amic acids were not inhibitory. The lack of inhibition of *R. pilimanae* urease by avidin suggests that a biotin containing enzyme[11] is not involved in the hydrolysis of urea in *R. pilimanae*.

Prior studies on the inhibition of urease were based on experiments using primary hydroxamic acids and a few cyclic hydroxamic acids have rigid conformations[5,7]. Observations made here with acyclic-secondary hydroxamic acids indicate that only primary hydroxamic acids sufficiently resemble urea to interact with urease to form an enzymatically inactive complex. Because not all hydroxamic acids are inhibitory, the inhibition by hydroxamic acids is not due simply to their chelating properties. The inhibition of urease by primary hydroxamic acids could be due to the formation of an inactive acyl-enzyme which is an analog of the normal carbamyl-enzyme intermediate formed from urea[12] or inhibition may be due to the complexing of the active site nickel[13] by a hydroxamic acid acting as a substrate analog. These latter proposals provide explanations for the lack of urease inhibition or nickel removal by dimethylglyoxime[14].

Hydroxamic acids are used or have been proposed for a number of clinical applications. The observations made here permit the rational design of drugs that possess the chelating properties of hydroxamic acids, which are useful for the alleviation of chronic iron toxicity[15], but will not inhibit ureases present in intestinal microbes. Also the chemical restrictions on the design of urease inhibitors, to prevent ammoniurea[16] or calculus deposition on dental surfaces[17], for example, have been more rigorously defined.

Table 2. Inhibition of jack bean and *Rhodotorula pilimanae* ureases with hydroxamic acids[a]

Additive	Concentration (M)	Relative activity of ureases from	
		Jack bean	*R. pilimanae*
None	-	100	100
Avidin	b	97	89
Rhodotorulic acid	1.1×10^{-5}	101	94
	1.1×10^{-3}	98	ND
	1.1×10^{-2}	97	101
Acetohydroxamic acid	1.7×10^{-6}	68	ND
	1.7×10^{-5}	21	23
	1.7×10^{-4}	4	10
Glycine hydroxamic acid	2.5×10^{-6}	73	ND
	2.5×10^{-5}	44	52
Propiono-hydroxamic acid	6.2×10^{-5}	15	21
N-Methylaceto-hydroxamic acid	4.9×10^{-5}	103	ND
	4.9×10^{-3}	99	97
N-Butylaceto-hydroxamic acid	7.1×10^{-6}	99	ND
	7.1×10^{-3}	96	100

[a] The effect of hydroxamic acids and avidin on ureases prepared from jack bean powder and *R. pilimanae* using the procedures of Kobashi et al.[6] *R. pilimanae* extracts contained 3.5 mg/ml of protein. The data represents an average of 2 separate determinations. ND indicates data not determined. [b] Sufficient avidin to bind 1 mg of biotin per mg of protein was incubated for 30 min prior to assay with the ureases.

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Induction of nuclear styrene monooxygenase and epoxide hydrolase in rat liver[1]

E. Garattini, G. Gazzotti and M. Salmona[2]

Laboratory for Enzyme Research, Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea, 62, I-20157 Milano (Italy),
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Summary. The apparent K_m and V_{max} of styrene monooxygenase and styrene epoxide hydrolase were determined in intact nuclear preparations from male rat liver after in vivo treatment with phenobarbital and β-naphthoflavone, which are known to induce microsomal cytochrome P-450 and cytochrome P-448 respectively. Treatment with phenobarbital does not alter the apparent K_m, but greatly increases the V_{max} of both nuclear styrene monooxygenase and styrene epoxide hydrolase. Almost the same pattern is observed for styrene monooxygenase after treatment with β-naphthoflavone, whereas the same treatment slightly increases both the V_{max} and K_m value of styrene epoxide hydrolase.

The presence of a metabolizing system capable of both activating and inactivating potentially carcinogenic and mutagenic substances in the endoplasmic reticulum of rat liver has long been known[3], whereas this system has only very recently been found in other cellular fractions. Its presence in relatively high concentrations has been demonstrated in plasma membrane, Golgi apparatus, and nuclear membrane[4]. Particular attention has been paid to the nuclear membrane localization[5-11] because of its spatial proximity to the genomic material which, at the moment, is considered the most likely terminal target for chemically induced mutagenic and carcinogenic effect[12-14].

A gas-chromatographic method for the simultaneous determination of styrene monooxygenase and styrene epoxide

Kinetic parameters of nuclear styrene monooxygenase and styrene epoxide hydrolase in rats after pretreatment with phenobarbital (PB) and β-naphtoflavone (β-NF)

	Apparent K_m (μM)		V_{max} (pmol/min/mg prot)		V_{max} hydrolase / monooxygenase
	Styrene monooxygenase	Styrene epoxide hydrolase	Styrene monooxygenase	Styrene epoxide hydrolase	
Control	169±39	18.8±6.6	123.2±3.6 (100)	367.6±29.1 (100)	2.98
PB	139±24	15.8±2.6	378.8±9.1 (308)*	900.3±27.0 (245)*	2.37
Control	151.8±35	11.5±1.6	130.2±18.5 (100)	393.1±11.7 (100)	3.01
β-NF	133.7±25	19.3±2.1*	297.6±25.6 (228)*	462.3±18.7 (117)*	1.55

* $p < 0.001$ in respect to controls with Student's t-test. The numbers in brackets represent the percentage of induction.

hydrolase enzymatic activities in intact nuclear preparations has been set up in our laboratory[15] and an initial characterization of the two enzymes has already been reported[16]. This paper presents the kinetic parameters of styrene monooxygenase and styrene epoxide hydrolase in rats after treatment with phenobarbital and β-naphthoflavone.

Material and methods. Male CD-COBS rats weighing 150–200 g were purchased from Charles River Italy, Calco (Co). The animals were injected i.p. with 2 daily doses of phenobarbital (Merck) in saline (40 mg/kg) for 3 consecutive days or with 1 daily dose of β-naphthoflavone (Aldrich) in corn oil (60 mg/kg) for 2 days. The control groups were treated with the respective vehicles alone. After the last injection the animals were fasted for 16 h until killed. Nuclear preparations were isolated according to the method of Bresnick et al.[7]. Incubation of isolated nuclei with styrene or styrene oxide and gas chromatographic analysis of their metabolic products, were carried out according to the method of Gazzotti et al.[15].

Results. The table shows the results of at least 2 different experiments. The apparent K_m and V_{max} values were determined at different protein concentrations for styrene monooxygenase and styrene epoxide hydrolase. The protein values ranged from 0.8 to 1.5 mg/ml for styrene monooxygenase and from 0.21 to 0.66 mg/ml for styrene epoxide hydrolase. As regards styrene monooxygenase, treatment with phenobarbital, a well known microsomal P-450 inducer, does not significantly alter the apparent K_m, whereas it causes an approximately 3-fold increase in the V_{max}. Likewise, treatment with β-naphthoflavone, a known P-448 inducer, almost doubled the V_{max} value, but had no significant effect on the apparent K_m.

A slightly different pattern was seen with styrene epoxide hydrolase. Phenobarbital treatment did not change the K_m value but it raised the V_{max} value about 2.5-fold. Treatment with β-naphthoflavone slightly increased the V_{max} value and caused a 1.7-fold increase in the apparent K_m.

The table also shows there was no difference between the styrene epoxide hydrolase: styrene monooxygenase ratios for phenobarbital treated animals and controls, whereas this ratio was reduced by the β-naphthoflavone treatment.

Discussion. The presence of a monooxygenase system on the nuclear envelope has been established recently by several authors[5–8] and its possible role in the metabolic activation of promutagens and procarcinogens has been assessed. This enzymatic system is similar to the one in the microsomal fraction[17]. The presence of a hydrolase system immunochemically equivalent to the one in the microsomal fraction, located on the nuclear membrane very close to the monooxygenase system, has been established as well[4,8], and its role in the metabolic deactivation of the toxic intermediates mentioned above has been investigated.

The biological relevance of these 2 nuclear systems seems to be related to their proximity to the genomic material. The nuclear monooxygenase system may well be one of the effective metabolic activators of pro-genotoxic substances and the nuclear hydrolase system one of the last barriers for the inactivation of genotoxic intermediates even though the role of the hydrolase system itself is not completely clear since it seems to have a toxifying as well as a detoxifying activity[18]. The data presented in this paper show that rat liver contains almost 3 times as much styrene epoxide hydrolase as styrene monooxygenase, as can be calculated from the V_{max} of the 2 enzymatic systems. This ratio is even higher if it is considered that the substrate K_m of styrene epoxide hydrolase is almost ten times that of styrene monooxygenase. Both systems are induced by phenobarbital pretreatment, as far as V_{max} is concerned, but their K_m are not altered, indicating that probably there is either a new synthesis or a decrease in protein degradation of these enzymes.

Moreover the styrene epoxide hydrolase-styrene monooxygenase ratio is only slightly altered by pretreatment with phenobarbital. The effect of pretreatment with β-naphthoflavone, in contrast, is more ambiguous; this compound does not affect the styrene monooxygenase system's affinity for its substrate, but it does seem to have a very slight effect on the K_m value of the styrene epoxide hydrolase system. The reverse pattern is seen with the V_{max} values of these 2 enzymatic systems, i.e. a 2-fold increase for styrene monooxygenase and a statistically significant but only small change for styrene epoxide hydrolase.

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2 To whom enquiries should be addressed.

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