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EFFECTS OF PYRAZOLE AND 3-AMINO-1,2,4-TRIAZOLE ON METHANOL AND ETHANOL METABOLISM BY THE RAT*

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Abstract—The metabolism of methanol-^{14}C and ethanol-1-^{14}C in rats was evaluated from the rates of $^{14}CO_2$ production. 3-Amino,1,2,4-triazole, a known catalase inhibitor, decreased by 10 and 35 per cent the rates of oxidation of ethanol and methanol, whereas pyrazole, an alcohol dehydrogenase inhibitor, decreased the rates 85 and 50 per cent respectively. However, the simultaneous use of both inhibitors gave the same effects produced by pyrazole alone. Thus the relative contributions *in vivo* to alcohol metabolism of rat liver alcohol dehydrogenase and catalase-mediated peroxidation, cannot be estimated only in this way. Rat liver alcohol dehydrogenase was purified 14·7 times. At pH 7·0 and 30°, the K_m for methanol was 340 mM and for ethanol 0·26 mM. The V_{max}/e was 2·36 nM for methanol and 22·3 nM for ethanol (NADH $\times$ U^{-1} $\times$ 1^{-1} $\times$ sec^{-1}). 3-Amino-1,2,4-triazole inhibited the purified enzyme with a K_i of 55 mM for methanol and 33 mM for ethanol. The K_i of pyrazole was 2·3 mM for methanol and 2·2 mM for ethanol. The amount of alcohol dehydrogenase present in rat liver, with the found kinetic constants, can account for the ethanol oxidation *in vivo*, but fails to account, as methanol dehydrogenase, for the observed pyrazole-sensitive methanol oxidation. A mechanism for the complete oxidation of methanol to CO_2 and water through the concerted action of catalase and alcohol dehydrogenase is suggested. 3-Amino-1,2,4-triazole in a dose of 1 g/kg decreases more than 90 per cent of the catalatic activity of catalase, but under certain conditions *in vitro*, only about 50 per cent of the peroxidative activity of catalase towards methanol and ethanol. Consequently, the degree of catalase-mediated peroxidation should not be controlled or estimated from the residual catalatic activity when using catalase inhibitors. Pyrazole, at a dose of 0·3 g/kg, does not affect catalase activity 1 hr after administration, but decreases it more than 90 per cent after 24 hr. This effect is completely prevented in the presence of alcohol.

THE LOW primary alcohols, methanol and ethanol, are oxidized *in vivo* to their corresponding aldehydes, acids and finally to carbon dioxide and water. There is evidence that the liver alcohol dehydrogenase (ADH; alcohol NAD$^+$ oxidoreductase, EC 1.1.1.1) is the main catalyst for the first oxidative reaction with ethanol but apparently not for methanol.[1–3] Two other mechanisms have been postulated for the oxidation of methanol and ethanol, but the importance of their contribution has not been clearly established. They are the catalase (H_2O_2 : H_2O_2 oxidoreductase, EC 1.11.1.6)-mediated peroxidation[4,5] and the oxidation by a microsomal system,[6,7] demonstrated only *in vitro* and not necessarily different from the catalase-mediated peroxidation.[8–11]

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Pyrazole is a potent inhibitor of ADH,[12] and 3-amino-1,2,4-triazole (AT) is a well known irreversible inhibitor of catalase.[13,14] Both compounds are active *in vivo* and have been used extensively in the study of alcohol metabolism,[15–30] raising many of the arguments held in support of the various estimates on the relative contribution of the different alcohol-oxidizing systems. Thus, in the rat, 50 per cent of methanol is supposed to be oxidized by liver ADH and 50 per cent peroxidatively by catalase.[27,29,30] Ethanol would be oxidized almost exclusively by rat liver ADH.[16,18,29]

We present now our observations on the effect of pyrazole and AT, administered separately or combined, on the oxidation of methanol and ethanol to carbon dioxide by the rat and on the activities *in vitro* of rat liver ADH and catalase. Our purpose has been to correlate observations *in vivo* and *in vitro* to characterize the role of the different alcohol-metabolizing systems in the rat. We confirmed the fundamental role attributed to rat liver ADH in ethanol metabolism and the value of pyrazole as its specific inhibitor. The evaluation of the role of catalase-mediated peroxidation with AT is subject to various considerations that restrict its value.

EXPERIMENTAL PROCEDURE

Non-fasted male Sprague–Dawley rats weighing 240–260 g and fed Purine rat chow were used for all the experiments.

The following reagents were employed: ethanol-1-^{14}C methanol-^{14}C and barium carbonate-^{14}C for instrument calibration, from New England Nuclear Corp.; ethanol p.a. and semi-carbazide chlorhydrate p.a. from Merck; 3-amino-1,2,4-triazole purum and pyrazole purum from Fluka; glycolic acid sodium salt from M.C. & B.; titanium oxisulphate as titanium sulphate basic wet cake from A.D. Mac Kay Inc., New York; DEAE cellulose, medium mesh, and NAD^+ grade III from Sigma.

Determination of alcohol oxidation in vivo. The rats received 1 g/kg, i.p., of methanol-^{14}C in a 10% (w/v) solution (10 μCi/ml) or ethanol-1-^{14}C in a 10% (w/v) solution (1 μCi/ml). AT was given i.p., 1 g/kg in a 5% (w/v) water solution, 1 hr before the alcohol. Pyrazole was given 30 min before the alcohol, i.p., 0·2 g/kg in a 4% (w/v) water solution. The control rats were injected with equivalent volumes of saline.

The excretion of $^{14}CO_2$ was measured by placing three rats simultaneously in a plastic cage, with an air inlet on one end, and connected at the opposite end through a $CaSO_4$ drying column to an ionization chamber coupled to an electrometer with continuous recording. The air flow was kept constant at 10, l/min. The system was calibrated with $BaCO_3$–^{14}C;[31,32] the drying column was shown to retain all the methanol or ethanol released into the air. Four experimental determinations were made for each set of conditions.

Determination of the catalase-mediated catalatic and peroxidative activities in liver. Catalatic activity was measured by detecting colorimetrically the disappearance of hydrogen peroxide with titanium sulphate.[33] The effect of AT on catalatic activity was determined by measuring the residual activity of liver homogenate prepared in 0·25 M sucrose 1 hr after administration of the inhibitor. The role of alcohols in the inactivation of catalase by AT was established by injecting methanol or ethanol, 1 g/kg, 30 min before or 30 min after AT. The effect of pyrazole on catalase activity

was studied 1 and 24 hr after administration of a single dose of 0·2 g/kg i.p. or 0·3 g/kg intragastrically.[34]

To study the effect of alcohol on the inactivation of catalase by pyrazole, the rats received pyrazole, 0·3 g/kg intragastrically, and methanol, 2 g/kg i.p.; the dose of alcohol was only partially eliminated after 24 hr, when livers were removed to determine catalase activity.

We developed a simple procedure *in vitro* to measure methanol-^{14}C or ethanol-1-^{14}C peroxidation in a system coupling the hydrogen peroxide production of an oxidase to the catalase-mediated peroxidation of alcohols. The labelled aldehyde produced was trapped and precipitated as its semi-carbazone and washed free of the residual labelled alcohol. A mixture was prepared by adding 1·0 ml liver homogenate in 0·25 M sucrose containing: 250 mg liver; 1·0 ml phosphate buffer, 0·2 M, pH 7·4; 1·0 ml of semicarbazide solution, 0·2 M at pH 7·4; 0·1 ml of sodium glycolate, 0·01 M; and 0·1 ml methanol-^{14}C or ethanol-1-^{14}C containing 1·0 μCi. The mixture was incubated at 37° with continuous shaking in an atmosphere of 95% O_2 and 5% CO_2. After 30 min, a 0·1-ml aliquot was placed on a dry 24 mm diameter Whatman 3 MM filter paper disk previously impregnated with 0·1 ml of a 45 per cent solution of $ZnSO_4$–7 H_2O. The disk was placed under vacuum in a desiccator for 10 min, washed in absolute ethanol and diethyl ether, 10 min each, and placed again under vacuum for 30 min. The disks were counted in 0·4% (w/v) BBOT (Packard) in toluene using a Nuclear Chicago Mark I liquid scintillation counter. Under these washing conditions, the labelled alcohol was completely removed, while the aldehyde was trapped as the corresponding semi-carbazone. Sodium glycolate, used as a substrate for peroxisomal L-alpha-hydroxy acid oxidase which generates hydrogen peroxide,[35] was omitted from the blanks. With this procedure, 10–15 samples can be removed during incubation, with the help of a thin polyethylene tube placed through the rubber stopper of the incubation flask. Constant production of aldehyde was detected during 40 min, decreasing thereafter.

Purification and assay of rat liver ADH. ADH assays in crude preparations were performed and the units expressed according to Reynier.[36] The enzyme was purified with a modification of the method developed by Reynier.[36] After chromatography on DEAE cellulose with Tris–phosphate, 5 mM at pH 8·0, in a column of 2·5 × 30 cm, the enzyme, which is not retained under these conditions, was dialysed overnight against sodium carbonate buffer, 20 mM at pH 9·5, and rechromatographed on DEAE cellulose with the same carbonate buffer in a 2·5 × 30 cm column; the enzyme, which is now retained, was eluted with a 0·0–0·5 M sodium chloride gradient in carbonate buffer. The fraction containing the main ADH peak was dialysed against Tris–phosphate, 5 mM at pH 8·0, and was found to keep its activity for several days at 4°. Proteins were determined by the procedure of Lowry *et al.*[37]

Determination of the rates of methanol and ethanol oxidation by purified ADH. The reduction of NAD^+ coupled to alcohol oxidation was determined fluorometrically according to Theorell and McKinley-McKee.[38] An Eppendorf fluorometer was used with excitation at 313 and 366 nm and a 400–3000 nm filter for the emission. All measurements were done in phosphate buffer with an ionic strength of 0·1, at pH 7·0, 30° and 0·5 mM NAD^+. Initial velocities were expressed as micromoles per litre and per second. Results were plotted according to Lineweaver–Burk,[39] in terms of e over v vs. the reciprocal of the substrate concentration.[38]

RESULTS

Alcohol oxidation in vivo. The pulmonary excretion rate of $^{14}CO_2$ after the administration of methanol-^{14}C (Fig. 1) reaches a steady state after 2 hr. The continuous recording of $^{14}CO_2$ production was usually maintained for approximately 4 hr, but constant rates of production were found in control animals when the period of

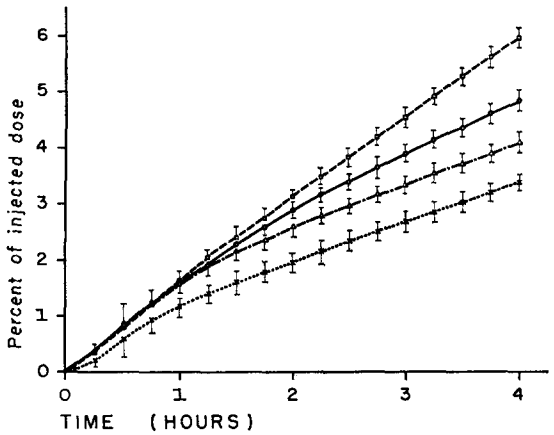

FIG. 1. Effect of 3-amino-1,2,4-triazole (AT), pyrazole, and AT plus pyrazole on the cumulative elimination of $^{14}CO_2$ after the administration of 1·0 g/kg of body wt of methanol-^{14}C. Controls (top, broken line) received saline. AT (continuous line) was given at a dose of 1 g/kg of body wt. Pyrazole (lines and dots) was given at a dose of 0·2 g/kg of body wt. The same doses were used for the combined administration (lower, dotted line). The S.D. values for each interval are indicated.

recording was prolonged up to 8 hr. AT caused a 35 per cent reduction in the rate of $^{14}CO_2$ production detected at the steady state period. Pyrazole reduced the rate at steady state to 50 per cent of control values. The simultaneous use of both inhibitors induced a relative decrease in the initial rate of oxidation, but under steady state conditions the maximal rate of $^{14}CO_2$ production was the same as that with pyrazole alone (Fig. 1).

When ethanol-1-^{14}C was used (Fig. 2), the maximal rate of oxidation was approximately 15 times higher than that for methanol, but it did not reach a clearcut steady state in control or AT-treated rats; in fact, both curves have an inflection point at approximately 2 hr. AT induced a 10 per cent decrease in the maximal rate of $^{14}CO_2$ production, and pyrazole reduced the $^{14}CO_2$ production rate in 85 per cent, giving a cumulative production curve identical to the one observed when both inhibitors were used simultaneously.

Catalatic and peroxidatic activities of catalase. The results summarized in Table 1 show that AT is effective in reducing catalase activity to about 8 or 9 per cent of the control value. This effect persists when the rats receive ethanol 0·5 hr after the injection of AT, but is completely prevented when the alcohol is given before, an observation already made by Nelson.[40]

Pyrazole, which proved equally effective when administered intraperitoneally or intragastrically, does not affect catalase activity 1 hr after administration, but reduces

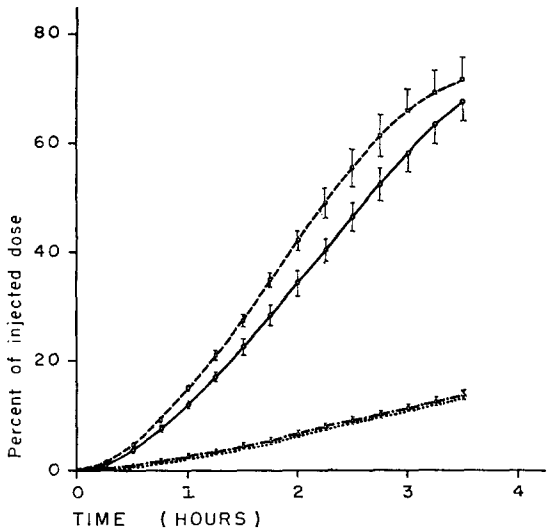

FIG. 2. Effect of 3-amino-1,2,4-triazole (AT), pyrazole, and AT plus pyrazole on the cumulative elimination of $^{14}CO_2$ after the administration of 1 g/kg of body wt of ethanol-1-^{14}C. Controls (top, broken line) received saline. AT (continuous line) was given at a dose of 1 g/kg of body wt. Pyrazole (lines and dots) was given at a dose of 0·2 g/kg of body wt. The same doses were used for the combined administration (lower, dotted line). The S.D. values for each interval are indicated. The two lower curves are identical.

its level to about 8 per cent of control values 24 hr after administration, a value somewhat lower than that originally described by Lieber *et al.*[34] However, we have found that when the rat receives methanol simultaneously, in a dose that is only partially eliminated and metabolized in 24 hr, the inhibitory effect of pyrazole is completely prevented.

TABLE 1. EFFECTS OF PYRAZOLE AND 3-AMINO-1,2,4-TRIAZOLE (AT) AND PROTECTIVE ROLE OF ALCOHOLS ON THE CATALATIC ACTIVITY OF RAT LIVER CATALASE*

	Catalase (units ± S.D.)	N†
Controls	62·7 ± 8·9	15
AT (1)‡	5·8 ± 3·2	5
AT (1) + ethanol (0·5) or methanol (0·5)	4·4 ± 0·8	4
AT (1) + ethanol (1·5) or methanol (1·5)	64·2 ± 7·9	4
Pyrazole (0·5)	62·3 ± 10·9	4
Pyrazole (24)	4·7 ± 3·0	8
Pyrazole (24) + methanol (24)	63·7 ± 8·3	4

* The following doses and routes of administration were used: AT, 1 g/kg of body wt i.p., in a 5% (w/v) water solution. Pyrazole, either 200 mg/kg of body wt i.p., or 300 mg/kg of body wt intragastrically, in a 4% (w/v) water solution. Ethanol, 1 g/kg of body wt in a 10% (w/v) solution. Methanol, 1 g/kg of body wt in a 10% (w/v) solution or twice this dose when given 24 hr before assaying for catalase.

† N represents the number of rats in each group.

‡ Figures in parentheses show the interval in hours between the administration of each drug and the autopsy.

The effect of AT on the peroxidative activity of the catalase present in liver homogenates is shown in Fig. 3. The rats had received one of the drugs 1 hr before preparing the homogenate. Under these conditions, pyrazole does not affect the peroxidative activity of catalase. But AT reduces the peroxidatic activity 53 per cent when assayed with methanol or 43 per cent with ethanol. The control rats received an injection of saline with the same interval.

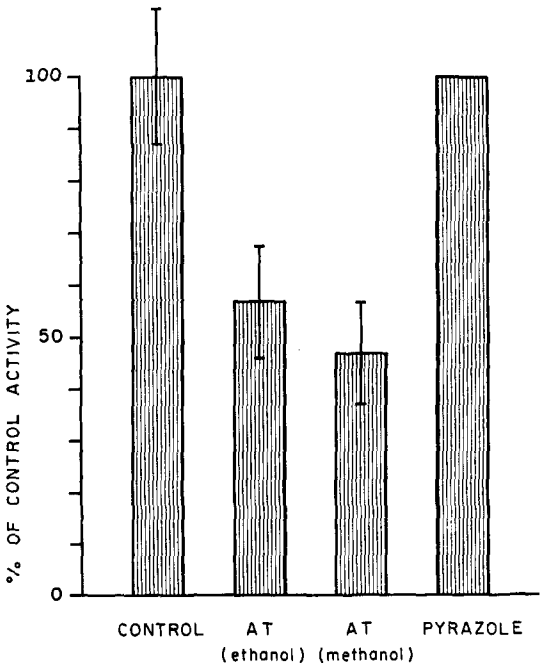

FIG. 3. Effect of pretreatment with AT and pyrazole on peroxidation by rat liver homogenates. Control shows activity in homogenates from rats that received saline. AT denotes the relative activity in homogenates prepared from rats receiving AT, 1 g/kg of body wt, 1 hr before and assayed with ethanol or methanol as substrate. Pyrazole denotes the activity in homogenates prepared from rats receiving 0·2 g/kg of body wt of the drug 1 hr before and assayed with ethanol as substrate. The S.D. values are indicated.

Purification of rat liver ADH. The yield and efficiency of the different steps in the purification of ADH are presented in Table 2. Rat liver was found to contain 743 $\pm$ 69 units of ADH/kg, but the purification is referred to the first supernatant obtained from the Ultra-Turrax liver homogenate, since crude homogenate determinations are affected by the variability of the blanks. Only after the second chromatography on DEAE cellulose (Fig. 4) did the fluorimetric method utilized for the rate of oxidation determinations give linear responses of activity versus time that were considered satisfactory to make a kinetic study of the enzyme. The extra step added to Reynier's procedure[36] was designed after determining that the enzyme, which was unstable at a pH below neutrality even in the presence of ethanol, remained stable for 24 hr in 20 mM carbonate or glycine buffers up to a pH of 10·5.

TABLE 2. PURIFICATION PROCEDURE FOR RAT LIVER ALCOHOL DEHYDROGENASE

	Protein (mg)	Activity (mU)	Specific activity (mU/mg)	Purification	Yield
First supernatant of whole homogenate	6000	57,000	9·5	1·0	100
Precipitate [35–70% $(NH_4)_2SO_4$]	4200	52,000	12·4	1·3	91
After filtration and first DEAE-chromatography	353·4	22,401	63·5	6·7	43
After second DEAE-chromatography	45·5	6338	139·4	14·7	11

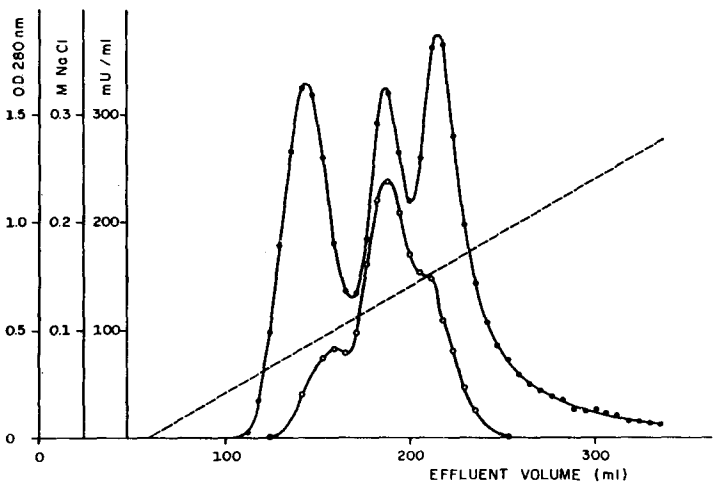

FIG. 4. Chromatography of rat liver ADH on DEAE cellulose with 20 mM sodium carbonate buffer, pH 9·5, and an eluting gradient of NaCl. The figure shows extinction at 280 nm (black dots); enzyme activity (open circles) and NaCl concentration (broken line).

Kinetics of purified rat liver ADH and inhibition with pyrazole and AT. The kinetic constants for the purified ADH are shown in Table 3. The K_m for methanol is three orders of magnitude higher than the K_m for ethanol, and the V_{max} for methanol is one-tenth the V_{max} for ethanol.

TABLE 3. KINETIC CONSTANTS FOR RAT LIVER ALCOHOL DEHYDROGENASE AT pH 7·0 AND 30°

Substrate	K_m (mM)	K_i-pyrazole (μM)	K_i-AT (mM)	V_{max}/e*	V_{max}/e pyrazole	V_{max}/e AT
Ethanol	0·26 (0·20–0·32)†	2·2 (1·9–2·5)	33·1 (22·2–58·5)	22·3 (21·0–24·0)	24·0 (22·0–26·0)	26·0 (22·0–31·0)
Methanol	340 (312–369)	2·3 (2·0–2·6)	55·4 (32·3–171·0)	2·36 (2·31–2·42)	2·70 (2·43–3·04)	2·28 (2·08–2·51)

* V_{max}/e are expressed as nM NADH × U^{-1} × 1^{-1} × sec^{-1}.
† 95% Confidence interval.

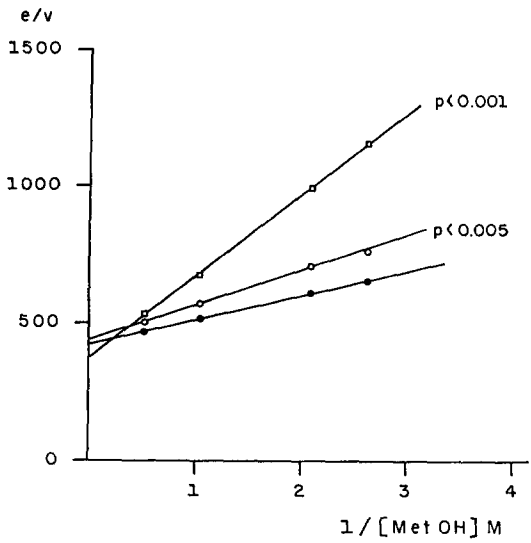

FIG. 5. Lineweaver–Burk plots of rat liver ADH with methanol as substrate. Without inhibitor, black dots; in the presence of 20·2 mM 3-amino-1,2,4-triazole, open circles; in the presence of 6·42 μM pyrazole, open squares. The P values indicate the significance of the differences in slope.

Pyrazole behaves as a competitive inhibitor with the same K_i for methanol and ethanol. AT also inhibits rat liver ADH, even when its K_i is rather high. The slopes of the Lineweaver–Burk plot in the presence of the inhibitors (Figs. 5 and 6) are statistically different from those of the corresponding controls.

Two batches of enzyme were prepared and used in at least three series of experi-

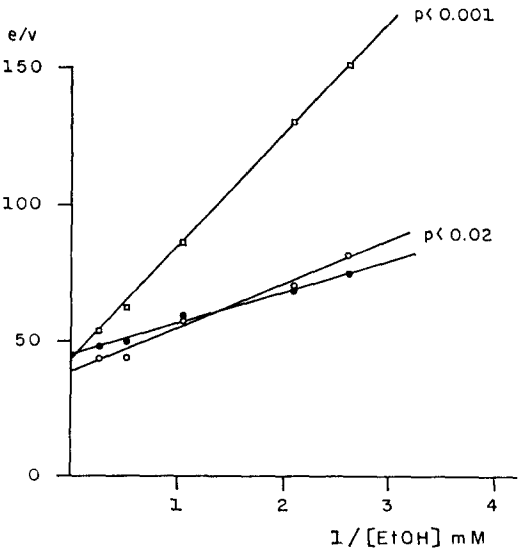

FIG. 6. Lineweaver–Burk plots of rat liver ADH with ethanol as substrate. Without inhibitor, black dots; in the presence of 20·2 mM 3-amino-1,2,4-triazole, open circles; in the presence of 6·42 μM pyrazole, open squares. The P values indicate the significance of the differences in slope.

ments from which all the results were pooled for statistical analysis to determine the kinetic constants.

DISCUSSION

We have confirmed previous reports that AT decreases the rate of CO_2 production from methanol by approximately 50 per cent,[24,27] and have also found a 10 per cent reduction in CO_2 production from ethanol after acute alcohol administration to non-fasted rats.

Our results are also in keeping with the reported effects of pyrazole, a very effective ADH competitive inhibitor, which reduces methanol and ethanol oxidation to CO_2 in approximately 50 and 80 per cent.[16,17,24,29]

In these reports, it has been assumed that an equivalent fraction of alcohol would be metabolized by ADH or by the catalase-mediated peroxidative system. However, in the absence of a demonstrated functional relationship between the two systems, our failure to find, either with methanol or with ethanol, an additive effect on the rate of CO_2 production when pyrazole and AT were used simultaneously casts doubt on the quantitative estimations of the reported relative contributions of ADH and catalase to alcohol metabolism.

Role of rat liver ADH. There is general agreement that rat liver ADH is responsible for the bulk of ethanol oxidation to acetaldehyde; nevertheless, Lieber and de Carli[19] have recently challenged this idea on the basis of the reduced activity of rat liver ADH at pH 7·0.

From the apparent Michaelis constants and the maximal velocities determined by us for purified rat liver ADH, and assuming an activity of 0·7 unit/g, we can determine the time required by the rat to eliminate a known dose of alcohol by applying the equation of Lundquist and Wolthers.[41] The equation is:

$$V_t = (C_0 - C) + K \ln \frac{C_0}{C}$$

K is the apparent Michaelis constant, V the maximal velocity, C the blood alcohol concentration, and t the time after administration of alcohol. The elimination times are 5 hr for ethanol and 80 days for methanol at a dose of 1 g/kg of body wt. Elimination time is defined as the time required to decrease C_0 by 95 per cent. Accordingly, rat liver ADH could account for the observed ethanol oxidation *in vivo*, but only for a minimal part of the oxidation of methanol.

The Lineweaver–Burk plots obtained with ethanol and with ethanol plus AT (Fig. 5) intercross in a pattern of competitive inhibition and stimulation (stimulating at high substrate concentration and inhibiting at low substrate concentration), a pattern originally described for AT and horse liver ADH by Theorell *et al.*[42]

The 10 per cent decrease induced by AT in the oxidation of ethanol when maximum rates of CO_2 production are compared (Fig. 2) might be due either to the interaction of AT with rat liver ADH that we are describing or to the interaction of AT with catalase. This latter possibility would fit better with our previous description of a 10 per cent reduction in blood ethanol clearance by AT in fed but not in fasted rats.[15]

The 50 per cent reduction in the rate of CO_2 production from methanol, induced by pyrazole, cannot be attributed to the inhibition of methanol oxidation to formaldehyde by rat liver ADH, since our purified enzyme shows negligible activity towards this substrate. However, Kendal and Ramanathan[43] and Abeles and Lee[44] have shown

that, in the presence of NAD^+, formaldehyde can be dismutated to formic acid and methanol by horse liver ADH. If this mechanism is active *in vivo* with rat liver ADH, pyrazole might be acting on the oxidation of formaldehyde to formic acid and not in the oxidation of methanol to formaldehyde.

The kinetic constants found for our purified ADH deserve further comment. A K_m value, particularly an apparent K_m, is a constant valid only for a determined set of conditions, such as temperature, ionic strength, pH, ions, cofactors and other enzymatic and non-enzymatic components of the incubation medium, a fact that must be considered when comparing different K_m values. The *in vitro* to *in vivo* extrapolation of an enzyme activity should be more accurate when the assays are carried out under conditions similar to those of the natural media. We worked at 30° instead of the usual 23·5°, and at neutral pH, which is not the optimum for the enzyme; NAD^+ and buffer strength were kept at currently used values. Under these conditions, only the more purified enzyme gives linear initial velocities suitable for kinetic analysis in the fluorometric assay.

The K_m found for methanol is high, making rat liver ADH very inefficient for methanol oxidation. Our value is one order of magnitude higher than that reported by Makar *et al.*[22] for a crude preparation from rat liver at pH 10 and 23°. We have found no other determination of rat liver ADH K_m with methanol; furthermore, the values for one species can not be generalized. Von Wartburg *et al.*[45] found equal rates of reaction for methanol and ethanol with human liver ADH. Mani *et al.*,[46] working with pure enzymes, found the following K_m values with methanol at pH 8·8 and 23·5°: human liver ADH, 6·8 mM; horse liver ADH, 39 mM; yeast ADH, 316 mM.

The K_m for ethanol and the K_i for pyrazole–ethanol we found are one-half the values reported by Reynier[36] for rat liver ADH, also at pH 7, but at 23·5° and using 0·2 mM NAD^+. Recently, Markovic *et al.*,[47] working with highly purified rat liver ADH, reported a K_m for ethanol 10-fold higher than the value we found; however, they worked at pH 10 and 23·5°. Kinetic constants for methanol were not reported for this enzyme.

The competitive inhibition and stimulation of rat liver ADH by AT, in the presence of ethanol, was established with the concentration the inhibitor would reach at the usual doses *in vivo*, assuming free diffusion. Since AT *in vitro* stimulates or inhibits depending on the ethanol concentration, and since the real concentration AT reaches in the liver during the experiments has not been determined, its effects *in vivo* are difficult to interpret.

Our results with ADH have a common feature with many of the attempts to correlate observations made *in vitro* and *in vivo*. They are based on a purified rat liver ADH which represents a small fraction of the total activity. Both the selection of a hypothetical isoenzyme and the modification of the native enzyme properties during purification could affect our conclusions.

Role of the peroxidative system. The finding by Lieber *et al.*[34] that pyrazole induces a striking decrease in rat liver catalase activity 24 hr after a single dose raised doubt about the specificity of this inhibitor for ADH. We have confirmed these observations but we have found that, in the presence of alcohol, pyrazole does not affect catalase (Table 1). Hence, there are no indications that some of the effects of this inhibitor might be due to its interaction with catalase and the peroxidative system.

However, the use of AT as a specific inhibitor of the peroxidative activity of catalase

can not be accepted on the following grounds: AT has been shown to interact with rat liver ADH and, on the other hand, the residual peroxidative activity cannot be deduced from the residual catalatic activity in a process that is not rate limited by the amount of the enzyme.

The rate-limiting factor for peroxidation is the hydrogen peroxide generation, while the catalase content of the liver is in excess[5,24,48] even to metabolize all the ethanol the rat can oxidize. The oxidase activity of rat liver[49] and the liver content of lactate during ethanol administration[50] could sustain a rate of hydrogen peroxide generation high enough to catalyse the oxidation of ethanol, provided that the partial pressure of oxygen itself does not become the limiting factor.

The discrepancy found between the degree of inhibition of catalatic and peroxidatic activities for methanol or ethanol (Fig. 3) can be explained by assuming that the maximal velocity in the control experiments was limited by the hydrogen peroxide generation rate. However, it should also be pointed out that it has not been proven that a molecule of catalase, whose covalent binding sites for AT[14,51] are partially saturated, will be equally affected in its catalatic and peroxidatic actions.

The role of peroxidation, if any, in the metabolism of ethanol by the rat after acute administration is a minor one. ADH can account for the rates of oxidation *in vivo*, and pyrazole shows, accordingly, a strong inhibition. But the role of peroxidation in the adaptive response to chronic alcohol administration has not been established. Lieber and de Carli[19] attribute this adaptive response to the microsomal ethanol-oxidizing system which has been demonstrated *in vitro*, but whose enzymological basis is a subject of controversy; several groups believe, considering, among others, the effect of AT, that the catalase-mediated peroxidation is essential to it.[8–11] The former authors[19] dismiss the role of peroxidation on ethanol metabolism, based upon an old review by Bartlett[52] and a study of von Wartburg and Papenberg[3] on ADH, which we think do not warrant the conclusion.

For methanol oxidation, the role of peroxidation seems to be well established on several lines of evidence.[24,27,28] Catalase inactivation, lack of activity of ADH, the effect of hydrogen peroxide-generating substrates and competitive inhibition by substrate analogues have been used. However, it is not clear to us at which oxidative step peroxidation is important, since not only the oxidation of methanol to formaldehyde is mediated by catalase, but also the oxidation of formaldehyde to formic acid[11] and the oxidation of formic acid to carbon dioxide and water.[8]

The characteristics of rat liver ADH and the lack of additive effect for AT and pyrazole, taken together with the fact that in methanol oxidation AT could block three different reactions and pyrazole, two (i.e. the oxidation of the alcohol and the dismutation of the aldehyde), have led us to postulate that the oxidation of this alcohol would be mediated by catalase in the first and maybe the third oxidative step, with ADH acting as a dismutase in the second step. Therefore, the concerted action of both enzymes would be required to oxidize methanol to carbon dioxide and water, a hypothesis which is consistent with our experimental results.

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