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KINETICS OF *IN VITRO* *O*-DEETHYLATION OF PHENACETIN AND 7-ETHOXYCOUMARIN BY RAT INTESTINAL MUCOSAL CELLS AND MICROSOMES

THE EFFECT OF INDUCTION WITH 3-METHYLCHOLANTHRENE AND INHIBITION WITH α-NAPHTOFLAVONE

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Abstract—A novel, sensitive (0.5 ng) assay for acetaminophen, using HPLC with selective electrochemical detection, enabled us to study rat small intestinal *O*-deethylation of phenacetin and compare it with corresponding 7-ethoxycoumarin-*O*-deethylation. Two *in vitro* systems, i.e. isolated intestinal mucosal cells and microsomal fractions thereof, were used to study kinetics for the *O*-deethylation of both substrates. K_m^{app}- and V_{max}-values are similar for 7-ethoxycoumarin- and phenacetin-*O*-deethylation. Apparent K_m-values varied between 50 and 70 μM in control rats and decreased after 3-methylcholanthrene pretreatment to 20–45 μM. V_{max}-values were increased by 3-methylcholanthrene pretreatment. *O*-Deethylation was inhibited equally in cells and microsomes by α-naphtoflavone, but is inhibited more markedly in intestinal preparations after pretreatment with 3-methylcholanthrene. It is suggested that 7-ethoxycoumarin and phenacetin are *O*-deethylated by different forms of cytochrome P-450 with almost identical K_m^{app} and that these enzymes have a different distribution along the villus.

The multiplicity of the cytochrome P-450 system is well established [1]. Different forms or populations of cytochrome P-450 vary in substrate specificity [2] and in their response to inducers [3] and inhibitors [4]. Although the different forms of cytochrome P-450 do vary in substrate specificity, there is considerable overlap [5], resulting in biphasic Michaelis–Menten kinetics. Substrates showing biphasic kinetics include benzo[*a*]pyrene [6], aniline [7], ethylmorphine [8], aminopyrine [9], acetanilide [10] and 7-ethoxycoumarin [11].

Recently it was shown by Boobis *et al.* [12] in human and rat liver microsomes that 7-ethoxycoumarin- and phenacetin-*O*-deethylation were better described by a biphasic plot than by monophasic kinetics. *In vitro*-inhibition by α-naphtoflavone and metyrapone further supported their hypothesis that the two phases of *O*-deethylase activity represent two different populations of cytochrome P-450.

Also in the gastrointestinal tract the cytochrome P-450 system has been extensively described and reviewed [13]. Referring to the inhibition studies in the liver, it seems likely that the small intestine exhibits a similar heterogeneity of cytochrome P-450. Thus, a high inhibition by α-naphtoflavone may indicate the participation of cytochrome P-448 induced by polycyclic hydrocarbons, whereas metyrapone-inhibition points to the involvement of a cytochrome P-450 responding to phenobarbital pretreatment [14]. The gastrointestinal monooxygenase system is highly adaptive to exogeneous inducers [15] and can reach liver activity on the basis of microsomal protein [13]. It was previously shown in our department by Klippert *et al.* [16] that in 3-methylcholanthrene pretreated rats some 53% of orally administered phenacetin was metabolized by the gut wall. Moreover, we were able to predict the intestinal oxidative metabolism of phenacetin from *in vitro* data obtained with isolated intestinal mucosal cells [17].

It was the purpose of this study to characterize phenacetin-*O*-deethylation by studying its enzyme kinetic properties and the influence of induction and inhibition of cytochrome P-450 (448). In this respect phenacetin was compared with 7-ethoxycoumarin as a known substrate of the intestinal cytochrome P-450 system [2]. *In vitro*-systems isolated intestinal mucosal cells and microsomal fractions thereof were used.

MATERIALS AND METHODS

Chemicals. NADP, glucose-6-phosphate (disodium salt), glucose-6-phosphate dehydrogenase (grade I) and 7-ethoxycoumarin (no. 407097) were purchased from Boehringer Mannheim GmbH. Hydroxycoumarin (Umbelliferon, no. 93979) and 3-methylcholanthrene (20), no. 66230, were products of Fluka AG, Buchs SG. β-Glucuronidase/arylsulphatase (type H_2) was obtained from Sigma. Acetaminophen (paracetamol) and phenacetin were obtained from Brocacef BV, Maarssen, The Neth-

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† Abbreviations used: ANF, α-naphtoflavone; 7-EC, 7-ethoxycoumarin; 7-HC, 7-hydroxycoumarin; 3-MC, 3-methylcholanthrene.

erlands. The last two products were recrystallized from ethanol–water prior to use. *N*-Butyroyl-*p*-aminophenol (no. 1887, K & K) was purchased from ICN Pharmaceuticals Inc., New York. All other chemicals and solvents were of analytical grade purity.

Animals and pretreatment. Adult male Wistar rats (Cpb: WU, TNO Zeist, The Netherlands) weighing approximately 250 g were used. Animals were administered 1.0 ml corn oil or 3-methylcholanthrene (3-MC), 20 mg/kg body weight, in 1.0 ml cornoil by stomach tube, 24 hr before preparation of intestinal mucosa cells and/or microsomes. Before and after pretreatment rats were allowed free access to tap-water and a commercially available diet (RMH-B, Hope Farms, Woerden, The Netherlands).

Isolation of mucosal cells and preparation of microsomes. After anaesthesia of the rat with ether, the small intestine was excised, flushed free of intestinal contents with ice-cold saline and intestinal mucosal cells were prepared by vibration of everted intestinal segments in EDTA-containing buffered saline, pH 7.4, as we described previously [17, 18]. Microsomes were prepared from these cells immediately after isolation [18]. Cell viability was measured by leakage of lactate dehydrogenase from the cell cytoplasm as described previously [18]. When kept on ice, cells showed viabilities between 80 and 90% at least 2 hours after isolation. During the 30 min incubation at 37° no significant loss of viability was detected.

Incubations. Microsomes or cells obtained from 0.25 g intestine were used per incubation vessel in a total volume of 3.0 ml. After a preincubation of 5 min at 37°, the reaction was started by the addition of 0.5 ml substrate in buffer/methanolic solution (final stubstrate concentration between 2 and 200 μM, final MeOH concentration 1.2%, v/v). Incubations were performed at 37° and stopped after 10 and 30 min for microsomes and cells respectively. Free *O*-deethylated substrate was measured immediately. Conjugated product was determined by a 48 hr incubation of 1.5 ml aqueous supernatant after chloroform-extraction with 1.5 ml acetate buffer (0.1 M, pH 5) containing 6000 FU β-glucuronidase/arylsulphatase and determination of the released phenolic aglycons. For inhibitor studies, α-naphtoflavone was dissolved in methanol and 30 μl added to incubations (final concentration 1–50 μM ANF), control incubations receiving solvent alone. α-Naphtoflavone (50 μM) had no effect on cell viability during 30 min incubation at 37° as compared with controls. The *O*-deethylation of 7-EC was assayed according to Greenlee and Poland [11].

Phenacetin-*O*-deethylase activity was determined

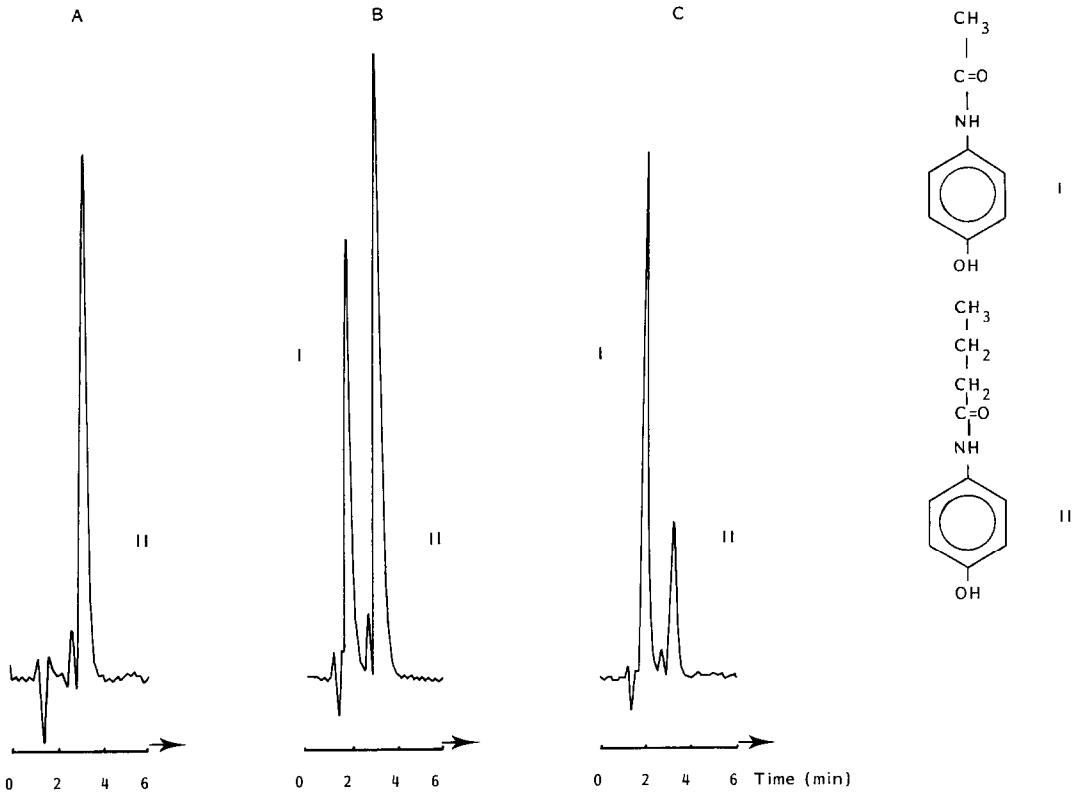

Fig. 1. Typical chromatograms of microsomal incubations with phenacetin of control and 3-methylcholanthrene pretreated rats, showing the electrochemical responses of acetaminophen (I, retention time 1.80 min) and *N*-butyroyl-*p*-aminophenol (II, 3.10 min) as internal standard (1 ng/μl injected volume). (A) Controls, 10 μM phenacetin (substrate concentration). (B) 3-MC, 10 μM phenacetin. (C) 3-MC, 200 μM phenacetin. Microsomal incubations were performed as described in the text. Injected volume was 100 μl for (A) and (B) and 50 μl for (C).

as the amount of acetaminophen formed with a novel assay, using High Pressure Liquid Chromatography (HPLC) with electrochemical detection (ECD). Incubations were stopped by putting the incubation vessels on ice and adding 0.32 ml 0.5 N NaOH, bringing the pH to 12. *N*-Butyroyl-*p*-aminophenol (Fig. 1) was added as an internal standard in the desired concentration range. Excess phenacetin and interfering substances from cells and/or microsomes were extracted into 10 ml dichloromethane (20 min). After centrifugation (5 min, 3000 rpm) 2.0 ml of the aqueous layer was transferred to a clean tube, 0.30 ml 0.5 N HCl was added (pH 5.0), and the acetaminophen extracted into 20.0 ml diethylether (20 min). 18.0 ml of the ethereal layer, after centrifugation, was reduced to dryness in a waterbath at 45° and dissolved in 500 μl mobile phase.

HPLC using electrochemical detection was performed using a 6000 A solvent delivery system and WISP 710 B automatic injection system from Waters Associated. The chromatographic column (30 × 0.46 cm, Lichrosorb 10 RP 18, Chrompack) was used at a head pressure of 3000 psi at a flow rate of 2.0 ml/min. The mobile phase consisted of a mixture of methanol:H_2O (40:60, v/v) brought to pH 2.5 with H_3PO_4. The detection system consisted of a self-constructed three-electrode potentiostatic wall-jet system as a modification of the Metrohm EA 1096 cell. The detector utilizes glassy carbon electrodes and a Ag/AgCl-electrode (Metrohm EA 442, KCl 3 mol/l) for reference. Peaks were monitored at a potential of 800 mV. Peak heights and retention times were measured electronically on a Waters Data Module. Injection volume was between 25 and 100 μl. Retention times for acetaminophen and the internal standard were 1.80 and 3.10 min, respectively (Fig. 1). The assay has a detection limit of 0.5 ng (S/N = 3) and a high reproducibility (S.D. 2–3%, 20 ng injected, 20 samples).

Measurement of cytochrome P-450 and microsomal recovery. Cytochrome P-450 was estimated by means of a dithionite difference spectrum [19] (ε = 100 mM^{-1} cm^{-1}) using an Aminco DW-1 UV-Vis spectrophotometer in the split beam mode.

Microsomal recovery was determined as described by Shirkey *et al.* [20], using the distribution of the marker enzymes succinic dehydrogenase [21], arylesterase [22] and lactate dehydrogenase [23] in all subcellular fractions. Observed activities were corrected for protein content, resulting in a microsomal recovery of 45%.

Experimental design. The experiment was designed as a three factorial (control vs 24 hr 3-MC pretreatment, cells vs microsomes and 7-ethoxycoumarin- vs phenacetin-*O*-deethylation) in three replicates. Cells or microsomes from two rats per treatment were pooled. All grouped data are presented as the mean ± S.E.M. (standard error of the mean), unless stated otherwise. K_m- and V_{max}-values were obtained initially by graphical analysis of Eadie–Hofstee plots. The values thus obtained were used as first estimates in an iterative programme based on non-linear least squares regression analysis (Duggleby [24]) to fit the equation: $V = V_{max}/(1 + K_m/[S])$.

RESULTS

Both 7-EC and phenacetin were *O*-deethylated by rat intestinal mucosal cells and microsomes. A typical chromatogram obtained from microsomal incubations with phenacetin is shown in Fig. 1. Both after induction and in control rats linear Eadie–Hofstee plots were obtained for the deethylation of 7-EC and phenacetin (Fig. 2A), in contrast to liver microsomes where non-linear plots were observed (Fig. 2B) as found previously by Boobis *et al.* [12].

Michaelis–Menten parameters were calculated in

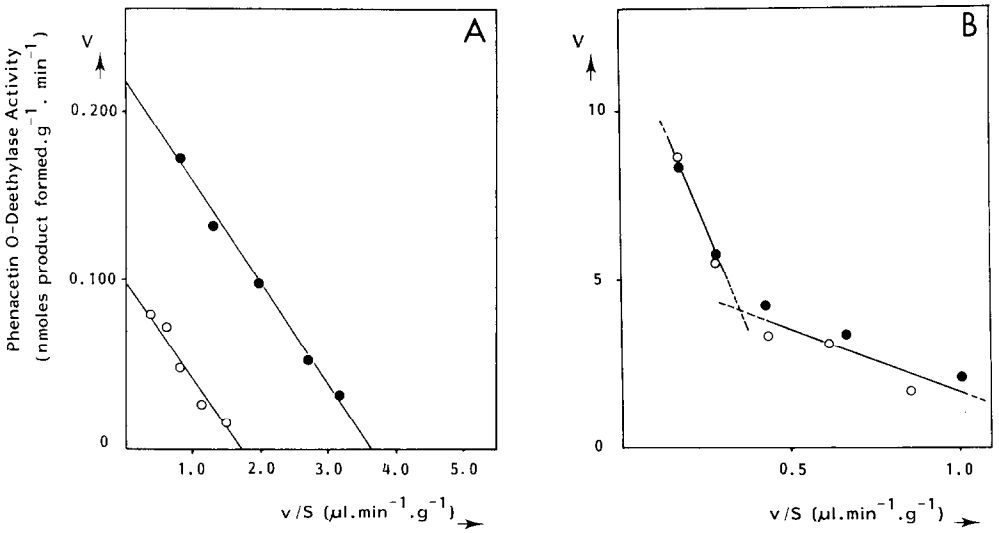

Fig. 2. Eadie–Hofstee plots for phenacetin-*O*-deethylase activity of (A) control rat intestinal cells (●) and microsomes (○); the values of one typical experiment, using cells or microsomes pooled from four animals are shown. Each value is the mean of three incubations. In at least two other experiments similar plots were obtained. (B) Control rat liver microsomal preparations; the values of two experiments, each using microsomal batches from two pooled livers, are shown (●, ○). Each value is the mean of 3 incubations. Note the different scale!

Table 1. Values for the Michaelis–Menten parameters of 7-ethoxycoumarin-*O*-deethylase and phenacetin-*O*-deethylase activities in mucosal cells and intestinal microsomes of control and 3-MC pretreated rats

Pretreatment	*In vitro*-method	7-Ethoxycoumarin K_m*	7-Ethoxycoumarin V_{max}†	Phenacetin K_m*	Phenacetin V_{max}†
Oil (24 hr)	Microsomes	47.7 ± 10.6	0.488 ± 0.04	74.2 ± 10.4	0.242 ± 0.04
	Cells	60.6 ± 4.6	0.215 ± 0.01	57.3 ± 3.4	0.163 ± 0.08
3-MC (24 hr)	Microsomes	21.7 ± 3.0	2.52 ± 0.12	46.3 ± 4.2	1.44 ± 0.20
	Cells	35.0 ± 3.9	2.54 ± 0.08	34.3 ± 4.2	4.20 ± 0.50

* K_m values in μM.

† V_{max} in nmoles (min·g intestine)$^{-1}$. Maximal velocity in microsomes was corrected for a recovery of 45% (Materials and Methods). The averaged values ± S.E.M. of two or three different experiments with different batches of cells or microsomes are shown.

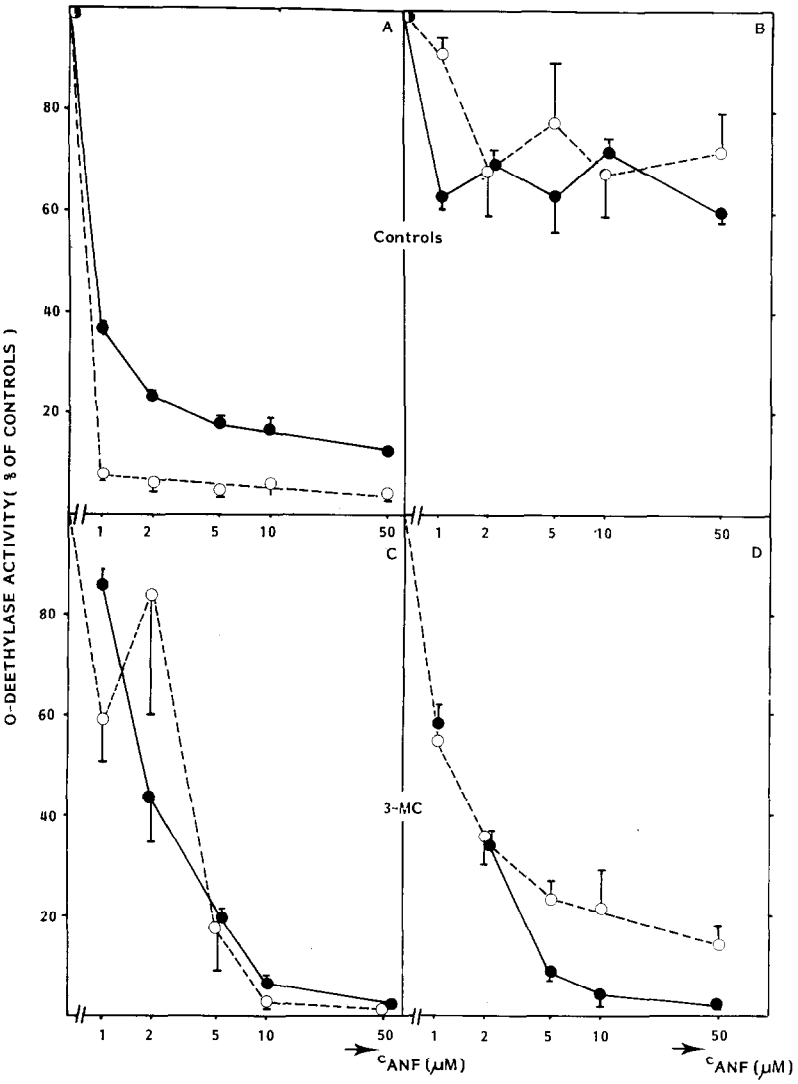

Fig. 3. Effect of α-naphtoflavone (ANF) on 7-ethoxycoumarin-*O*-deethylase (A, C) and phenacetin-*O*-deethylase (B, D) activity in intestinal mucosal cells of control (A, B) and 3-MC pretreated (C, D) rats. Incubations were performed at 5 (○) and 200 μM (●) substrate concentrations, as described in the text. The results are the mean of duplicate experiments with two different cell batches of two rats each. Bars indicate the standard error of the mean (S.E.M., $n = 4$). If bars are not indicated S.E.M. was smaller than the symbol used.

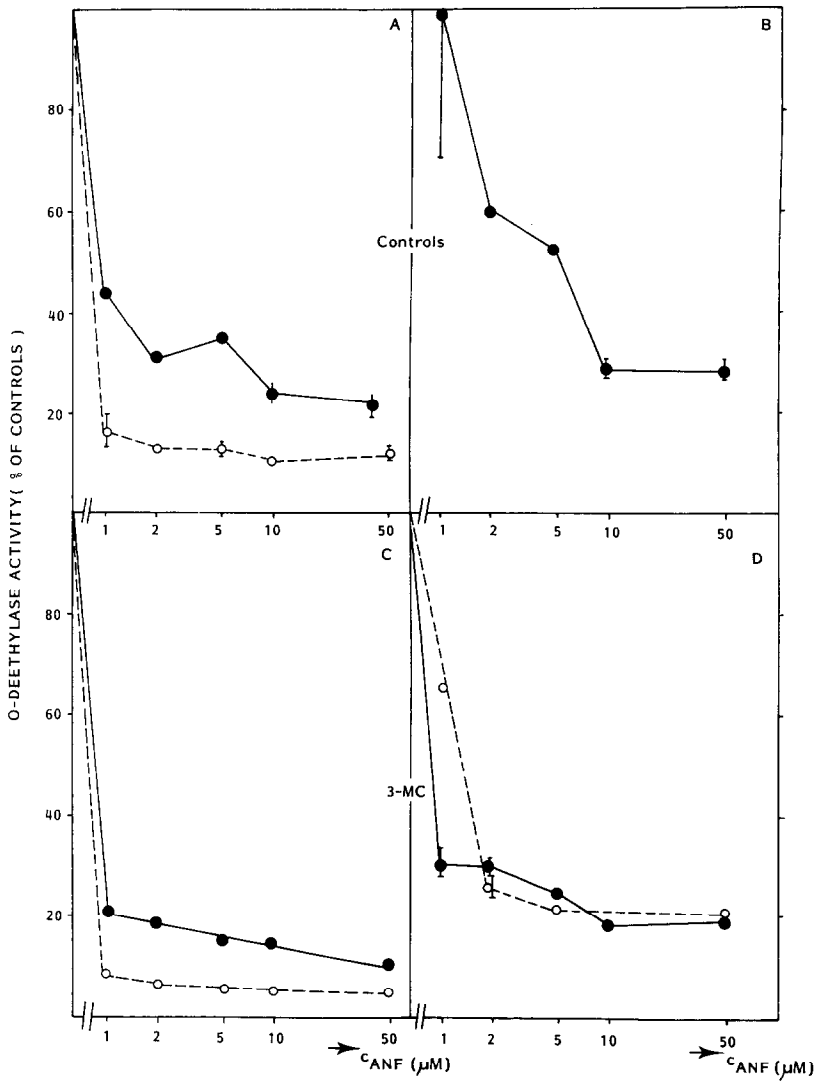

Fig. 4. Effect of α-naphtoflavone (ANF) on 7-ethoxycoumarin-*O*-deethylase (A, C) and phenacetin-*O*-deethylase (B, D) activity in intestinal microsomes of control (A, B) and 3-MC pretreated (C, D) rats. Incubations were performed at 5 (○) and 200 μM (●) substrate concentrations as described in the text. Results are the mean of duplicate experiments with two different microsomal batches of four rats each. Bars indicate the S.E.M. If bars are not indicated S.E.M. was smaller than the symbol used.

control and 3-MC pretreated rats. Results are shown in Table 1. Apparent K_m values in cells did not differ significantly from microsomes ($P > 0.05$, analysis of variances). The affinity of the monooxygenase system is increased for both substrates after pretreatment of rats with 3-MC: all K_m-values are lowered significantly ($P < 0.01$ and $P < 0.05$ for 7-EC and phenacetin respectively, Student's *t*-test for paired samples).

V_{max}-values for both dealkylations are increased significantly ($P < 0.01$, analysis of variance) but differ depending upon the substrate. In control cells the V_{max} values for 7-EC were lower as compared with microsomal deethylation, after correction for 45% recovery. After 3-MC pretreatment cells metabolized phenacetin faster (V_{max}) than microsomes but for 7-EC no difference was observed between cells and microsomes.

Additional proof that *O*-deethylation kinetics are not biphasic was obtained by studying the possible selective effect of an *in vitro*-modifier of monooxygenase (P-448) activity, α-naphtoflavone (ANF). ANF (and metyrapone) was used by Boobis *et al.* [12] to distinguish between the two different phases in 7-EC- and phenacetin-*O*-deethylation. Incubations were performed at 5 and 200 μM substrate concentration to distinguish between the two possible components with different affinity for the substrates. The effects of ANF on phenacetin- and 7-EC-*O*-deethylation in control and 3-MC pretreated rats are presented in Figs. 3 and 4 for cells and microsomes, respectively.

No marked differences were observed between the effect of ANF on 7-EC-*O*-deethylation at the two substrate concentrations in control or 3-MC pretreated cells and/or microsomes. In a particular treatment it should be noted, however, that at nearly all ANF-concentrations percentual inhibition of 7-

EC-*O*-deethylase in both *in vitro* systems at 5 μM is significantly greater ($P < 0.01$, paired Student's *t*-test) than at the higher (200 μM) substrate concentration. Fifty per cent inhibition in control cells and microsomes already occurred below 1 μM ANF-concentration (Figs. 3A, 4A). After 3-MC pretreatment IC_{50} is increased in cells to about 2 μM (Fig. 3C) while in microsomes no change in IC_{50} was observed (Fig. 4C). In microsomes after 3-MC pretreatment inhibition at the same substrate concentration is significantly stronger at all data points ($P < 0.05$, paired Student's *t*-test), consistent with the specific P-448 enhancement by 3-MC and a specific inhibition of P-448 by ANF. In cells, however, an increased inhibition by 3-MC pretreatment is only observed at ANF-concentrations above 5 μM reaching 100% at 50 μM ANF, while in control cells complete inhibition at 200 μM 7-EC never is attained.

Also for phenacetin no differential inhibition for the two substrate concentrations was observed. In contrast with 7-EC-*O*-deethylation, however, no more than 40% inhibition was reached with ANF concentrations up to 50 μM in control cells incubations (Fig. 3B). In control intestinal microsomes phenacetin-*O*-deethylation at 5 μM was too low (< 10 pmol min^{-1} g $intestine^{-1}$) even without ANF to quantitatively study ANF-inhibition at this substrate concentration. At 200 μM phenacetin concentration (Fig. 4B) 50% inhibition is attained at about 5 μM ANF, and maximal inhibition is greater (70%) then in corresponding cell incubations. After pretreatment of rats with 3-MC, phenacetin-*O*-deethylase was inhibited more strongly in cells (Fig. 3D, 80–100% inhibition at 50 μM ANF) as well as microsomes (Fig. 4D, 80% inhibition at 50 μM ANF).

In Fig. 5, the effect of phenacetin on 7-EC-*O*-deethylation in control microsomes is presented graphically in an Eadie–Hofstee plot. Phenacetin appears to be a competitive inhibitor of 7-EC-*O*-deethylase in microsomes from control as well as from 3-MC (data not shown) pretreated rats. A Dixon plot (K_m^{app}/V_{max} vs concentration of inhibitor) was used to determine the K_i-values to be 90 and 61 μM in control and 3-MC pretreated microsomes, respectively.

DISCUSSION

In this study rat intestinal mucosal cells and microsomal fractions thereof *O*-deethylate phenacetin and 7-EC according to simple monophasic Michaelis–Menten kinetics. Biphasic deethylation was previously reported for mouse [11], rat [12, 25, 26, 27] and human [12] liver for 7-EC and in human and rat liver [12] for phenacetin. There is good evidence from these studies that the two phases of *O*-deethylase activity represent different forms or populations of cytochrome P-450.

However, using enzyme kinetic data in this study we could not distinguish between possible different components responsible for 7-EC- and phenacetin-*O*-deethylation.

Kinetic parameters for 7-EC-*O*-deethylation (Table 1) are well in agreement with K_m- and V_{max}-values of 62 μM and 0.123 nmoles/min mg

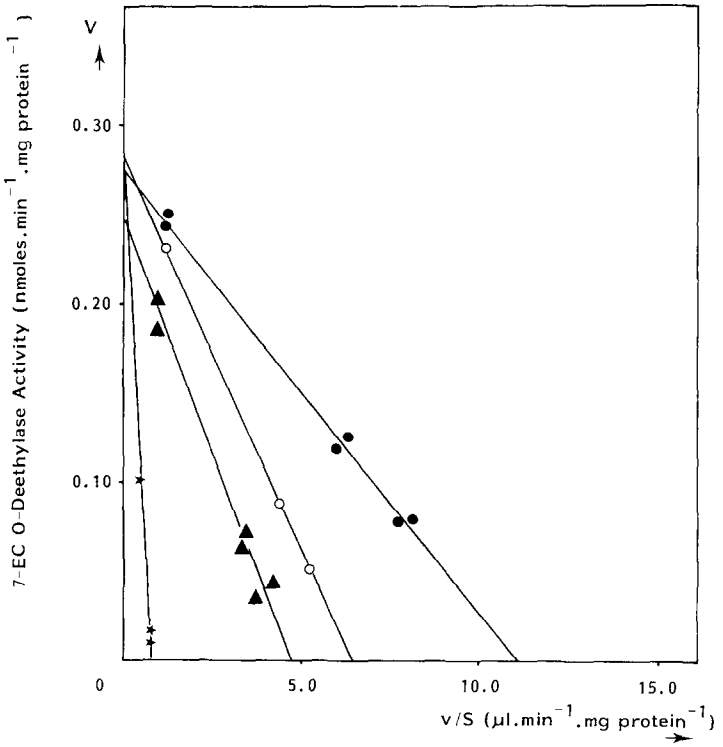

Fig. 5. Eadie–Hofstee plots for 7-ethoxycoumarin-*O*-deethylase in control rat intestinal microsomes (●) and its competitive inhibition by phenacetin *in vitro* at concentrations of 50 μM (○), 100 μM (▲) and 1 mM (*).

microsomal protein found by Shirkey *et al.* [28]. Using our average protein content (see [18]) of 2–3 mg protein/g intestine, we find a microsomal V_{max} of 0.10–0.15 nmoles/min·mg microsomal protein. A five-fold enhancement of 7-EC-*O*-deethylation was observed after 3-MC pretreatment. This is in agreement with a 390 ± 180% increase of 7-EC-*O*-deethylation in intestinal microsomes found by Shirkey *et al.* [28].

A comparison between apparent K_m values of cells and microsomes revealed no significant differences (Results). V_{max}-values, if corrected for 45% microsomal recovery, differed between cells and microsomes. Surprisingly, these differences depend upon the nature of the substrate. In control rats the microsomal V_{max} considerably exceeds the V_{max} for cells for 7-EC-*O*-deethylation, whereas 24 hr after 3-MC pretreatment no difference is apparent. For phenacetin deethylation, on the other hand, a V_{max} difference is observed only in 3-MC treated rats. Here the cells appear to be more active, whereas no difference could be demonstrated in controls.

This observation indicates that, most likely, the homogenisation procedure is not only incomplete (45% microsomal protein recovery) but also shows a preference for cells with a higher 7-EC-*O*-deethylation capacity in control cells: most likely to be villus tip cells [29]. In control rats, it appears that the distribution of 7-EC and phenacetin deethylation over the villus differs somewhat. This difference is more pronounced in 3-MC treated rats. Apparently induction of 7-EC deethylation is more prominent in cells remote from the villus tip. This is even more apparent for phenacetin deethylation, because the (villus tip) microsomes appear to *O*-deethylate phenacetin more slowly than the entire cell population. In Fig. 6, a schematical representation of this hypothesis is given. Observations in previous publications on the comparison of cells and microsomes [18] and different cell preparations [30] led to the same conclusion: 3-MC induction is mainly localized at cells on the lower part of the villus.

In vitro inhibition by ANF was used previously to differentiate between two distinct types of benzo[*a*]pyrene-monooxygenase [31], 7-EC-*O*-deethylase [12] and phenacetin-*O*-deethylase [12] in rat liver microsomes. No such a cytochrome P-450 pattern responsible for the studied deethylation reactions was observed in rat intestinal cells and/or microsomes (Figs. 3 and 4). We did find that ANF inhibits *O*-deethylation more markedly in cells and microsomes of 3-MC pretreated rats than in control preparations. This was found previously for benzo[*a*]pyrene [32] and 7-EC [28] in rat liver and intestinal microsomes, respectively. The different inhibition patterns of ANF on the deethylation of 7-EC and phenacetin in cells (Figs. 3A, B) and, albeit less pronounced, in microsomes (Figs. 4A, B) suggest there might be a difference in populations of cytochrome P-450 responsible for their metabolism, as suggested before [12] for rat liver. IC_{50}s for ANF-inhibition of both deethylations tend to be higher in cells as compared with microsomal fractions thereof. There are a number of physicochemical factors such as solubility, diffusion barriers and non-specific binding that can explain such a difference. Inhibition with ANF is consistent with data found by Grafström and Stohs [33] who examined the influence of various inhibitors on ethoxyresorufin and 7-EC metabolism (1 mM substrate concentration) in rat small intestinal cells and microsomes. They also observed similar effects in both *in vitro* systems. ANF concentrations necessary to obtain 50% inhibition of ethoxyresorufin deethylase activity varied from 0.05 μM in control and

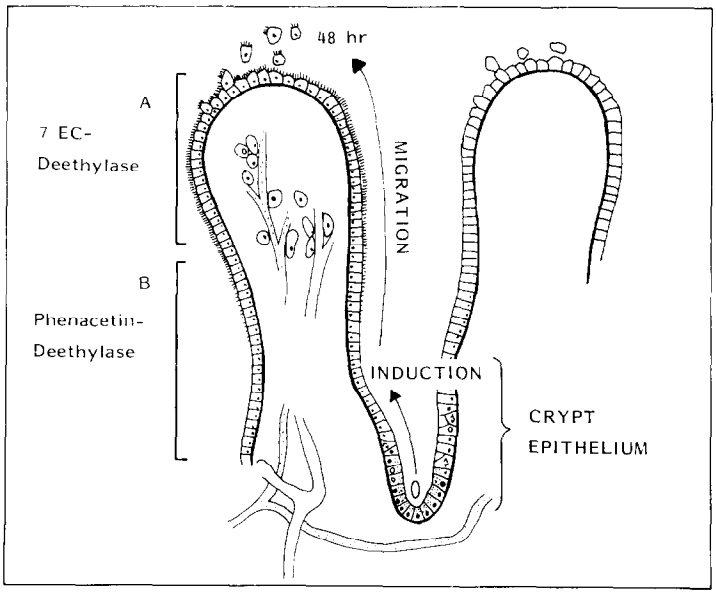

Fig. 6. Schematical drawing of hypothetical localization of two cytochrome P-450 mediated *O*-deethylase activities along the intestinal villus. 7-EC-*O*-deethylase is mainly localized in the older, mature villus tip cells (A), which are readily homogenized. Phenacetin-*O*-deethylase is localized in the migrating and dividing cells of the side-walls and crypts (B), where induction of the monooxygenase system is supposed to take place.

3-MC pretreated microsomes to about 2 μM in whole cells from 3-MC treated rats. 7-EC-*O*-deethylase in microsomes was inhibited by ANF in a manner similar to ethoxyresorufin deethylase. At 10 μM ANF deethylase-activity was completely abolished.

On the other hand ANF has been previously shown to stimulate benzo[*a*]pyrene metabolism in intestinal microsomes and cells [34, 35] from control rats and inhibit its disposition in 3-MC treated specimens. These differences in response for benzo[*a*]pyrene, ethoxyresorufin and now 7-EC and phenacetin indicate that in the intestinal mucosa different forms of cytochrome P-450 may be present. It was previously proposed that the 3-methylcholanthrene inducible form of cytochrome P-450 (P-448) might be the only form present in the small intestine of the rat [36]. However, other investigators using intestinal microsomes [34] and isolated mucosal cells [37] observed significant differences between control and 3-MC induced forms of cytochrome P-450.

This study presents evidence that in control as well as in 3-MC pretreated rats different cytochrome P-450 subspecies are present in the intestinal mucosa. This is based primarily on the hypothesis that the relative quantity of these different forms of cytochrome P-450 is not distributed uniformly along the length of the villus in control rats (Fig. 6). The latter is supported by the findings of Porter *et al.* [38] studying in detail the villus-to-crypt gradient of aryl hydrocarbon hydroxylase (AHH) using 10 sequentially isolated cell fractions. AHH-activity (a known parameter for cytochrome P-448, [1]) was highest in the midvillus region (40% lower at the villus tip), while cytochrome P-450 is well known from other studies [29] to be highest in the villus tip.

In addition, inhibition data with ANF in control and 3-MC pretreated animals suggest that the *O*-deethylation of both 7-EC and phenacetin is catalysed by more than one form of cytochrome P-450. Also phenacetin was a competitive inhibitor of the *O*-deethylation of 7-EC with a K_i very similar to its K_m. This would suggest that either the major form of cytochrome P-450 metabolizing phenacetin is the same as that metabolising 7-EC or two different forms are apparent, having almost identical K_m-values. No definite decision can be made with present data. If overlapping is 90% or more, also monophasic Eadie–Hofstee plots will be obtained in control rats. However, inhibition data with ANF suggest that in 3-MC pretreated rats anomalies in the Eadie–Hofstee plots could be expected. In view of the monophasic nature of the kinetics of the two enzyme reactions both in control and 3-MC pretreated rats, it seems more likely that two different forms of cytochrome P-450 have almost identical K_ms.

More studies comparing two or more xenobiotics with various inducers are necessary to discern the different localization of different cytochrome P-450 subspecies in intestinal mucosa and its relevance in the biotransformation process.

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