These results show the presence of adenyl cyclase in baker's yeast, *Sacch. cerevisiae*, which is clearly necessary if cyclic AMP is to exert a physiological control. The specific activity of the Mn-dependent cyclase in whole homogenates is about 20 times greater than that of the Mg-dependent cyclase in *Sacch. fragilis*.[4] Both enzymes have low pH optima and are only weakly stimulated by NaF, in contrast to many mammalian enzymes.[13] In *Sacch. fragilis* sphaeroplasts 80 % of the total activity can be located in the plasma membrane. Our results suggest a considerable part of the total activity is so located in *Sacch. cerevisiae*, and much of the activity found in other particulate fractions probably originates from the plasma membrane which is known to fragment during disruption of this hard-walled yeast, and also precipitate in these fractions.[5,11]

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Thiourea, an Effective Inhibitor of the Non-Enzymatic Ethanol Oxidation in Biological Extracts

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The determination of acetaldehyde in biological extracts is complicated by the formation of this compound in blood and tissue precipitates when ethanol is present.[1,2] This acetaldehyde is formed by a non-enzymatic oxidation of ethanol by means of a radical chain reaction mechanism. A semidehydroascorbate peroxy radical has been hypothesized to act as an electron acceptor in the reaction.[2] The reactive radicals are produced by the autoxidation of ascorbic acid. Since thiourea is an effective inhibitor of the latter reaction,[3-5] acting as a free radical acceptor and breaking the radical chain reaction,[6-8] it was thought that it should block the non-enzymatic oxidation of ethanol. The protective effect of thiourea can be looked upon as a type of antioxidation.[6,9]

It has now been found that the non-enzymatic ethanol oxidation in the supernatant of fresh liver homogenate precipitated by perchloric acid (PCA), is completely inhibited by 25 – 50 mM thiourea (Table 1). If the incubation time at 65°C was 15 – 20 min, and the homogenate concentration ≤ 20 % (w/v), a 25 mM thiourea concentration was enough to inhibit all ethanol oxidation in the sample. Even a 8 mM thiourea concentration was able to inhibit 80 – 90 % of the ethanol oxidation.

Thiourea was also added to samples containing 1.0 mM ascorbic acid and 20 mM ethanol and again was found inhibit non-enzymatic ethanol oxidation (Table 1). The fact that thiourea is effective in both liver extracts and in pure ascorbic acid solutions is strong evidence that ascorbic acid is involved in both reactions.[2]

An alternative explanation for the above results could be that thiourea interacts with the acetaldehyde present in the sample. In order to test this hypothesis thiourea was added to solutions containing acetaldehyde. As Table 2 illustrates, 100 mM thiourea had no effect on the acetal-

Table 1. Effect of thiourea on non-enzymatic ethanol oxidation in (A) the supernatant of PCA-precipitated rat liver homogenate (pH< 1) and (B) an ascorbic acid solution. The ascorbic acid was dissolved in 0.1 M HCl-citrate buffer, pH 3.0. The ethanol concentration was 20 mM both in the ascorbic acid solution and the liver supernatant. All values are the means from three experiments.

Thiourea (mM)	Incubation time at 65° (min)	Acetaldehyde formed (nmol/ml incubation solution)
(A) 20 % (w/v) liver extract:		
–	15	20
–	60	41
8	15	2
8	60	8
25	15	0
25	60	1
50	60	0
(B) 1.0 mM ascorbic acid solution:		
–	15	36
1	15	5
13	15	1
25	15	0
50	15	0
100	15	0

dehyde level in the sample. On the other hand, a 950 mM thiourea solution can have a significant effect on the acetaldehyde level (Table 2). Thus it is recommended that the thiourea concentration used to prevent the ethanol oxidation should not be higher than 100 mM. The effect of thiourea (100 mM) on a 20 mM ethanol solution was also tested, but no effect was found. Unpublished work in this laboratory also indicate that the determinations of lactate and pyruvate (by the enzymatical method of Hohorst *et al.*[10]) were unaffected of a 25 mM thiourea concentration.

To test the stability of acetaldehyde in the supernatant of PCA-thiourea precipitated liver homogenate, acetaldehyde was added to liver extract containing 25 mM thiourea and the samples were kept for different lengths of time at 4°C or at 25°C and then analyzed. Table 3 shows that the acetaldehyde level in the supernatant was very stable at both temperatures, regardless of the presence of 25 mM thiourea. Thus thiourea in the initial experiments apparently inhibited the ethanol oxidation, rather than interacting with the acetaldehyde.

Table 2. Effect of thiourea on the acetaldehyde level at pH 5. The concentrations of acetaldehyde, measured after incubation, in nmol per ml of the incubation medium are given as means ± S.E.M. with the number of determinations in parentheses.

Thiourea (mM)	Incubation time at 65° (min)	Acetaldehyde formed (nmol/ml)
44 μM acetaldehyde present in the sample:		
–	30	43.5 ± 0.5 (5)
100	30	43.0 ± 1.5 (5)
89 μM acetaldehyde present in the sample:		
–	15	89.0 ± 7.9 (7)
950	15	75.0 ± 5.1 (7)

Table 3. The stability of the acetaldehyde in PCA-precipitated rat liver extract. The extract (20 % w/v) was kept for different lengths of time at 25° and 4° before the analysis. The samples were incubated at 65° for 15 min before the head-space gas was analyzed. The values are the means of two experiments.

Time of "incubation" (h)	Acetaldehyde found (nmol/ml incubation solution) in		
	$PCA-H_2O$ (control)	PCA-extract without thiourea	PCA-extract with 25 mM thiourea
0	44	44	43
22 (at 25°)	39	39	38
47 (at 25°)	33	32	32
47 (at 4°)	40	39	40

Non-enzymatic ethanol oxidation does not occur only at higher temperatures.[2,3] If rat liver extract (20 % w/v) was incubated for 55 h with 10 mM ethanol at room temperature (25°), there was a clear acetaldehyde formation in the sample (27 nmol/ml). The identical procedure with 25 mM thiourea produced only insignificant amounts of acetaldehyde (< 1 nmol/ml). In both cases, to prevent ethanol oxidation during production of the gas phase needed

for gas chromatographic analysis, thiourea was added to the samples.

Butt and Hallaway [11] have reported that thiourea slowly disappears in an ascorbic acid solution, due to the hydrogen peroxide generated by the autoxidation of ascorbic acid. However, with the parameters used in the present experiments, no decrease in the inhibitory effect of thiourea was found (Table 1).

It was found that the non-enzymatic ethanol oxidation in fresh rat liver, extract, containing 0.3 – 0.6 mM ascorbic acid, was completely inhibited by 25 – 50 mM thiourea. The required concentration of thiourea depends primarily on the incubation time and the ascorbic acid level in the tissue sample. In blood samples which have a low ascorbic acid content, a much lower thiourea concentration was needed to inhibit the ethanol oxidation.

Experimental. The concentrations of acetaldehyde and ethanol in the samples were determined in a Perkin-Elmer automatic F 40 head-space gas liquid chromatograph with a hydrogen flame detector. The column used was 15 % polyethyleneglycol on celite (60/100). Each bottle in which the head-space was produced had 0.5 ml of the sample, containing either liver extract or ascorbic acid and 20 mM ethanol, added. The bottles were placed in a thermostatically controlled water bath at 65°C and kept at equilibrium for at least 15 min prior to the automatic analysis of the head-space gas. The analytical procedure was standardized with samples of diluted acetaldehyde and ethanol. The acetaldehyde was determined by using fresh redistilled acetaldehyde (50 μM) as a reference standard. The acetaldehyde concentration in the standard was checked daily with 0.025 ‰ *tert.*butanol as an internal standard.

The thiourea (of reagent grade from E. Merck, Darmstadt, Germany) was dried at 90°C for 12 h before the use to eliminate traces of volatile compounds which interfere with the acetaldehyde determination. The stability of thiourea in a 0.6 N perchloric acid (PCA) solution was tested but no decreased inhibitor effects was found even if the PCA-thiourea solution was one to two weeks old.

The rat liver was quickly removed from the animal after decapitation, and 5 g of the liver was homogenized in 20 ml 0.6 N PCA containing different amounts of thiourea. The precipitate was centrifugated, and 0.5 ml of the supernatant was analyzed by gas chromatography after incubation with 20 mM ethanol. The thiourea can also be added directly to the acid supernatant after homogenization if the homogenization in PCA and the subsequent centrifugation are carried out quickly at a temperature of + 4°C.

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On the Existence of Aqueous Penta- and Hexaimidazole Complexes of Copper(II)

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In an earlier emf-investigation,[1] equilibria

$$Cu^{2+} + n\ C_3H_5N_2^+ \rightleftharpoons Cu(C_3H_4N_2)_n^{2+} + n\ H^+ \quad (1)$$

were studied at 25°C and in 3.0 M (Na)ClO_4. It was then found that, Z_n, the