Note

Inducibility of Rat Liver Drug-Metabolizing Enzymes as an Index of Food-Screening for Safety Assessment

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Knowledges about the toxicities and permissible limits of various chemical compounds, which may contaminate food, have been accumulated. Chemical analyses for particular toxic compounds have also been advanced. However, practical test-system to detect contamination of food with these chemical compounds is insufficient. Difficulty is due to an infinite number of foods to be tested for contamination with innumerable toxic compounds. It may be impossible to predict what kind of toxic compounds may contaminate what kind of food. This may be a reason why we could not prevent such damages caused by the contamination of edible oil with polychlorinated biphenyl (1968 in Japan) and the contamination of feed with polybrominated biphenyl (1973 in USA). If a food-screening method which is responsive to various toxic compounds is devised, test-system to estimate food safety may be improved. The drug-metabolizing enzymes in liver microsomes are known to be induced by more than 200 kinds of drugs, insecticides, carcinogens and other chemicals.¹⁾ This communication is concerned with the possibility of utilization of inducibility of rat liver drug-metabolizing enzymes as a method of food-screening for safety assessment.

As shown in Fig. 1, amount of liver microsomal cytochrome P-450 determined by the method of Omura and Sato²⁾ is higher in rats fed commercial stock diet (CLEA CE-2) than in rats fed a synthetic diet. Similar results were also obtained in cytochrome *b₅* level which was determined as described by Omura and Takesue.³⁾ In these experiments, the composition of macronutrients of the synthetic diet was made quantitatively the same as that of the stock diet by mixing 26.6% casein, 4.9% rapeseed oil, 4.4% cellulose powder, 7.2% salt mixture, 55% α -starch and 2% vitamin mixture. This fact may indicate that the similar observation by Brown *et al.*,⁴⁾ in which N-demethylation activity of liver homogenate was found to depend on the nature of the diet, was not due to difference in composition of nutrients of diets. In addition, no differ-

FIG. 1. Variation of Liver Microsomal Cytochrome P-450 by Feeding of Natural Diet or Synthetic Diet.

Male wistar rats (100~120 g) were previously fed the commercial stock diet (CLEA CE-2) for 8 days *ad libitum*. After determining the amounts of cytochrome P-450 on the zero day, rats were divided into two groups. Rats of the first group were fed the commercial stock diet (●) and rats of the second group were fed the synthetic diet (○). On the 7th day, 8 rats of the second group, which had been fed the synthetic diet, were given the commercial stock diet again (▲) and remaining 8 rats were given the synthetic diet continuously. Slaughter of rats and change of diets were made 9:30 AM and 5:00 PM, respectively, on the day indicated. Preparations of liver microsomes and determination of cytochrome P-450 were made as described by Omura and Sato²⁾ with minor modification. Means for 4 animals are presented with standard deviations.

ence was observed between rats fed the synthetic diet and the commercial stock diet in food intake, body weight and liver weight throughout the experiments. These facts suggest that the difference in amounts of the cytochromes are not due to nonspecific effects of diets.

Although the composition of macronutrients of two diets in above experiments were made quantitatively the same, it is possible that a qualitative difference in a nutrient may affect on amounts of the drug-metabolizing enzymes. Among macronutrients, protein may be the most probable nutrient which causes the qualitative difference of diet. Table I-(A) shows the amounts of the cytochromes of rats fed synthetic diet in which 26.6% casein is replaced with zein or egg protein. When the amounts were indicated as n moles per 100 g body weight, both cytochrome P-450 and *b₅* levels did not change by protein quality of diets. On the other hand, significant increases of the cytochromes were observed by addition of an inducer, phenobarbital, to the synthetic diet. These results may indicate that the difference in amount of the cytochromes between rats given the synthetic diet and the commercial stock diet may be caused by some inducer(s) contaminated in ingredients of the commercial stock diet.

In order to explore technical problems in the practical use of this induction for food safety assessment,

TABLE I. LIVER MICROSOMAL CYTOCHROME P-450 AND
CYTOCHROME b_5 OF RATS FED VARIOUS DIETS

Rats were preliminarily fed the synthetic diet with casein so as to reduce the effect of previous diet. After this preliminary feeding, diets were changed to the diets to be tested at 5:00 PM on the zero day. On the 8th day, amounts of cytochrome P-450 were determined as described in Fig. 1. Cytochrome b_5 was measured by a modification of the method of Omura and Takesue.³⁾ Means for 4 animals are presented with standard deviations.

Experiment	Diet	Cytochrome P-450 (n mol/100 g body weight)	Cytochrome b_5
(A)	Synthetic diet with		
	Casein	74.5 $\pm$ 2.9	41.1 $\pm$ 2.2
	Zein	70.0 $\pm$ 6.0	37.4 $\pm$ 3.8
	Egg protein	74.0 $\pm$ 3.5	42.1 $\pm$ 1.5
	Casein + Phenobarbital (100 ppm)	145.0 $\pm$ 20.6	63.1 $\pm$ 4.1
(B)	Test-diets of		
	Defatted soy bean meal	90.6 $\pm$ 8.3	45.1 $\pm$ 3.5
	Corn gluten meal	98.3 $\pm$ 6.2	51.3 $\pm$ 2.0
	Fish meal	99.8 $\pm$ 2.6	49.6 $\pm$ 4.1

a model experiment was performed preparing test-diets with crude proteins. Defatted soy bean meal, corn gluten meal and fish meal were selected as samples. These crude proteins are on market for supplements of protein in feed. For the preparation of the test-diet, all protein sources were supplied with individual meals. The amounts of other insufficient nutrients were computed from the composition of individual meal and were supplemented with ingredients for the synthetic diet so as to have the same composition of the commercial stock diet. As shown in Table I-(B), amounts of the cytochromes of rats fed these test-diets were higher than those of rats fed the synthetic diet. This result may indicate that the drug-metabolizing enzymes are responsive to some inducer(s) contaminated in these crude proteins.

Induction of cytochrome P-450 was also found to be responsive to more than 1 ppm of tetrachlorinated biphenyl (PCB), *p, p'*-dichloro-diphenyl-trichloroethane (DDT), γ -benzenehexachloride (BHC) and 3,4-benzpyrene added to the synthetic diet. Although the sensitivity of this method for detection of toxic compounds in food may be lower than chemical method for particular compound, an advantage of utilization of inducibility of the drug-metabolizing enzyme is that contamination with various inducers can be tested by one effort. In addition, a contamination of unknown toxic compound(s) may be detected since biological index is used in this method.

These advantages of this method lead a disadvantage that the contaminated compound(s) cannot be identified even if a food causing a remarkable induction of the drug-metabolizing enzyme is found. This disadvantage may be conquered to a certain extent by following two analyses. 1) In profiles of time course

of inductions, oral administration of PCB did not reach the maximal induction of cytochrome P-450 and b_5 within 8 days. However, DDT elevated the amounts of the cytochromes to the maximal value within 4 days. Profile of time course of induction by BHC was a middle of those of PCB and DDT. 2) The patterns in inductions of multiple forms of cytochrome P-450,³⁾ which were detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis of liver microsomal protein, were characteristic among rats fed synthetic diet containing PCB, DDT and BHC.

Insufficient correlation between the toxicities and the inducibilities in certain compounds may be a problem of this method. This resembles the correlation between carcinogens and mutagens.⁶⁾ Therefore, we must await an accumulation of a lot of data for correlation between the toxicities and the inducibilities of various compounds. By these studies, the limit of application of this method for food safety assessment will be made clear.

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