Biochemical Pharmacology, Vol. 37, No. 17, pp. 3245–3249, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

EFFECT OF NUTRITIONAL IMBALANCES ON CYTOCHROME P-450 ISOZYMES IN RAT LIVER

Z. AMELIZAD,* J. F. NARBONNE,†‡ C. R. WOLF,§ L. W. ROBERTSON‖ and F. OESCH*

* Institute for Toxicology, University of Mainz, Federal Republic of Germany; † Laboratoire de Toxicologie Alimentaire, Université de Bordeaux I, Avenue des Facultés, 33405 Talence Cedex, France; § Imperial Cancer Research Fund, Laboratory of Molecular Pharmacology and Drug Metabolism, Edinburgh, U.K.; and ‖ Graduate Center for Toxicology, University of Kentucky, Lexington, U.S.A.

(*Received* 19 *February* 1988; *accepted* 22 *February* 1988)

Abstract—Male Sprague–Dawley rats were fed for six weeks either a control diet containing 22% casein (C) and 5% fat (F) or a low-protein diet (6% C, 5% F) or high-lipid diet (30% C, 30% F). A group of rats received a control diet containing 50 ppm of Phenoclor DP6. Three major forms of cytochrome P-450, UT 50, BP 3a and MC 2 were purified from livers of DP6-fed rats and only two forms, UT 50 and PB 3a, were purified from control and dietary groups. The amino acid composition and the catalytic activities towards all substrates tested were only significantly modified in the purified UT 50 P-450 isozyme from rats fed the low-protein diet. The N-terminal sequence analysis shows that cytochrome P-450 UT 50 (from control group) and UT 501 (from low-protein group) are two distinct proteins.

Cytochrome P-450 systems catalyze the metabolism of a wide variety of hydrophobic compounds. The presence of a given pattern of cytochrome P-450 isozymes differing from each other in substrate specificity and catalytic activity greatly affects the metabolism of the compounds such as endogenous substrates, environmental pollutants and carcinogens, and thereby represents one level of control in the production of reactive mutagenic and carcinogenic intermediates [1–3]. It is well-established that changes in the level and activity of cytochrome P-450 isozymes occur following exposure to numerous xenobiotics [4–7]. However, it has been shown that the diet composition can influence the level of total cytochrome P-450 as well as microsomal monooxygenase activities [8–11]. We have previously demonstrated an alteration in the cascade of enzymatic reactions of benzo(*a*)pyrene metabolism and in the ability of liver fractions to activate this compound to mutagenic metabolites in rats under unbalanced nutritional conditions [12].

In protein deficiency some cytochrome P-450 characteristics were changed [13, 14] and it is therefore conceivable that not only xenobiotics but also imbalances in the relative amount of normal food constituents may differentially influence the pattern of the cytochrome P-450 isozymes.

In this study we have compared the characteristics of the two major forms of cytochrome P-450 in liver of untreated rats either in normal or in unbalanced dietary conditions. These characteristics were also compared to those of isozymes from livers of rats treated with a strong mixed-type PCB inducer.

‡ Author for correspondence.

METHODS

Materials. Phenoclor DP6 was obtained from Prodelec Co, France; ^{3}H benzo(a)pyrene from Centre d'Etude Nucléaire de Saclay, Gif/Yvette, France; Sepharose 4B and DEAE-cellulose from Pharmacia, Emulgen 911 from Kao-Atlas Ltd, Japan and experimental diets were purchased from Usine d'Alimentation Rationnelle, Villemoisson/Orge, France. Other chemicals were of high purity and were obtained from commercial sources.

Nutritional conditions and xenobiotic treatment. Experimental conditions were as previously described [12]. Male Sprague–Dawley rats (70–80 g) were fed a standard (Co) diet (22% casein, 5% lard) for one week and then randomized in 4 groups of 10 animals. Group 1 was fed the Co diet, group 2 was fed a high-lipid (Hl) diet (30% lard), group 3 was fed a low-protein (LP) diet (6% casein) for 6 weeks. Group 2 was fed the Co diet during 2 weeks and with a Co diet containing 50 ppm of a DP6 (3.5 mg/kg/day) for the last 4 weeks. Rats from all experimental groups were fasted 16 hr before sacrifice and then killed by decapitation. Hepatic microsomes were prepared as described [15].

Purification of cytochrome P-450. Liver microsomal cytochrome P-450 (about 300 nmol) was solubilized with sodium cholate, and purified by affinity chromatography on ω-aminooctylsepharose 4B as described [16, 17]. The specific content of this fraction was 17 nmol of P-450 per mg of protein with a recovery of approximately 45% of the total microsomal cytochrome P-450. The purified fractions of P-450 eluted from the column with 0.08% Emulgen 911 were immediately pooled and dialyzed overnight and applied on a DEAE-52 cellulose column pre-

viously equilibrated with a 20% glycerol buffer. Cytochrome P-450 UT 50, P-450 PB 3a and P-450 MC 2 were sequencially eluted and then each one separately applied on a hydroxylapatite column. The eluted fractions from this column showed a single protein staining band when analyzed by SDS-polyacrylamide gel electrophoresis. The specific contents were 14–19 nmol of cytochrome P-450 per mg of protein.

Purification of NADPH-cytochrome P-450 reductase. The microsomal enzyme was purified to a specific activity of 21500 units/mg of protein for reconstitution of the catalytic activities of cytochrome P-450. Reductase was extracted from the remaining proteins after the cytochrome P-450 extraction on a ω-aminooctylsepharose 4B column and further purified by affinity chromatography on a 2′,5′-ADP-sepharose 4B column as reported [18]. One unit of reductase is defined as that amount catalyzing the reduction of 1.0 nmol of cytochrome *c*/min [19].

Assay methods. The concentration of cytochrome P-450 was determined from the CO reduced difference spectrum [20]. Protein was measured by the method of Lowry *et al.* [21] and SDS polyacrylamide slab-gel electrophoresis was performed as described by Laemmli [22]. Antibodies to the purified proteins were raised in New Zealand white rabbits. Purified proteins (200 μg in 1 ml Freunds complete adjuvant) were administered, followed by 3 further administrations of 150 μg purified enzyme in 1 ml incomplete adjuvant, at 2 week intervals. Double immunodiffusion was carried out as described [23]. The conditions used for the reconstitution of monooxygenase activity consisted of (per ml) 0.5 nmol cytochrome P-450, 50 units of cytochrome P-450 reductase and 0.1 mg dilauryl-phosphatidyl-choline [24]. A direct fluorimetric assay for 7-ethoxycoumarin [25], a spectrophotometric assay for ethylmorphine and benzphetamine demethylation [26], zoxazolamine hydroxylase [27] and the radiometric assay for benzo(*a*)pyrene hydroxylation [28] were used. Ten μg $MgCl_2$ were present in all assays. Amino acid analysis was performed using a Kontron analyser on the purified cytochrome samples after lyophylization for 5 hr in $-10°$ and treatment with 5.7 N HCl at 110° for 24, 48 and 72 hr. Tryptophan values were determined after hydrolysis with 4N methanesulphonic acid containing 0.2% 3-(2-amino-ethyl)-indole at 118° for 21 hr. Values were corrected using lysozyme as a standard [29]. Cysteine values were obtained after performic acid oxidation as described [30].

Statistical analysis. Guilloteau and coworkers [31] suggested that the amino acid composition of 2 proteins *i* and *j* be compared using the χ^2 which was defined for 18 amino acids, according to the formula:

$$\chi^2 = 18 \sum_{k=1}^{k=18} \frac{(AA\,ik - AA\,jk)^2}{\frac{AA\,ik + AA\,jk}{2}}$$

AA *ik* and AA *jk* are the respective percentage of amino acid *k* in the sum of the amino acid assayed in the proteins *i* and *j*. Comparisons between groups were performed using Dunnett's multiple comparison analysis [32].

N-terminal sequence analysis. Five hundred μg/mg protein from each purified cytochrome P-450 isozyme were dialyzed for 3 days against 0.2% trifloroacetic acid and freeze-dried for 8 hr. The samples were used to determine N-terminal sequences [33].

RESULTS

The three forms of cytochrome P-450 purified in this study can be compared to the nomenclature of forms described in the literature as follows: cytochrome P-450 PB 3a was described as the major cytochrome P-450 induced in rat liver by phenobarbital [34] and was identical, or structurally-related, to that described by Ryan *et al.* [35] as cytochrome P-450b based on the criteria of molecular weight, N-terminal sequence and the specificity for all substrates investigated so far, named recently P-450 1131 [36]. Cytochrome P-450 UT 50 correspond to the form *i* published by Haniu *et al.* [37] with 50% homology in N-terminal sequence. Cytochrome P-450 MC 2 is identical to P-450c published by Botelho *et al.* [18] with recent nomenclature published by Nebert *et al.* named P-450 IAI [36].

SDS-gel electrophoresis of microsomal and purified cytochromes P-450

From the liver microsomes of rats treated with Phenoclor DP6 were isolated the following forms of cytochrome P-450 with SDS-gel electrophoretic mobilities corresponding to the minimum molecular weights indicated in brackets in terms of daltons: P-450 UT 50 (50,000), P-450 PB 3a (52,000) and P-450 MC 2 (56,500). From the animals fed either the Co diet (not receiving DP6) or the unbalanced diets, two cytochromes P-450 were in each case purified, P-450 UT 50 and P-450 PB 3a.

Reactivity with antibodies

The immunoidentification of the cytochromes P-450 purified from the experimental groups was carried out using cross-reactivity with antibodies which had been raised against gel electrophoretically homogeneous cytochromes P-450 UT 50, PB 3a and MC 2 purified from Aroclor 1254-treated rats. The results indicate that antisera to cytochrome P-450 UT 50 and P-450 PB 3a react with purified enzymes from all experimental groups. Antisera against cytochrome P-450 MC 2 react with P-450 MC 2 from DP6-treated rats and with microsomes from LP group when a high concentration (×10) of this cellular fraction is used. This result is according to the increased ability of 9000 *g* supernatant of livers from rats fed a low protein diet to activate benzo(*a*)pyrene to mutagenic metabolites as previously found [12]. Thus the data reported here show that unbalanced diets did not change the immunospecificity of purified forms of cytochrome P-450.

Catalytic properties of purified cytochrome P-450

Table 1 shows the catalytic activities of cytochrome P-450 isozymes from experimental groups towards several substrates. As expected, ethoxycoumarin,

Table 1. Metabolism of various substrates by cytochrome P-450 isozymes

P-450 isozymes	P-450 UT 50				P-450 PB 3a				P-450 MC 2
Treatment	Co	HL	LP	DP6	Co	HL	LP	DP6	DP6
Substrate	nmol/min/nmol cytochrome P-450								
Benzphetamine	4.12	4.20	2.70*	4.60	84.00	84.20	74.10	77.50	5.40
7-Ethoxycoumarin	0.30	0.35	0.10*	0.45	3.80	4.10	2.80*	4.2	15.20
7-Ethoxyresorufin	0.34	0.37	0.22*	0.38	0.48	0.42	0.40	0.38	8.40
Benzo(*a*)pyrene	0.10	0.10	0.05*	0.25*	0.80	0.90	0.60	0.82	20.10
Zoxazolamine	1.20	1.00	0.80*	1.20	4.00	4.20	4.20	4.50	9.00
Ethylmorphine	21.00	16.00	7.10*	22.00	64.00	63.60	63.50	66.00	ND

Catalytic activities of purified cytochrome P-450 isozymes from rats either fed a control diet (Co) or a high lipid diet (HL) or a low protein diet (LP) or a control diet containing 50 ppm phenoclor DP6 (DP6). Catalytic activity is expressed as nmol of product formed per min per nmol of cytochrome P-450 determined under conditions in which 0.5 nmol cytochrome P-450, 50 units NADPH cytochrome *c* reductase and optimal dilauryl phosphatidyl choline concentrations were used in all experiments. Results with triplicate incubations differed by 20% or less. The effects of the dietary treatments on the catalytic activity of the purified cytochrome P-450 UT 50 and PB 3a were compared using Dunnett's method for multiple comparisons. Activities marked with an asterisk are significantly different from the control values at a level of significance $P < 0.01$.
ND = not detected.

benzo(*a*)pyrene and zoxazolamine are preferentially metabolized by cytochrome P-450 MC 2 but P-450 UT 50 and P-450 PB 3a also shows detectable activity for these substrates. Likewise, the *N*-demethylation of benzphetamine and ethylmorphine is catalyzed most efficiently by cytochrome P-450 PB 3a. As is apparent in Table 1, the catalytic activities of cytochrome P-450 UT 50 and PB 3a are not significantly modified byHL diet. For cytochrome P-450 UT 50 from LP group the metabolism of the various substrates is less efficient than for the same isozyme purified from Co group (from 33 to 67% of decrease). The catalytic activities towards ethoxycoumarin and benzo(*a*)pyrene of cytochrome P-450 PB 3a from LP group is slightly decreased (−25%). Cytochrome P-450 UT 50 from DP6-fed group catalyzes the metabolism of benzo(*a*)pyrene more efficiently than UT 50 isozyme from the Co group. In contrast, the catalytic activities of cytochrome P-450 PB 3a remain unchanged by DP6 induction. Thus the catalytic activities of cytochrome P-450 PB 3a are less affected than cytochrome P-450 UT 50 through nutrition and induction.

Amino acid composition

The effect of nutritional imbalances and DP6 induction on amino acid composition of purified forms of cytochrome P-450 is shown in Table 2. The difference calculated (using the χ^2 as described in Methods) for the various experimental groups compared to the control group was very significant ($P < 0.001$) only for P-450 UT 50 from the LP group,

Table 2. Amino acid composition of the purified forms of rat liver cytochrome P-450

Diet and	P-450 UT 50				P-450 PB 3a				P-450 MC 2
treatment	Co	HL	KP	DP6	Co	HL	LP	DP6	DP6
Amino acid	No. of residues/molecule								
Asx	44	40	40	42	42	42	40	42	47
Thr	22	37	26	26	24	24	28	26	28
Ser	25	25	21	21	22	28	22	26	36
Glx	40	27	17	37	38	37	36	37	46
Pro	13	21	12	24	24	23	23	24	23
Gly	32	33	33	30	40	41	40	42	34
Ala	25	24	23	19	19	17	17	18	30
Val	27	25	31	22	36	30	40	34	34
Met	11	11	11	11	11	11	11	11	11
Ile	25	25	35	22	26	26	30	24	30
Leu	49	50	60	47	54	54	53	56	52
Tyr	17	16	10	16	16	16	14	14	15
Phe	29	30	51	30	32	34	38	33	35
Lys	35	33	30	33	28	28	25	28	28
His	11	12	11	11	14	16	13	14	14
Arg	20	18	17	17	17	17	16	19	22
Trp	1	1	1	1	1	1	1	1	1
Cys	3	3	3	3	5	5	5	5	8
Total	435	438	439	435	448	448	450	450	494

Assay conditions as described in Materials and Methods. Each value represents the data from hydrolyses performed at 24, 48 and 72 hr. Tryptophan and cysteine values were determined separately.

Table 3. N-terminal sequences of purified cytochrome P-450

Cytochrome	Apparent molecular weight	Residue number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
PB 3a	52,000	Met	Glu	Pro	Ser	Ile	Leu	Leu	Leu	Leu	Ala	Leu	Leu	Val	Gly	Phe	Leu	Leu	Leu	Leu	Val	
PB 3a1	52,000	Met	Glu	Pro	Ser	Ile	Leu	Leu	Leu	Leu	Ala	Leu	Leu	Val	Gly	Phe	Leu	Leu	Leu	Leu	Val	
UT 50	50,000	Met	Asp	Leu	Val	Leu	Val	Leu	Ala	Leu	Thr	Leu	Gly	Phe	Val	Ile	Tyr	Tyr	Phe	Val		
UT 501	50,000	Met	Asp	Leu	Ala	Asp	Val	Ala	Ala	Leu	Glu	Leu	Ile	Leu	Leu	Ile	Ile	Leu	Leu	Leu	Gly	
MC 2	57,000	Pro	Ser	Val	Tyr	Gly	Phe	Pro	Ala	Phe	Thr	Ser	Ala	Thr	Glu	Leu	Leu	Leu	Ala	Val	Val	Val

N-terminal sequence analysis were carried out as described in Materials and Methods on purified isozymes of cytochrome P-450 from rats either fed a control diet (UT 50 and PB 3a) or a low protein diet (UT 501 and PB 3a1) or a control diet containing 50 ppm Phenoclor DP6 (MC 2).

with the amino acids valine, isoleucine, leucine and phenylalanine significantly increased and with glutamine and tyrosine decreased. Cytochrome P-450 UT 50 from the Co group contained 40% hydrophobic, 31% polar, 14% negatively charged and 15% positively charged amino acids. In P-450 UT 50 from LP group the level of hydrophobic amino acids was increased to 48% and the polar amino acids decreased to 26%. Moreover, for this isozyme, the positive/negative charged amino acid ratio was 1.07 for the Co group and 1.36 for the LP group. Thus, the LP diet seems to lead to changes in cytochrome P-450 isozyme characteristics. In order to provide more information about this difference the N-terminal sequence of the purified cytochrome P-450s from Co and LP groups were determined. For this comparison isozymes from LP group were named UT 501 and PB 3a1.

N-terminal analysis

As shown in Table 3 the N-terminal sequence was the same for P-450 PB 3a and PB 3a1 whereas the sequence of UT 501 was significantly different compared to UT 50. In this case, 11 amino acids were different from the 20 analyzed. These last results give a confirmation on a difference between isozymes UT 50 and UT 501.

DISCUSSION

Cytochrome P-450 UT 50 (but not cytochrome P-450 PB 3a) was dramatically altered by the low-protein diet with severe decrease in all the catalytic activities tested. In contrast, the characteristics of this isozyme are not affected either by the high-lipid diet or by PCB-induction. The major change consisted of an increase in the content of hydrophobic amino acid residues. However, the cross-reaction with the specific antibodies was not modified. But all other results reported here show that UT 50 and UT 501 are two distinguished proteins. Changes in amino acid composition are particularly interesting because the implication of hydrophobic amino acids as membrane insertion signals in the N-terminal regions [33]. Moreover, these results can partly explain the altered interaction between cytochrome P-450 and cytochrome P-450 reductase from low-protein-fed rats previously reported in reconstituted system assays [14].

Acknowledgements—This work was supported by grant from the MRES and SFB 302. We thank Prof. J. Fothergill and B. Dunbar, Biochemistry Department, University of Aberdeen, U.K. for N-terminal sequence analysis.

REFERENCES

1. Guengerich FP, Dannan CA, Wright ST, Martin MV and Kaminsky LS, Purification and characterization of liver microsomal cytochromes P-450: electrophoretic, spectral, catalytic and immunochemical properties and inducibility of eight isozymes isolated from rats treated with phenobarbital or β-naphtoflavone. *Biochemistry* **21**: 6019–6030, 1982.
2. Conney AH, Pharmacological implications of microsomal enzyme induction. *Pharmacol Rev* **19**: 317–366, 1967.

3. Gelboin HV, Benzo(*a*)pyrene metabolism, activation and carcinogenesis: role and regulation of mixed function oxidases and related enzymes. *Physiol Rev* **60**: 1107–1166, 1980.
4. Jakoby WB, *Enzymatic Basis of Detoxication*. Academic Press, New York, 1980.
5. Lu AYH and West SB, Multiplicity of mammalian microsomal cytochromes P-450. *Pharmacol Rev* **31**: 277–295, 1980.
6. Wolf CR, Cytochrome P-450s: polymorphic multigene families involved in carcinogen activation. *Trends Genet* **2**: 209–214, 1986.
7. Adesnik M and Atchison M, Genes for cytochrome P-450 and their regulation. *CRC Crit Rev Biochem* **19**: 247–305, 1986.
8. Nerurkar M, Campbell TC and Hayes RJ, The reconstitution of hepatic microsomal mixed oxidase activity with fractions derived from weaning rats fed different levels of proteins. *J Nutr* **108**: 678–686.
9. McLean AEM and McLean EK, The effect of diet and 1,1,1,-trichloro 2,2-bis-(*p*-chlorophenyl)ethane (DDT) on microsomal hydroxylation enzymes and on sensibility of rats to carbon tetrachloride poisoning. *Biochem J* **100**: 564–571, 1966.
10. Bodile MUK, Hayes JR and Campbell TC, Effect of protein deficiency on the inducibility of the hepatic microsomal drug-metabolizing enzyme system—II. Effect on enzyme kinetics and electron transport system. *Biochem Pharmacol* **22**: 1125–1132, 1973.
11. Czygan P, Greim H, Garro A, Schaffner F and Popper H, The effect of dietary protein deficiency on the ability of isolated hepatic microsomes to alter the mutagenicity of a primary and a secondary carcinogen. *Cancer Res* **34**: 119–123, 1974.
12. Cassand P, Bonnamour D, Grolier P, Pelissier MA, Amelizad Z, Albrecht R and Narbonne JF, The effect of dietary imbalances on the activation of benzo(*a*)pyrene by the metabolizing enzymes from rat liver. *Mutat Res* **191**: 67–72, 1987.
13. Amelizad Z, Narbonne JF and Oesch F, Nutritional influence on some cytochrome P-450 characteristic. *Biochem Pharmacol* **34**: 383–384, 1985.
14. Amelizad Z, Narbonne JF, Daubèze M, Bonnamour D and Oesch F, Monooxygenase activity of systems reconstituted with fractions from rats fed standard and low protein diets. *Biochem Pharmacol* **35**: 3169–3171, 1986.
15. Van der Hoeven T and Coon MJ, Preparation and proproperties of partially purified cytochrome P-450 and reduced nicotamide adenine dinucleotide phosphate–cytochrome P-450 reductase from rabbit liver microsomes. *J Biol Chem* **249**: 6301–6310, 1974.
16. Imai Y and Sato R, An affinity column method for partial purification of cytochrome P-450 from phenobarbital induced rabbit liver microsomes. *J Biochem* **75**: 689–697, 1974.
17. Suzuki M, Purification and properties of cytochrome P-450 from liver microsomes of rat treated with PB or 3MC. *Kumamoto MED J* **31**: 35–37, 1978.
18. Yasuko CI and Masters BSS, Some properties of a detergent-solubilized NADPH cytochrome *c* (cytochrome P-450) reductase purified by biospecific affinity chromatography. *J Biol Chem* **251**: 5337–5344.
19. Philips AH and Langdon RG, Hepatic triphosphopyridine nucleotide-cytochrome *c* reductase: isolation, characterization and kinetic studies. *J Biol Chem* **237**: 2652–2660, 1962.
20. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes "solubilisation". *J Biol Chem* **239**: 2370–2378.
21. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with folin reagent. *J Biol Chem* **193**: 265–275, 1951.
22. Leammli UK, Cleavage of structural properties during the assembly of the head of bacteriophage T_4. *Nature (Lond.)* **227**: 680–685.
23. Ouchterlony O, Antigene antibody reactions in gels. *Acta Pathol Microbiol* **26**: 507–515, 1949.
24. Hammer CT and Wills ED, The role of lipid components of the diet in the regulation of the fatty acid composition of the rat liver endoplasmic reticulum and lipid peroxidation. *Biochem J* **174**: 585–599.
25. Jacobson M, Levin W, Poppers PJ, Wood AW and Conney AH, Comparison of the o-daxicylation of 7-ethoxycoumarin and hydroxylation of benzo(*a*)pyrene in human placenta (effect of cigarette smoking). *Clin Pharmacol Ther* **16**: 701–710, 1974.
26. Lu AHY, Somogyi A, West S, Kuntzman R and Cooney AH, Pregnenolone-16-carbonitrile: a new type of inducer of drug metabolizing enzyme. *Arch Biochem Biophys* **152**: 457–462, 1972.
27. Tomaszewski JE, Jerina DM, Levin W and Conney AH, A highly sensitive radiometric assay for zoxazolamine hydroxylation by liver microsomal cytochrome P-450 and P-448: Properties of the membrane-bound and purified reconstituted system. *Arch Biochem Biophys* **176**: 788–798, 1976.
28. Depierre JW, Moron MS, Johannesen KAM and Ernster L, A reliable sensitive and convenient radioactive assay for benzopyrene monooxygenase. *Anal Biochem* **63**: 470–484, 1975.
29. Liu TY, Elliot SD and Hayward J, The presence of a group A variant-like antigene in Streptococci of other group with special reference. *Meth Enzymol* **25**: 44–45, 1972.
30. Hirs CHW, Performic acid oxidation. *Meth Enzymol* **11**: 197–203, 1967.
31. Guilloteau P, Sauvant D and Patureau-Mirand P, Methods of comparing amino acid composition of proteins: application to undigested proteins in the preruminant calf. *Ann Nutr Metab* **97**: 457–469, 1983.
32. Dunnett CW, New table for multiple comparisons with a control. *Biometrics* **20**: 482–491, 1964.
33. Botelho LH, Ryan DE and Levin W, Amino acid compositions and partial amino acid sequences of three highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital or 3-methylcholanthrene. *J Biol Chem* **254**: 5635–5640, 1979.
34. Pyerin W, Taniguchi H, Horn F, Oesch F, Amelizad Z, Friedberg T and Wolf CR, Isozyme-specific phosphorylation of cytochromes P-450 and other drug metabolizing enzymes. *Biochem Biophys Res Commun* **142**: 885–892, 1987.
35. Ryan DE, Thomas PE, Korzeniowski D and Levin W, Separation and characterization of highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital and 3-methylcholanthrene. *J Biol Chem* **254**: 1365–1374, 1979.
36. Nebert DW, Adesnik M, Coon MJ, Estabrook RW, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Kemper B, Levin W, Phillips IR, Sato R and Waterman MR, The P-450 gene superfamily: recommended nomenclature. *DNA* **6**: 1–13, 1987.
37. Haniu M, Ryan DE, Iida S, Lieber Cs, Levin W and Shively JE, NH_2-terminal sequence analysis of four rat hepatic microsomal cytochromes P-450. *Arch Biochem Biophys* **235**: 304–311, 1984.
38. Botelho LH, Ryan DE, Yuan PM, Kutny R, Shively JE and Levin W, Amino-terminal and carboxy-terminal sequence of hepatic microsomal cytochrome P-450d, a unique hemoprotein from rats treated with isosafrole. *Biochemistry* **21**: 1152–1155, 1982.