFO system to graded dietary protein levels, where purified whole egg protein, i.e. free from limiting amino acids, was used, and the induction of the MFO system by PB was examined concurrently.

EXPERIMENTAL

Animals and diets. Male Sprague-Dawley rats (Clea, Tokyo, Japan), 6 weeks of age, weighing 180–194 g and 186–204 g, were used in experiments 1 and 2, respectively. The compositions of basal and experimental diets were fundamentally according to the AIN-76 purified diet for rats (19, 20). The composition of the basal diet was described in a previous paper (21) and those of the experimental diets (g/kg diet) are shown in Table 1. Rats were fed the basal diet for 3 d and then randomly assigned to the experimental groups. Food and water were available ad libitum. Throughout the experiments, all rats were individually housed in stainless steel, wire-bottomed cages under controlled temperature (22 ± 1°C), humidity (50–60%) and light (12 h light/dark cycle). Food intake and body weight gain were measured four times per week.

Experiment 1. Groups of seven to ten rats were fed the experimental diet containing 0, 5, 10, 20 or 40% of purified whole egg protein (purity: 97.8%; Taiyo Kagaku Co., Ltd., Yokkaichi, Japan) for 16 d. After fasting overnight, the rats were anesthetized with intraperitoneal injection of sodium pentobarbital and blood was collected by heart puncture. The livers were promptly excised, washed with isotonic saline (9 g sodium chloride/liter), weighed, and then perfused with ice-cold isotonic saline via the portal vein. The livers were stored at −80°C until analysis of the MFO activity. Serum was prepared by centrifugation and used for the determination of leucine aminopeptidase (LAP) activity and albumin/globulin (A/G) ratio.

Experiment 2. Groups of fourteen to sixteen rats were fed the experimental
Table 1. Composition of diets for experiments 1 and 2.

| Ingredient                     | Experiment 1                  | Experiment 2                  |
|--------------------------------|-------------------------------|-------------------------------|
|                               | Protein level (%)             | Protein level (%)             |
|                               | 0    | 5       | 10    | 20    | 40    | 5    | 10    | 15    | 20    |
| Whole egg protein<sup>1</sup>  | 0    | 50      | 100   | 200   | 400   | 50   | 100   | 150   | 200   |
| α-Cornstarch                  | 650  | 600     | 550   | 500   | 450   | 600  | 550   | 500   | 450   |
| Glucose<sup>2</sup>           | 153  | ←      | ←     | ←     | ←     | 153  | ←     | ←     | ←     |
| Cellulose powder              | 50   | ←      | ←     | ←     | ←     | 50   | ←     | ←     | ←     |
| Soybean oil                   | 100  | ←      | ←     | ←     | ←     | 100  | ←     | ←     | ←     |
| Mineral mixture (AIN)<sup>3</sup> | 35 ←      | ←     | ←     | ←     | 35   | ←     | ←     | ←     | ←     |
| Vitamin mixture (AIN)<sup>3</sup> | 10 ←      | ←     | ←     | ←     | 10   | ←     | ←     | ←     | ←     |
| Choline bitartrate            | 2    | ←      | ←     | ←     | ←     | 2    | ←     | ←     | ←     |

<sup>1</sup>Purified whole egg protein (Taiyo Kagaku Co., Ltd., Yokkaichi, Japan).  
<sup>2</sup>Dextrose (Sanmatsu Chemicals, Tokyo, Japan).  
<sup>3</sup>Compositions of mineral and vitamin mixtures are described in refs. 19 and 20.

diet containing 5, 10, 15 or 20% of purified whole egg protein for 16 d. Half of the rats of each group were intraperitoneally injected sodium phenobarbital (PB) dissolved in isotonic saline at the level of 80 mg/kg of body weight for three successive days before killing. The rest of the rats in each group were injected only isotonic saline. After fasting overnight, the rats were decapitated and treated as described in experiment 1.

**Analytical procedures.** Liver microsomes were prepared as described previously (22). The MFOs as measured by the contents of cytochromes P-450 and b, and the activities of NADPH-cytochrome c reductase (EC 1.6.2.5), aminopyrine N-demethylase and aniline hydroxylase were analyzed as described previously (21, 22). Liver microsomal protein was measured by the method of Lowry et al. (23). Serum LAP activity was measured using L-leucine-p-nitroanilide as substrate (24). Serum albumin concentration was determined by the method of Doumas et al. (25). Globulin concentration in serum was obtained as the value subtracted albumin concentration from serum total protein concentration measured by the biuret method (26).

**Statistical analysis.** After confirming the homogeneity of variance of data of the examined groups employing Bartlett test, statistical significance of differences between mean values was assessed by analysis of variance (ANOVA) coupled with Duncan's multiple-range test at the 5% level of significance (27).

**RESULTS**

**Experiment 1**

Effects of graded levels from deficiency to excess of dietary whole egg protein...
Table 2. Effect of graded levels of dietary whole egg protein on food intake, body weight gain, liver weight, leucine aminopeptidase activity and albumin/globulin ratio in serum, and liver microsomal protein content of rats.1

| Protein level (%) | 0    | 5    | 10   | 20   | 40   |
|------------------|------|------|------|------|------|
| Food intake, g/16 d | 160±26a | 297±15b | 341±25c | 321±37bc | 302±21b |
| Body weight gain, g/16 d | −40±6a | 39±10b | 104±23c | 114±23c | 98±24c |
| Liver weight | 5.4±0.8a | 7.0±1.1b | 9.2±1.4c | 9.0±1.1c | 8.8±0.9c |
| g/100 g body weight | 3.1±0.4a | 2.8±0.3b | 2.9±0.2bc | 2.8±0.2b | 2.9±0.2b |
| Serum component | | | | | |
| LAP2 activity, U/L | 244±22a | 188±12b | 143±16c | 138±13c | 152±28c |
| A/G3 ratio | 0.80±0.08a | 0.99±0.08b | 0.99±0.04b | 0.99±0.04b | 0.96±0.05b |
| Liver microsomal protein | 16.4±3.7a | 18.0±3.3ab | 20.4±2.2bc | 23.8±5.0d | 21.9±4.9ed |

1 Rats were fed diets containing different levels of whole egg protein for 16 d ad libitum. Values are means±SD of seven to ten rats. Mean values within the same row that are not followed by a common superscript letter are significantly different as assessed by analysis of variance and Duncan's multiple-range test (p<0.05). 2 LAP, leucine aminopeptidase. 3 A/G, albumin/globulin.

on the liver microsomal MFO system were examined in experiment 1. Food intake, body weight gain, liver weight, LAP activity and A/G ratio in serum, and liver microsomal protein content are presented in Table 2. Food intake of the 10% group was highest and significantly higher than those of the other three groups of 0, 5 and 40%. There was no significant difference between the 10 and 20% groups. Body weight gain and total liver weight increased as the dietary protein level increased and they reached a plateau at 10% level. Liver weight per 100 g of body weight was not so much different among the groups. The serum LAP activity decreased with increasing dietary protein level, reaching a plateau at 10% level. There were no significant differences in the serum A/G ratio among the groups except the protein-deficient group in which the value was significantly low. The liver microsomal protein content increased as the dietary protein level increased, showing the highest value at 20% level, but no significant difference was recognized between the 20 and 40% groups.

Figure 1 and Table 3 show the liver microsomal cytochrome P-450 content and the changes in the activity of other MFOs, respectively. The cytochrome P-450 content did not change significantly with increasing dietary protein level and almost plateaued even at 5% level. The cytochrome b3 content increased slightly as the dietary protein level increased and its content of the 40% group was significantly higher than those of the other groups. The NADPH-cytochrome c reductase activity showed a similar change to that of cytochrome P-450 content, showing a significant difference only between the protein-deficient and 40% groups. The
Table 3. Effect of graded levels of dietary whole egg protein on cytochrome \( b_5 \) content and activities of NADPH-cytochrome c reductase, aminopyrine \( N \)-demethylase and aniline hydroxylase in liver microsomes of rats.1

| Protein level (%) | Experiment 1 |
|-------------------|--------------|
|                   | 0 | 5 | 10 | 20 | 40 |
| Cytochrome \( b_5 \) content \( \text{nmol/mg protein} \) | 0.30±0.04<sup>a</sup> | 0.31±0.03<sup>ab</sup> | 0.33±0.03<sup>ab</sup> | 0.34±0.05<sup>b</sup> | 0.40±0.05<sup>c</sup> |
| NADPH-cytochrome \( c \) reductase activity \( \text{nmol/} \text{(min} \cdot \text{mg protein)} \) | 79.33±8.53<sup>a</sup> | 87.67±12.62<sup>ab</sup> | 94.61±14.64<sup>ab</sup> | 91.95±15.98<sup>ab</sup> | 104.04±24.45<sup>b</sup> |
| Aminopyrine \( N \)-demethylase activity \( \text{nmol/} \text{(min} \cdot \text{mg protein)} \) | 9.24±2.21<sup>a</sup> | 9.32±1.37<sup>a</sup> | 8.64±0.92<sup>a</sup> | 8.39±1.25<sup>a</sup> | 8.99±1.85<sup>a</sup> |
| Aniline hydroxylase activity \( \text{nmol/} \text{(min} \cdot \text{mg protein)} \) | 0.15±0.04<sup>a</sup> | 0.19±0.04<sup>ab</sup> | 0.20±0.04<sup>ab</sup> | 0.25±0.07<sup>bc</sup> | 0.31±0.10<sup>c</sup> |

1 Values are means±SD of seven to ten rats. Mean values within the same row that are not followed by a common superscript letter are significantly different as assessed by analysis of variance and Duncan’s multiple-range test (\( p < 0.05 \)).
Fig. 1. Effect of graded levels of dietary whole egg protein on liver microsomal cytochrome P-450 content of rats. Values are means±SD of seven to ten rats. Mean values that are not followed by a common superscript letter are significantly different as assessed by analysis of variance and Duncan's multiple-range test (p<0.05). (experiment 1)

aminopyrine N-demethylase activity was not influenced by dietary protein levels. The aniline hydroxylase activity slightly increased with increasing dietary protein level and it showed a similar change to that of the cytochrome b5 content.

**Experiment 2**

In experiment 2, effects of an induction of the liver microsomal MFO system by PB treatment were examined when rats were given the graded levels of dietary whole egg protein. Food intake, body weight gain, liver weight and liver microsomal protein content are indicated in Table 4. Food intakes of both untreated and PB-treated 5% groups tended to be lower but there were no significant differences compared with those of the respective other three groups. Body weight gains of the two 5% groups were significantly lower than those of the respective other three groups. Total liver weight showed a similar change to the body weight gain, but liver weights per 100 g of body weight were conversely higher in both the untreated and PB-treated 5% groups. No significant differences were observed in the liver microsomal protein content among the four untreated groups, but in the PB-treated groups its content of the 5% group was significantly lower than those of the other three groups. Liver microsomal protein contents of the PB-treated groups were generally higher than those of the untreated groups.

As shown in Fig. 2, the liver microsomal cytochrome P-450 content of the untreated groups was not influenced by dietary protein levels as observed in experiment 1. In the PB-treated groups, on the other hand, the 15% group showed the highest value and the 5% group the lowest. The PB treatment significantly induced the cytochrome P-450 content as clearly demonstrated by the higher values of the PB-treated groups than those of the untreated groups.
Table 4. Effects of graded levels of dietary whole egg protein and phenobarbital treatment on food intake, body weight gain, liver weight and liver microsomal protein content of rats.1

| Protein level (%) | Experiment 2 |         |         | PB2-treated groups3 |
|------------------|--------------|---------|---------|---------------------|
|                  | Untreated groups | 5 | 10 | 15 | 20 | 5 | 10 | 15 | 20 |
|                  |              |  |  |  |  |  |  |  |  |
| Food intake, g/16d | 332±18a | 367±39ab | 356±37ab | 354±24ab | 346±50ab | 376±37b | 365±26ab | 373±24b |
| Body weight gain, g/16d | 56±11a | 116±16bc | 123±22bc | 119±21bc | 53±15a | 117±21b | 115±15bc | 133±16bc |
| Liver weight | 8.6±1.1a | 10.0±1.3bc | 10.0±1.0b | 9.1±0.9abc | 10.3±1.0bcd | 11.4±1.3de | 11.7±1.2c | 12.3±0.7e |
| g/100 g body weight | 3.2±0.3a | 3.0±0.3ab | 3.0±0.2ab | 2.8±0.2b | 3.9±0.4a | 3.5±0.2a | 3.5±0.2d | 3.6±0.2d |
| Liver microsomal protein | 18.8±4.4a | 19.5±3.1ab | 19.5±3.0a | 19.4±4.6ab | 22.5±2.3b | 26.2±4.6c | 27.7±5.4c | 27.5±2.6c |

1Rats were fed diets containing different levels of whole egg protein for 16d ad libitum. Values are means±SD of seven to eight rats. Mean values within the same row that are not followed by a common superscript letter are significantly different as assessed by analysis of variance and Duncan’s multiple-range test (p<0.05). 2PB, phenobarbital. 3Rats of each group were injected intraperitoneally sodium phenobarbital at the level of 80mg/kg of body weight for three successive days before killing.

Table 5 presents the data of the other MFOs measured. There were no significant differences in the cytochrome b5 contents and the activities of NADPH-cytochrome c reductase, aminopyrine N-demethylase and aniline hydroxylase among the four untreated groups. The cytochrome b5 content of the PB-treated 5% group was slightly but significantly lower than those of the other three PB-treated groups. The activities of NADPH-cytochrome c reductase and aminopyrine N-demethylase were unchanged among the PB-treated groups. The PB treatment slightly increased the aniline hydroxylase activity with increasing dietary protein level but showing a significant difference only between the 5 and 20% groups. The PB treatment markedly induced the cytochrome b5 content and the activities of NADPH-cytochrome c reductase, aminopyrine N-demethylase and aniline hydroxylase as judged from the higher values of the respective PB-treated groups than
Fig. 2. Effects of graded levels of dietary whole egg protein and phenobarbital (PB) treatment on liver microsomal cytochrome P-450 content of rats. Open bars indicate PB-untreated groups and bars with slanting lines PB-treated groups. Values are means ± SD of seven to eight rats. Mean values that are not followed by a common superscript letter are significantly different as assessed by analysis of variance and Duncan's multiple-range test (p<0.05). (experiment 2)

those of the respective untreated groups.

DISCUSSION

The general indices obtained manifesting nutritional effects of purified whole egg protein such as the growth response and the LAP activity and A/G ratio in serum were in accordance with our previous consequences (28, 29).

When the liver microsomal MFO system was not induced by PB, the content of cytochrome P-450, a pivotal enzyme in the MFO system, plateaued even at the 5% level. Hence, even 5% level of dietary whole egg protein can maintain the cytochrome P-450 content at an ordinary level. The cytochrome b$_5$ content slightly increased gradually as the dietary protein level increased and its content of the 40% group, an excess level group, was significantly higher than those of the other lower level groups. Cytochrome b$_5$ exerts its function in the metabolism of fatty acids, desaturation and elongation, and cholesterol biosynthesis, and as an electron donor to cytochrome P-450 (30). Therefore, an excess level of dietary protein might partly be associated with an enhancement of such functions of cytochrome b$_5$. However, the increase of its content was at best modest, and the minor change is unlikely to be of any physiological significance. The differences of cytochrome b$_5$ content among groups from 5 to 20% were actually insignificant in experiment 1 and in the PB-untreated groups of experiment 2.

The dietary levels of whole egg protein did not influence the NADPH-cytochrome c reductase activity, except the protein-deficient group with lower activity. This was also the case in the PB-untreated groups of experiment 2. The
Table 5. Effects of graded levels of dietary whole egg protein and phenobarbital treatment on cytochrome b5 content and activities of NADPH-cytochrome c reductase, aminopyrine N-demethylase and aniline hydroxylase in liver microsomes of rats.1

| Protein level (%) | Untreated groups | PB2-treated groups3 |
|-------------------|------------------|---------------------|
|                   | 5  | 10  | 15  | 20  | 5  | 10  | 15  | 20  |
| Cytochrome b5 content | 0.29±0.03a | 0.33±0.03a | 0.31±0.04a | 0.32±0.03a | 0.47±0.05b | 0.53±0.05c | 0.55±0.07c | 0.54±0.03c |
| NADPH-cytochrome c reductase activity | 78.14±18.06a | 91.00±13.8a | 73.43±11.65a | 81.00±15.86a | 192.43±21.24b | 190.38±18.24b | 191.88±32.36b | 197.50±19.50b |
| Aminopyrine N-demethylase activity | 10.27±3.27a | 12.90±3.17a | 10.87±1.82a | 10.96±2.08a | 19.29±3.69b | 20.28±2.27b | 20.67±2.43b | 19.70±2.92b |
| Aniline hydroxylase activity | 0.51±0.15a | 0.75±0.11a | 0.59±0.11a | 0.70±0.24a | 1.42±0.42b | 1.61±0.26c | 1.71±0.35c | 1.81±0.30c |

1Values are means±SD of seven to eight rats. Mean values within the same row that are not followed by a common superscript letter are significantly different as assessed by analysis of variance and Duncan’s multiple-range test (p<0.05). 2PB, phenobarbital. 3Rats of each group were injected intraperitoneally sodium phenobarbital at the level of 80 mg/kg of body weight for three successive days before killing.

Aminopyrine N-demethylase activity also was not influenced by dietary protein levels in experiment 1 and in the PB-untreated groups of experiment 2. However, the aniline hydroxylase activity increased slightly with increasing dietary protein level and it showed a similar change to that of the cytochrome b5 content. Accordingly, excess level of dietary protein increases reaction promoted by specific
species of cytochrome P-450 which facilitate aniline hydroxylation. Nevertheless, the aminopyrine N-demethylase activity and content of total cytochrome P-450 were not influenced by excess level of dietary protein. This suggests that dietary protein excess causes a change in the relative abundance of cytochrome P-450 species or enhances only the activity without increasing enzyme protein synthesis of specific species of cytochrome P-450 which metabolize aniline. Protein excess employing casein as dietary protein source has been shown to increase the activity of some of the liver microsomal MFOs, cytochrome b₅, aniline hydroxylase (11) and p-nitrobenzoate reductase (17), not to alter cytochrome P-450 (11,17) and N-demethylase (11), and to decrease biphenyl 4-hydroxylase (17) when the MFOs are not induced by xenobiotics such as PB. These observations coincide with our present results. Hence, dietary protein excess exerts differential effects on the MFOs. However, as shown in Table 3, the increase is at best modest and the minor variation is unlikely to be of any important physiological significance.

Concerning the effect of protein quantity on the activity of liver microsomal MFO system, it has been shown so far that diets with lower levels of casein (5–12% by weight) decrease the activities of liver microsomal MFO system as measured by cytochrome P-450, O-deethylase, O-demethylase, N-demethylase and hydroxylase compared with a diet containing a normal level of casein (20% by weight) (2,4,8,9,11,13–15). This discrepancy between these and our results appears to depend on protein quality, where casein has limiting amino acids and whole egg protein no limiting amino acids. Truex et al. (31) and Edes et al. (32) indicate that specific amino acid deficiencies as well as those of sulfur amino acids cause unique alterations in the liver microsomal MFO activities. Magdalou et al. (5) also show that the decrease in liver microsomal cytochrome P-450 content caused by protein depletion can be restored with repletion of a diet with balanced amino acid mixtures as protein source, whereas a diet with amino acid mixtures restricted in sulfur amino acids is unable to restore the content to the same extent. In addition, a rice diet added with lysine and threonine enhances the liver microsomal MFO activity compared with the diet without those amino acids (33). Furthermore, a diet containing gluten or gelatin is reported to decrease the liver microsomal MFO activity when compared with a diet containing casein at the same level (18,34).

These observations imply that whole egg protein, which has no limiting amino acids and even abounds in sulfur amino acids, can maintain the liver microsomal MFO system with the lower dietary level owing to enhanced utilization of its constituent amino acids. This may be the reason why even the 5% level of dietary whole egg protein can maintain the liver microsomal MFO system untreated by PB. On the other hand, it has been observed that protein deficiency and feeding a rice diet without addition of its limiting amino acids alter the proportions of major fatty acids and phospholipids in liver microsomes, and the liver NADPH regeneration rate (33,35). This may indicate that poor quality protein having limiting amino acids decreases the activity of liver microsomal MFO system through the mechanisms mentioned above.

J. Nutr. Sci. Vitaminol.
When the liver microsomal MFO system was induced by PB treatment in experiment 2, the cytochrome P-450 reflected most specifically the dietary protein levels, where its content was highest at the 15% level. A similar response pattern of the cytochrome P-450 is also observed in rats given PCB (13). The other MFOs in the PB-treated groups, as shown in Table 5, also reached the highest level up to the 15% dietary protein level. These MFOs other than cytochrome P-450 did not vary so much in response to the dietary protein levels from 5 to 20%. This would be due, at least in part, to the reason that whole egg protein is of very good quality free from limiting amino acids. From these results, the 15% level of dietary whole egg protein can maintain the activity of liver microsomal MFO system induced by PB at its maximum level. This level is a little higher than the optimal dietary level of whole egg protein ever obtained by usual nutritional indices (28).

Cytochromes P-448, a distinct family of cytochromes induced typically by 3-methylcholanthrene, direct the metabolism of chemical carcinogens towards the formation of reactive intermediates (36), and similarly, the cytochromes P-450 induced by ethanol, preferentially catalyze the activation of nitrosamines and some aromatic amines (37,38). In contrast, cytochromes P-450 induced by PB exhibit primarily a deactivating role, leading also to the ultimate detoxication of chemical carcinogens (36). Therefore, it is important to retain a dietary condition to be able to evoke the maximum detoxication ability of the liver microsomal MFO system to cope with the various situations that induce the MFO system. It is observed that cytochrome P-450 responds most sensitively to dietary protein level (2, 4, 5). In this respect, it seems to be appropriate to assess the cytochrome P-450 content in a nutritional evaluation of dietary protein level associated with oxidative detoxication ability of rats.

In our present studies we obtained the dose response of the liver microsomal MFO system as measured by cytochromes P-450 and b5, NADPH-cytochrome c reductase, aminopyrine N-demethylase and aniline hydroxylase to the graded levels of dietary purified whole egg protein. As a result, the MFO system plateaued at the 5% dietary protein level when the MFO system was not induced by xenobiotics such as PB. However, the protein level to attain the maximum activity of MFO shifted to the 15% level when the MFO system was induced by PB, suggesting that PB treatment increased requirement of dietary protein for the induction of MFO system. When liver microsomal MFOs are induced by xenobiotics, membrane proteins of endoplasmic reticulum are synthesized in addition to the biosynthesis of enzyme proteins of the MFO system. Therefore, dietary protein requirement increases to adapt to such a condition, leading to the shift of the response peak of cytochrome P-450 from 5 to 15% dietary protein in the PB-treated rats. All living bodies respond to various external conditions including stress with a considerable range. The nutritional indices hitherto used are presumed to show a minimal requirement for such a response, while the physiological index as herein used a maximal requirement. Therefore, it is necessary to supply 15% level of dietary whole egg protein as protein source to enhance the metabolic functions up to the
upper limit of the ranges at any occasions. The 15% dietary level of whole egg protein, equivalent to 14.1 protein calories %, is very close to the optimum protein calories % of 12 to 14 that has been considered to be ideal for rats (28).

On the other hand, the flux through the MFO system is what really determines the in vivo rate of disposal of xenobiotics. This flux is not always proportional to the concentration of one or more of the constituent enzymes of the MFO system. Accordingly, our future investigations will be focused to obtain an optimal flux at graded whole egg protein intake in addition to a comparison of the induction of liver microsomal MFO system between PB and 3-methylcholanthrene, a distinct inducer of cytochromes P-448.

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