Hepatic p-nitrophenetole O-deethylation Activity in Mice depends on Diet Composition

by *Jesper Bo Nielsen*[1], *Ole Andesen*[1] and *Per Svendsen*[2]
[1]Department of Environmental Medicine, Odense University, Odense Denmark.
[2]Biomedical Laboratory, Odense University, Odense Denmark.

Introduction:

The toxicity of many chemicals depends on the activity of the hepatic microsomal mixed function oxidase (MFO) system. Some compounds need metabolic activation to exert their effect, and others are detoxified by this enzyme system, which is characterized by its broad substrate specificity (*Sipes & Gandolfi* 1986). A large number of different forms of the protein moiety determining the substrate specificity has been demonstrated in humans and various experimental animals (*Lu & West* 1980). The different forms of MFO may be more or less specifically induced in animals exposed to drugs, pesticides, industrial chemicals or natural products (Dannan et al. 1983). Thus, comparison of results from animals living under different environmental conditions can be misleading. Great efforts have therefore been invested in removal or control of these potential inducers or inhibitors of MFO in the environment of experimental animals. Effects of different factors in the physical environment (e.g. temperature, light) on MFO activity have been studied (*Fuhrman & Fuhrman* 1961, *Nair & Casper* 1969). Several studies report effects of various bedding materials (*Vesell* 1967, *Nielsen et al.* 1986). But despite the fact that the importance of nutrition on the metabolism of xenobiotics is widely recognized, still no serious attention is given to animal diets when designing or interpreting data from toxicological studies. A number of publications have been concerned with the effect of a particular dietary component on MFO activity (*Parke & Ioannides* 1981, *Wattenberg* 1983).

The present study investigated the effects of various commercial diets, of protein and fat levels in semisynthetic diets, and of fiber content and type on the hepatic O-deethylation of p-nitrophenetole in mice. The O-deethylase is part of the MFO system. The significance of measuring this particular enzyme activity is that factors affecting the MFO system most likely will affect several other enzyme activities as well.

Materials and Methods:

All experiments were performed with 4-5-week-old outbred SPF mice (Bom: NMRI) kept in standard type III cages (maximum 12 animals) with beech-wood bedding in animal rooms with 20 air changes per hour, temperature 21 ± 1° C, relative humidity 55 ± 5% and light/dark periods 12/12 with 1/2 h twilight. The animals had permanent access to water and food. Cages and bedding were changed twice a week.

Three commercial diets, Altromin (Brogården, Gentofte, Denmark), Ewos (Ewos, Silkeborg, Denmark) and Kemovit (Kemovit, Fredericia, Denmark), as well as 12 semisynthetic diets were used.

Semisynthetic diets contained casein as protein source, corn and potato starch as carbohydrate source and soy oil as fat. The composition of these diets is shown in Table 1. Equal and adequate amounts of vitamins and minerals were added to the diets. When different amounts of wheat bran were used, the diet composition was corrected for the content of available fat, carbohydrate and protein in bran.

After 5 weeks ad lib feeding on the various diets, animals were killed by cervical disloca-

Table 1. Composition of semisynthetic diets used in the study of effects of diet on hepatic 0-deethylation of p-nitrophenetole in mice. Equal and adequate amounts of vitamins and minerals were added to the diets.

experim.	group	1	2	3	4	5	6	7	8	9	10
Protein	energy %	10	20	20	20	40	20	20	20	20	20
	weight %	9,3	18.2	18.7	21.2	37.4	19.8	17.0	18.9	15.6	10.3
Fat	energy %	10	5	10	30	10	10	10	10	10	10
	weight %	4.2	2.0	4.2	14.2	4.2	4.4	3.8	4.1	3.1	1.5
Carbon-hydrates	energy %	80	75	70	50	50	70	70	70	70	70
	weight %	74.8	68.1	65.4	52.9	46.7	69.1	59.5	67.6	60.2	48.2
Cellulose	weight %	7.0	7.0	7.0	7.0	7.0	2.0	15.0	0.0	0.0	0.0
Wheat-bran	weight %	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	7.0	15.0
Total energy	(KJ/kg)	15889	15438	15893	17993	15889	16789	14459	16591	15197	12996

tion and livers removed. All subsequent operations were performed at 0 to 4° C. Microsomal pellets were isolated by differential centrifugation as described by *Omura and Sato* (1964). Cytochrome P-450 O-deethylation of p-nitrophenetole was assayed according to the O-demethylation assay described by *Konat and Clausen* (1971) except that p-nitrophenetole was used instead of p-nitroanisole to avoid an inhibitory effect of formaldehyde (Imai 1979). Total protein was determined according to *Lowry et al.* (1951) using bovine serum albumine as standard. No correlation between diet and total amount of microsomal protein was observed. Enzyme activity was calculated as µmol substrate metabolized per min per mg microsomal protein (mU/mg). The intra-assay standard deviation was below 5%. However, due to interassay differences, enzyme activities from separate experiments can not be compared unless the results are related to MFO activities in reference groups present in both experiments. The activities of O-deethylation of p-nitrophenetole in the experimental groups showed an approximate gaussian distribution, and the results were therefore compared using Student's t-test (two-sided, significance limit 0.05).

Results:

Hepatic O-deethylation of p-nitrophenetole in mice kept on semisynthetic diets with three different protein levels (Table 2, results from the 3 experimental groups 1, 3, 5) showed no correlation between enzyme activity and energy contribution from protein in the diet. The influence of a high fat content (30% energy from fat)

Table 2. Hepatic O-deethylation of p-nitrophenetole in mice (Bom: NMRI) kept on semisynthetic diets with three different protein contents for 5 weeks.

Percent energy from protein	n	enzyme activity (mU/mg) mean ± s.d.
10	8	0.640 ± 0.198
20	8	0.538 ± 0.141
40	8	0.575 ± 0.085

Table 3. Hepatic O-deethylation of p-nitrophenetole in mice (Bom: NMRI) kept on semisynthetic diets with three different protein contents for 5 weeks.

Percent energy from fat	n	enzyme activity (mU/mg) mean ± s.d.
5	8	0.563 ± 0.181
10	8	0.538 ± 0.141
30	8	0.719 ± 0.153*

*$p < 0.05$, compared with groups given diets with 5% and 10% of the energy contributed by fat.

Table 4. Hepatic O-deethylation of p-nitrophenetole in mice (Bom: NMRI) kept on semisynthetic diets with three different amounts of two types of fiber (diets number 4 and 8-12 in table 1, 8 animals per group). Standard diets were corrected for energy contribution from available carbohydrate, fat and protein in the fiber.

fiber content (%)	enzyme activity (mU/mg) wheat-bran	cellulose	
2	0.987 ± 0.164	1.045 ± 0.406	n.s.
7	0.975 ± 0.217	0.976 ± 0.319	n.s.
15	0.690 ± 0.219*	0.689 ± 0.134*	n.s.

*$p < 0.05$, compared to groups with dietary fiber levels of 2% and 7%. n.s., no significant difference between enzyme activities in groups given wheat-bran or cellulose.

Table 5. Hepatic O-deethylation of p-nitrophenetole in mice (Bom: NMRI) kept on three commercial feedings.

product	n	enzyme activity (mU/mg) mean ± s.d.
Altromin	28	0.445 ± 0.139*
Ewos	16	0.526 ± 0.116
Kemovit	12	0.575 ± 0.128*

*$p < 0.01$

in the diet significantly increased the hepatic O-deethylation of p-nitrophenetole compared to the enzyme activity in mice kept on a diet with 5% of the energy contributed by fat, whereas an increase in energy contribution from fat from 5% to 10% did not affect the O-deethylation of p-nitrophenetole (Table 3, results from the 3 experimental groups, 2, 3, 4). The dietary fiber content also affected the hepatic O-deethylation of p-nitrophenetole (Table 4). When the amount of fiber was increased from 7% to 15%, the O-deethylation of p-nitrophenetole was decreased about 30%, regardless of whether the fiber was cellulose or from wheat bran. Reduction of the fiber content from 7% to 2% did not affect the enzyme activity. Comparison of hepatic O-deethylation of p-nitrophenetole in animals kept on diets with equal amounts of wheat bran or cellulose fibers showed no difference in enzyme activities at the three different levels.

Table 5, comparing hepatic O-deethylation of p-nitrophenetole in mice kept on three commercial diets, shows that the enzyme activity was dependent on the feed: animals kept on Kemovit had a 30% higher enzyme activity than animals kept on Altromin, whereas the difference between enzyme activities in mice kept on Ewos and Altromin was only 18%.

Discussion:

The different isozymes comprising the MFO system are not induced by the same drug, or at the same time or to the same degree. Therefore, measured effects of environmental factors on the hepatic MFO activity demonstrate a quantitative relation only to the metabolism of a specific chemical, namely the substrate of the *in vitro* assay. But since the isozymes of the MFO system are often induced by and able to metabolize several different compounds, an inducer may induce a number of isozymes and thus affect a number of different enzyme reactions. The lack of effect of a chemical on one enzyme reaction does however not exclude effects on other enzyme reactions. Effects of diet composition on chemical toxicity have been reported in several cases (*Parke & Joannides* 1981, *Wattenberg* 1983). Animal and human data indicate that low protein diets diminish the hepatic enzymatic degradation rates of antipyrine and theophylline compared to diets with higher protein contents (*Kato et al.* 1968, *Andersen et al.* 1979). Furthermore, the potent liver carcinogen dimethylnitrosamine, which is bioactivated by the MFO, is far less hepatoxic in rats kept on a protein free diet than in rats kept on a normal diet, presumably due to reduced hepatic levels of microsomal enzymes (*Czygan et al.* 1974). These studies on effects of protein content on chemical toxicity have almost exclusively compared results from protein-deficient with protein-sufficient animal groups. The modifying effect of a high protein diet on the toxicity of a chemical may, however, not always be mediated through the hepatic MFO activity (*Singletary et al.* 1984).

A diet with a high fat content has previously been shown to induce a higher cytochrom P-450 actvity than a fat-free diet (*Caster el al.* 1968, *Norred & Wade* 1973). The metabolic ra-

tes for aniline hydroxylation, hexobarbital oxidation and heptachlor epoxidation were in one investigation shown to be enhanced 31%, 80% and 160% respectively (*Wade & Norred* 1976). Other investigations, on the contrary, tend to show that diets high in polyunsaturated fats decrease the concentration of hepatic cytochrome P-450 due to degradation of microsomal membranes resulting in a loss of cytochrome P-450 (*Sipes & Gandolfi* 1986). Carcinogenicity studies show that tumor induction in the mammary gland of rats is decreased when the animals are fed low-fat diets but is augmented on high-fat diets (*Reddy et al.* 1980).

The influence of dietary fiber on colon cancer incidence in animal models is wellknown (*Burkitt et al.* 1972, *Clinton et al.* 1978, *Watanabe et al.* 1978). But the effects of dietary fiber on the metabolism of toxicants in the liver have only been studied to a very limited extent. The influence of dietary fibers on the metabolism of the pesticide lindane in rats was a markedly altered metabolism of lindane resulting in a significant change in the proportions of the excreted metabolites (*Chadwick et al.* 1978). The biochemical background for the changed metabolism was, however, not resolved in the study.

The results obtained in this study with different protein diets cannot be directly compared to the studies referred to earlier (*Kato et al.* 1968, *Andersen et al.* 1979, *Czygan et al.* 1974), since some of their experimental groups were protein deficient. The effect of dietary fiber content on hepatic O-deethylation of p-nitrophenetole reported here not only confirms earlier observations of the influence of dietary fiber on metabolism, but actually measures the effect on enzyme activity. The effect of dietary fat content on hepatic O-deethylation of p-nitrophenetole observed in this study agrees with results of earlier experiments, and stresses the importance of controlling this dietary factor in experimental studies with animals.

The consequence of studies showing effects of dietary factors on the liver enzyme activity should be an approach towards more controlled dietary conditions for experimental animals. At the moment toxicological and pharmacological animal studies are carried out in different laboratories using different feeds, and direct comparisons of results are often made. The potential risk of misleading conclusions on the basis of such comparisons is illustrated by the difference in hepatic O-deethylation of p-nitrophenetole observed in the present study among animals kept on three different commercially available diets. A direct comparison of the commercial diets in relation to the hepatic MFO activity was not possible due to lack of information on the compositions of the diets. In conclusion we urge commercial firms to cooperate to achieve standardization of animals feeds with regard to fat, fiber and protein type and content.

Summary:

The present study investigated effects of various commercial diets, of protein and of fat levels in semisynthetic diets, and of fiber content and type on hepatic O-deethylation of p-nitrophenetole in mice. A high fat content increased and a high fiber content decreased the hepatic p-nitrophenetole O-deethylation activity. The activity in mice kept on different commercial feedings differed significantly. The level of hepatic microsomal cytochrome P-450 activity affects the toxicity of many compounds. Thus, standardization of commercial feeds for experimental animals used in toxicological and pharmacological studies is needed to avoid the potential risk of misleading conclusions.

Sammendrag

Effekten af forskellige kommercielle foderblandinger, af protein- og fedtindhold i semisyntetiske diæter samt af fiberindhold og type på leverens p-nitrophenetol 0-deethylase-aktivitet er undersøgt i mus. Et højt fedtindhold forøgede og et højt fiberindhold mindskede leverens p-nitrophenetol 0-deethylase-aktivitet. Der fandtes signifikant forskel i den hepatiske p-nitrophenetol 0-deethylase-aktivitet hos mus fodret med forskellige kommercielle foderblandinger. Enzymaktiviteten i leverens microsomale cytrochrom P-450 system er af betydning for toksiciteten af mange stoffer. Standardisering af kommercielle foderblandinger til forsøgsdyr anvendt i toksikologiske og farmakologiske studier er derfor nødvendig for at imødegå den potentielle risiko for misfortolkninger af forsøgsresultater.

Yhteenveto / K. Pelkonen
Tutkimuksessa selvitettiin eri kaupallisten rehujen vaikutuksia, sekä puolisynteettisten rehujen proteiini - ja rasvapitoisuuksien ja kuitukitoisuuden ja -tyypin vaikutuksia maksan p-nitrofenetolin 0-de-etylaatioaktiivisuuteen. Korkea rasvapitoisuus nosti ja korkea kuitupitoisuus laski maksan p-nitrofenetoli 0-de-etylaatioaktiivisuutta. Eri kaupallisilla rehuilla ruokituissa hiirissä aktiivisuus oli merkitsevästi erilainen. Maksan sytokromi P-450-aktiivisuus vaikuttaa monien aineiden myrkyllisyyteen. Näinollen tulisi toksikologisissa ja farmakologisissa kokeissa oleville koe-elaimille annettavat kaupalliset ruhut vakioida, jotta vältyttäisiin harhaanjohtavilta päätelmiltä.

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Correspondence to Jesper Bo Nielsen, Department of Environmental Medicine, Odense University, J. B. Winsløws Vej 19, DK-5000 Odense C, Denmark.