recent studies[15], it is clear that the method used for ethanol administration plays an important role in studies such as these. In fact, while Banerjee et al.[7] reported marked modifications in the density of β-receptors in the brain during both ethanol-treatment and ethanol-withdrawal, Hunt et al.[15] failed to observe any significant modification of the concentration of these receptors in most brain areas of rats rendered dependent after a 4 day oral administration of ethanol. In this connection, it is interesting to note that brain benzodiazepin receptors have also been reported to be unaffected in rats subjected to an ethanol treatment similar to that used here[16].

From this study, we can conclude that the chronic ethanol inhalation treatment presently used does not alter the density and affinity of cardiac α- and β-receptors. Moreover, since hydrolysis of cardiac triglycerides depends on a catecholamine-sensitive lipase[17], this study also indicates that the triglyceride accumulation that we have recently found in rat heart after the same ethanol inhalation treatment[18] does not result from an ethanol-induced alteration of the cardiac β-receptors.

1 Acknowledgments. This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale. We wish to thank Mr M. Clément for skilled technical assistance in performing chronic ethanol inhalation treatments.

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Urease inhibition by hydroxamic acids[1]

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Summary. Ureases from jack beans and *Rhodototorula pilimanae* were observed to be inhibited by primary hydroxamic acids but were not inhibited by acyclic-secondary (N-alkyl) hydroxamic acids.

During nutritional studies with the yeast *Rhodotorula pilimanae* it was observed that urea would serve as a nitrogen source in cultures deprived of iron. During iron deprivation *R. pilimanae* and related yeasts produce millimolar quantities of their iron transport agent, the hydroxamic acid rhodotorulic acid[2-4]. Urease inhibition and the retardation of microbial growth on urea by hydroxamic acids are well known[5,6], yet paradoxically *R. pilimanae* thrives (table 1) in low-iron cultures with urea as the sole nitrogen source in spite of the presence of millimolar concentrations of rhodotorulic acid. To resolve this problem the effect of a number of hydroxamic acids on *R. pilimanae* and jack bean ureases was determined.

Ureases were assayed spectrophotometrically and the inhibition by hydroxamic acids determined[7]. N-Methylhydroxylamine was obtained from Aldrich Chemical Co. while N-butylhydroxylamine was prepared by reducing the corresponding oxime[8]. Rhodotorulic acid was isolated from low iron *R. pilimanae* (ATCC 26423) cultures[2], glycine hydroxamic acid, jack bean powder, and avidin were from Sigma Chemical Co., while acetohydroxamic acid, propionohydroxamic acid, N-methylacetohydroxamic acid, and N-butylacetohydroxamic acid were prepared by acylation of the hydroxylamines[9]. Hydroxamic acid concentrations were determined spectrophotometrically with 5 mM $Fe(ClO_4)_3$ in 0.1 M $HClO_4$ using typical hydroxamic acid extinction coefficients[2,10]. For cultures of *R. pilimanae* grown with urea, 1.7 g/l was substituted for the ammonium contained in the normal culture medium[2]. Urea solutions were filter sterilized. *R. pilimanae* extracts were prepared from cells grown on urea containing medium for 2 days from a 1% inoculum by grinding with alumina, extracting with 0.02 M potassium phosphate 0.002 M EDTA, pH 6.5, and centrifuging at 14,000 × g for 20 min. Avidin and hydroxamic acid solutions were prepared with the same buffer (pH readjusted to 6.5).

Both jack bean and *R. pilimanae* ureases were found to be inhibited (table 2) by primary (unsubstituted nitrogen) hydroxamic acids while secondary (n-substituted) hydrox-

Table 1. The effect of nitrogen source and iron on the formation of rhodotorulic acid and cell yield in *Rhodotorula pilimanae* cultures[a]

N source[b]	Fe[c]	Rhodotorulic acid concentration (mM)	cell yield[d]
Urea	−	3.23	7.8
Urea	+	0.02	9.6
Ammonium	−	3.83	8.2
Ammonium	+	0.01	10.1

[a] Determinations were made after 3 days of growth at 23 °C from a 1% inoculum. The data represents an average of 3 separate determinations. [b] Cultures were prepared with 0.8 g of nitrogen per liter as urea or ammonium acetate. [c] A negative (−) sign indicated no iron salts were added to the cultures while a plus (+) indicated 5 mg of iron as ferric citrate was added per liter of culture. [d] Cells were washed twice with 1% saline and the resulting pellets were dried to a constant weight at 90 °C.

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.

1 This project was supported by a Lamar University organized research grant.
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Induction of nuclear styrene monooxygenase and epoxide hydrolase in rat liver[1]

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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