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EFFECT OF PYRAZOLE ON RAT LIVER CATALASE*

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Abstract—Pyrazole alone does not affect catalase activity *in vitro*; however, its administration *in vivo* produces irreversible inhibition of catalase in rat liver and kidney but not in blood. The inhibition in the liver, after a 70 mg/kg single dose of pyrazole, follows first-order kinetics with a half-life of 8 hr. The activity reaches a minimum at 28 hr followed by gradual recovery at a rate corresponding to a half-life of 1·19 days. This value agrees with previous half-life determinations for rat liver catalase; therefore it is taken as evidence that the irreversibility of the inhibition demonstrated *in vitro* is also maintained *in vivo*. The inhibition of catalase is mediated by a product from the metabolism of pyrazole by the microsomal mixed-function oxidase system. This active pyrazole derivative presumably reacts with catalase hydrogen peroxide complex I, and not with the native catalase, in a process that can be prevented by alcohol. It is shown that pyrazole, a drug also used as an alcohol dehydrogenase inhibitor, is eliminated from the liver in a simple exponential process with a half-life of 3·45 hr, which agrees with its reported effects on ethanol metabolism *in vivo*.

PYRAZOLE has been used extensively in studies of alcohol metabolism, both *in vivo* and *in vitro*, because of its activity as a competitive inhibitor of liver alcohol dehydrogenase (alcohol NAD^+ oxidoreductase, EC 1.1.1.1).[1] A single administration depresses ethanol oxidation for 35–50 hr[2] or 66 hr.[3] However, the specificity of its effect has been questioned after finding that the activity of rat liver catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6), another enzyme involved in the oxidation of alcohols, is greatly reduced 23 hr after the administration of pyrazole.[4] We have shown recently that the effect of pyrazole on alcohol metabolism is not due to catalase inhibition. This inhibition was completely prevented by the presence of alcohol and, furthermore, it was not detected during the first hr, when pyrazole was administered before the alcohol.[5]

The contrast between the rapid effect of pyrazole on alcohol dehydrogenase and the delayed inhibition of catalase, the prolonged effect of a single dose upon ethanol metabolism, and the prevention of the inhibition of catalase by the presence of alcohol prompted us to study its elimination from the organism and the nature of its interaction with catalase.

EXPERIMENTAL

Male Sprague–Dawley rats weighing 240–300 g, without previous fasting, were used. A single dose of pyrazole was administered intraperitoneally to each rat in a

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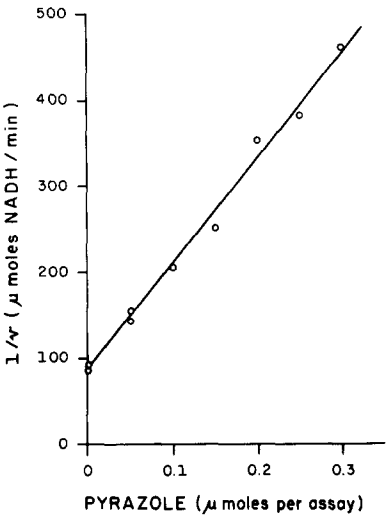

FIG. 1. Pyrazole determination by inhibition of yeast alcohol dehydrogenase.

neutralized water solution containing 10 or 20 mg/ml. Controls received an equivalent volume of saline.

Determination of pyrazole. The concentration of pyrazole in blood and liver homogenates or cell fractions was measured biochemically in a system based on the inhibition of alcohol dehydrogenase. The reduction of NAD^+ was followed during 10 min at 340 nm in a mixture kept at 25° and containing 13·5 mM ethanol, 6·35 mU/ml of yeast alcohol dehydrogenase, 0·93 mM NAD^+, 10 mM sodium pyrophosphate buffer (pH 8·8) and a 0·1 ml aliquot of the sample (0·05 to 0·30 μmole pyrazole) in a final volume of 2·91 ml. The interference by enzymes contained in the sample was eliminated by heating it at 60° for 20 min and submitting it to a short centrifugation to eliminate precipitated proteins. This procedure inactivates alcohol dehydrogenase completely and gives 100 per cent recovery of pyrazole when added to blood or liver homogenates at a concentration equivalent to 340 mg/kg of body weight. The reciprocal of the velocity of NAD^+ reduction is proportional to the concentration of the inhibitor (Fig. 1).

The temporal course of blood and liver pyrazole concentration was determined in rats that received a single dose of 340 mg (5 m-moles) per kg. At different intervals, under ether anesthesia, 4–6 ml of blood was collected from the aorta and the livers were removed. Therefore, the determinations in blood and liver were made with the same set of animals.

Incubation of pyrazole with catalase in vitro. Liver homogenate, prepared and diluted 1/10 in 0·25 M sucrose, was incubated at 25°, with or without 300 mg/l. of pyrazole, and the endogenous catalase activity was determined at hourly intervals for 5 hr. In another set of experiments, liver catalase prepared according to Leighton *et al.*[6] was concentrated on a hydroxyapatite column after adsorption in phosphate buffer (pH 7·0, 0·01 M) containing 0·1% ethanol and elution with the same buffer at a 0·1 M concentration. The enzyme was further purified following the method of Price *et al.*[7] from step 6 on. This catalase, later referred to as purified catalase, containing 4 hema-

tins/mole as determined by the pyridine hemochromogen method,[8] was incubated with pyrazole in a system described by Margoliash *et al.*[9] for the incubation with 3-amino-1,2,4-triazole. Five ml of potassium phosphate buffer (0·1 M, pH 7·0) containing 0·1 M pyrazole and 0·46 mg of purified catalase was dialyzed against 25 ml of the same buffer, containing 0·1 M pyrazole and 4 mM hydrogen peroxide, but without catalase. The dialysis was carried on for 40 hr at 37° with continuous shaking; samples were removed periodically to determine catalase activity. A parallel control experiment was run with 0·1 M 3-amino-1,2,4-triazole instead of pyrazole.

Intraperitoneal dialysis of catalase. To detect circulating catalase inactivators in rats treated with pyrazole, the following procedure was developed: dialysis sacs containing 0·8 mg of purified catalase in 3·3 ml of 76 mM potassium phosphate buffer, pH 7·0, with or without addition of 10 units of glucose oxidase, were prepared. Each sac was placed into the abdominal cavity of a rat, under ether anesthesia, that had received 300 mg/kg of pyrazole or saline 1 hr before. After 24 hr, the sacs were removed and the catalase activity of the contents was determined.

Incubation of pyrazole with catalase in a microsomal system. The production of pyrazole metabolites capable of inactivating catalase by the action of the drug-metabolizing system was studied *in vitro* under conditions similar to those used by Orrenius[10] for the oxidative demethylation of animopyrine. The incubation mixture consisted of 2 ml of a solution containing 5 mM nicotinamide, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 0·5 mM $NADP^+$ and 5 mM DL-isocitrate, plus 0·2 ml of liver microsomal fraction prepared in 0·25 M sucrose and containing 40 mg/ml of protein, 10 μl of purified catalase solution (5·7 mg/ml), 50 μl of 0·5 M pyrazole and 10 μl of isocitric dehydrogenase solution (50 units/ml). In some experiments, $NADP^+$ and the dehydrogenase were omitted. In another series, 1·0 mM SKF-525A was added to the complete system. Pyrazole was omitted in the blanks. Incubation was carried on for 2 hr in Warburg flasks at 37 with continuous shaking under an atmosphere of air, N_2 or 80% CO–20% O_2. To remove the air, the flasks were flushed for 10 min and only then were the pyrazole and the isocitric dehydrogenase, initially placed into the side arm, mixed with the rest of the contents to start the reaction. Aliquots for catalase determination were removed at different time intervals or at the end of the incubation.

Pyrazole-induced spectral changes of cytochrome P-450. The spectral changes induced by 17 mM pyrazole or 18 mM aniline were determined manually in a Gilford Bekman DU single-beam spectrophotometer. The drugs were added to microsomes prepared in 0·25 M sucrose, washed with 1·19% (w/v) KCl and resuspended in 0·1 M phosphate buffer, pH 7·4, at approximately 2 mg/ml.

Cell fractionation and enzyme determinations. Tissue homogenates, microsomal fractions or the entire fractionation procedure carried on to determine the subcellular localization of pyrazole in liver were made according to de Duve *et al.*[11] Enzyme activities were determined according to Baudhuin *et al.*[12] For the determination of catalase activity, the sample in 0·1 ml was added to 0·1 ml of a 2% (w/v) solution of Triton X-100 in a test tube placed into an ice bath at 0°. Five ml of cold substrate mixture containing 1·4 mM hydrogen peroxide, 20 mM imidazole buffer, pH 7·0, and 1 mg/ml of bovine serum albumin was squirted into the test tube in order to mix rapidly; after 2–4 min, the reaction was stopped by the addition of 3 ml of a $\frac{2}{3}$ saturated solution of titanium oxysulfate in 2 N H_2SO_4.

The extinction of the yellow complex formed with the residual hydrogen peroxide was read at 405 nm after placing the tubes for 10 min in water at room temperature. The reaction is first order with respect to the substrate.

Protein determinations were always made according to Lowry *et al.*[13] with bovine serum albumin as standard.

Materials. Twice crystallized yeast alcohol dehydrogenase, glucose oxidase type V, isocitric dehydrogenase type IV, DL-isocitric acid type I, NAD^+ grade III, $NADP^+$ and bovine serum albumin (fraction V) were obtained from Sigma Chemical Co. Pyrazole, 3-amino-1,2,4-triazole and hemin chloride cryst. were from Fluka A.G. SKF-525A was obtained from the National Cancer Institute, U.S.A. Titanium oxysulfate was purchased as titanium sulfate (basic wet cake) from A. D. McKay, New York. Most of the other reagents used were p.a. from E. Merck AG, Darmstadt.

CO was prepared by dropwise addition of formic acid into continuously stirred concentrated sulfuric acid at 70° and washed through sulfuric and potassium hydroxide. The 80% CO–20% O_2 mixture was prepared with the help of a Clark oxygen cathode. Nitrogen was used directly as supplied.

RESULTS

Observations after administration of pyrazole in vivo. The inhibition of rat liver catalase activity, 24 hr after a single injection, is practically maximum for a 50 mg/kg dose (Fig. 2). The catalase activity in the kidneys is also sensitive to pyrazole, in contrast with red blood cell catalase which is not affected, as shown in Table 1; it can also be seen that the activity in control rats is lower than in other experimental series (Figs. 2 and 3). In fact, periodic oscillations in the activity of rat liver catalase have been detected in our laboratory, making it always necessary to run control and experimental determinations simultaneously.

The concentration of pyrazole in blood and liver after the administration of 340 mg/kg is shown on a semilogarithmic plot in Fig. 4. The elimination curves, fitted by the least squares method, follow first-order kinetics, with a much faster decay in the liver than in the plasma. The elimination constant (k_e) for the liver, per hr, is

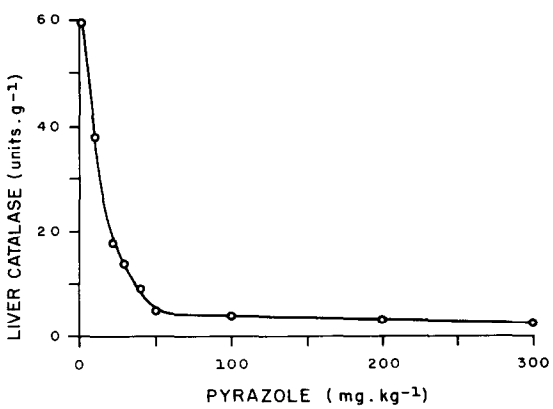

FIG. 2. Dose–response curve for the inhibition of catalase 24 hr after a single administration of pyrazole. The points are averages of four animals for the control and two for the rest.

TABLE 1. LIVER, KIDNEY AND BLOOD CATALASE ACTIVITY 24 HR AFTER THE ADMINISTRATION OF PYRAZOLE

	Controls	Pyrazole (300 mg/kg)	P
Liver	36·9 ± 11·4*	2·26 ± 0·82	<0·001
Kidney	13·9 ± 1·7	0·25 ± 0·15	<0·001
Blood	2·26 ± 0·42	2·45 ± 0·49	NS

* Units/g of tissue. Average of four animals ± S.D.
NS = not significant.

0·201, whereas the blood k_e is 0·0275, giving a half-life of 3·45 hr in the liver and 25·2 hr in the blood.

In a liver cell fractionation experiment done 30 min after the administration of 340 mg/kg of pyrazole, the drug was completely recovered in the soluble fraction with no detectable activity in the nuclear, mitochondrial, light mitochondrial or microsomal fraction (Fig. 5). Cytochrome oxidase was used as a marker for the mitochondrial fraction; acid phosphatase and L-α-hydroxyacid oxidase, a lysosomal and a peroxisomal enzyme, were used as markers for the light mitochondrial fraction; glucose 6-phosphatase was used as a marker for microsomes.

The temporal course of the irreversible inhibition of liver catalase by pyrazole, irreversible since it is not affected by dilution or by the washing of the catalase-containing liver cell particles (the peroxisomes), is shown in Fig. 3. The curve fitted to the experimental points, shows a minimum of activity 28 hr after the administration of pyrazole, followed by a slow recovery. The irreversible inhibition of catalase follows first-order kinetics, as shown by the straight line obtained in the semilogarithmic plot of residual catalatic activity vs time (Fig. 6). The slope of the line corresponds to a half-life of about 8 hr. The kinetics of the recovery of catalatic activity can be analyzed with the procedure used by Price *et al.*[7] for the recovery of catalase activity after inhibition with 3-amino-1,2,4-triazole; the assumption is made that steady state conditions are maintained, i.e. the zero-order rate constant of synthesis

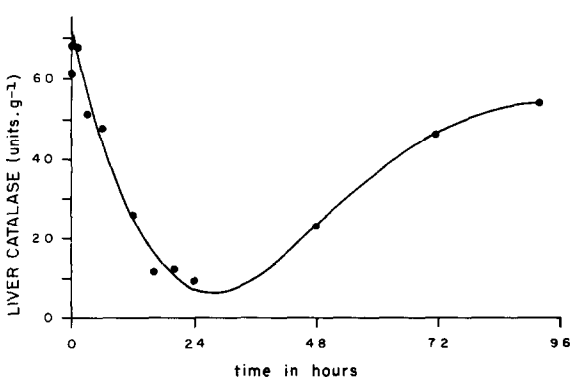

FIG. 3. Change in time of liver catalase activity after a single administration of pyrazole (70 mg/kg). Forty animals were used with each point representing the average of two to six. The fitted curve was calculated with a fourth degree polynomial regression.

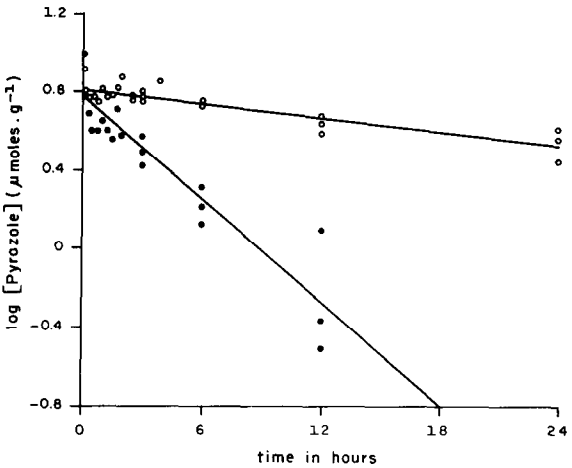

FIG. 4. Semilogarithmic plot of the concentration of pyrazole in the blood (open circles) and the liver (solid circles) against time, after administration of 5 m-moles (340 mg)/kg of pyrazole. Each point corresponds to a different animal. Correlation coefficients are −0·892 for the blood and −0·926 for the liver.

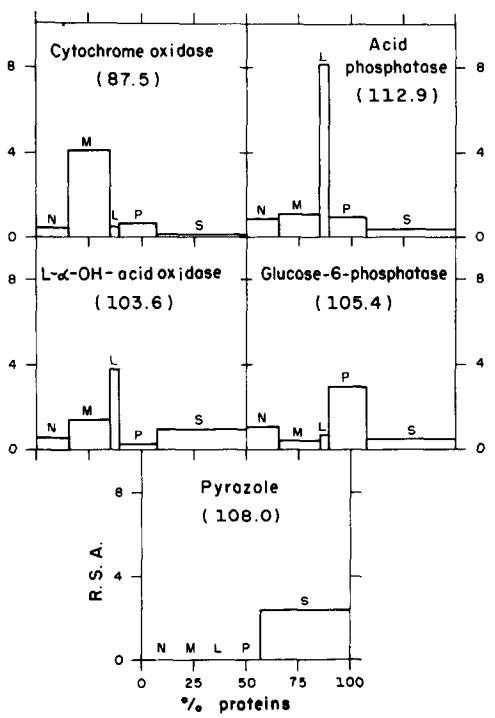

FIG. 5. Liver cell fractionation and subcellular distribution of pyrazole. Relative specific activity on the ordinates, corresponding to the ratio per cent of enzyme activity over per cent of protein in the fraction, is plotted vs cumulative per cent of protein in the nuclear, mitochondrial, light mitochondrial, microsomal and soluble fractions. Figures in parentheses represent fractionation recoveries.

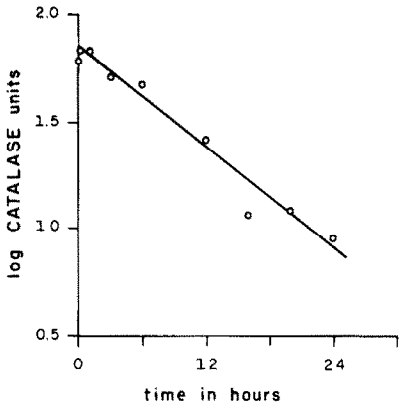

FIG. 6. Semilogarithmic plot of the decrease in catalase activity vs time after a single dose of pyrazole (70 mg/kg). Each point represents the average of two to six animals.

(k_s) and the first-order rate constant of degradation (k_d) do not change. Then C_p, the active catalase after pyrazole, is given by the following equation:

$$C_p = \frac{k_s}{k_d}(1 - e^{-k_d t}) \quad (1)$$

From the relation $C_n = (k_s/k_d)$, expressing the steady state concentration of catalase in normal rats, it can also be established that $\ln(C_n - C_p) = \ln C_n - k_d t$. In Fig. 7, using decimal logarithms, this relation is plotted against time after pyrazole administration for the values that correspond to recovery of the activity. The slope of the fitted straight line corresponds to a half-life for rat liver catalase of 1·19 days (95 per cent confidence interval, 0·84 to 2·00).

Nature of the interaction of pyrazole with catalase. The incubation of a liver homogenate for 5 hr at 25° revealed an exponential decay of the endogenous catalatic activity with a half-life of 2·5 hr. This rate of spontaneous inactivation was not modified by the presence of pyrazole.

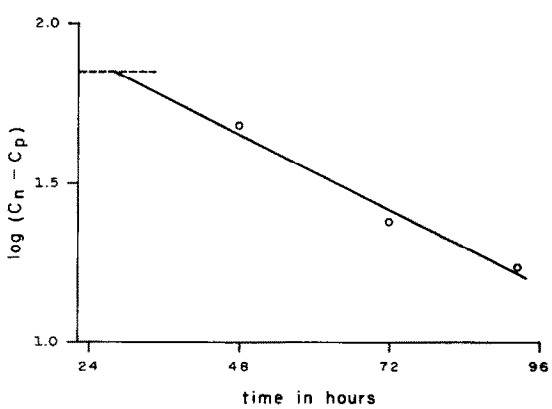

FIG. 7. Temporal course of the log of the difference between normal liver catalase activity (C_n) and the activities found in pyrazole-treated rats (C_p). The curve was made from ten animals. Each point represents the average of two or four.

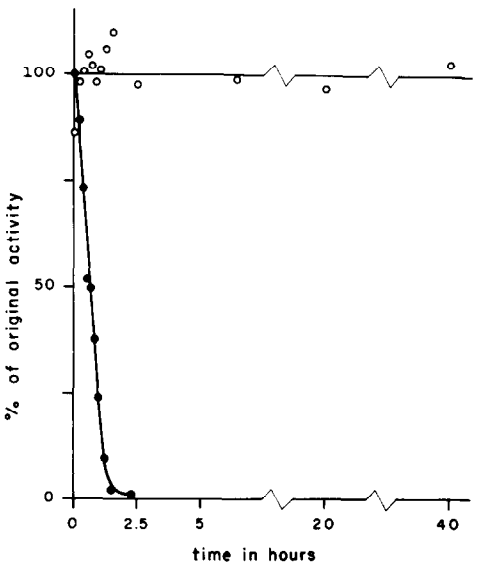

FIG. 8. Effect of pyrazole (open circles) or 3-amino-1,2,4-triazole (solid circles) on the activity of purified catalase dialyzed against a medium containing hydrogen peroxide.

Purified catalase incubated with pyrazole, under conditions in which 3-amino-1,2,4-triazole inactivates the enzyme, showed no inhibition even after 40 hr (Fig. 8). Since pyrazole *per se* does not inhibit catalase, the assumption was made that the inhibition could be due to a derivative of pyrazole. In order to test this hypothesis, dialysis bags containining purified catalase in buffer, with or without glucose oxidase to generate hydrogen peroxide from diffusing glucose and oxygen, were implanted in control or pyrazole-treated rats. Table 2 shows that, after 24 hr, catalase is almost completely inhibited in the rat treated with pyrazole, but only when glucose oxidase is present in the dialysis bag.

The previous experiments failed to show direct inhibition of catalase by pyrazole, but gave evidence that the administration of pyrazole induces the production *in vivo* of a dialyzable molecule capable of inactivating catalase in the presence of a hydrogen peroxide generator. A plausible hypothesis to explain the presence of such a molecule is to assume it to be a product of the metabolism of pyrazole by the drug-metabolizing mixed-function microsomal liver oxidases. Drugs metabolized by this system bind to the microsomal cytochrome P-450, inducing characteristic spectral

TABLE 2. PURIFIED CATALASE IMPLANTED INTRAPERITONEALLY IN RATS TREATED WITH PYRAZOLE

Rats	Addition of glucose oxidase	Catalase activity after 24 hr (units/ml)	Inactivation in presence of glucose oxidase (%)
Control	−	1·545	0·0
	+	1·510	2·3
Pyrazole-treated	−	1·738	0·0
	+	0·005	99·7

TABLE 3. INACTIVATION *in vitro* OF CATALASE INCUBATED WITH PYRAZOLE AND LIVER MICROSOMES

Incubation conditions	Gas phase	N	Per cent inactivated in presence of pyrazole*	P†
Complete system	Air	14	58·6 ± 6·0‡	
Complete system	80% CO–20% O_2	4	44·8 ± 3·9	<0·001
Complete system	N_2	4	40·0 ± 9·0	<0·001
Complete system plus SKF-525A (1 mM)	Air	5	40·9 ± 2·2	<0·001
Complete system minus $NADP^+$ and ICDH	Air	6	−8·2 ± 1·9	<0·001

* The inactivation is calculated relative to the activity found after incubation of catalase in complete system, without pyrazole, for 2 hr. This activity, taken as 0 % inactivation, corresponds in fact to 70 ± 9·9 per cent of the activity present at $t = 0$ and has been considered as a blank.

† When compared to complete system with air.

‡ Average value of N determinations ± S.D.

changes.[14] A typical type II spectral change was induced by pyrazole (Fig. 9) confirming a previous report of Rubin *et al.*;[15] aniline used as a control also gave a type II spectrum, with a maximum of extinction at 430 nm and a minimum at about 390 nm.

The incubation of pyrazole with liver microsomes, in a system supplied with a generator of NADPH, led to the inactivation of catalase, as shown in Fig. 10. About 70 per cent of the activity of the catalase added to the microsomal system is lost after 2 hr in the presence of pyrazole, while only 20 per cent disappears in the control experiment.

The characteristics of the inactivation of catalase *in vitro* in a system containing pyrazole, microsomes and a source of NADPH are summarized in Table 3. In the absence of pyrazole, the activity of catalase decreases by 29·8 ± 9·9 per cent in a process that is partially dependent on the presence of the NADPH generating system, as judged from the apparent activation of catalase in its absence. The activity of catalase at the end of the 2-hr incubation period has been used as a reference value to

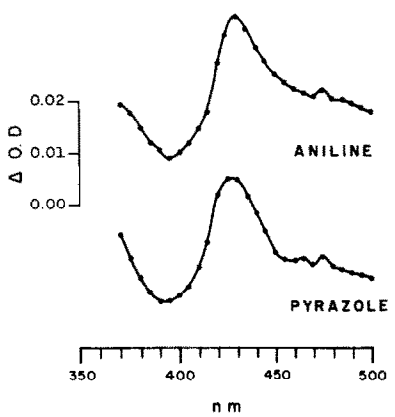

FIG. 9. Cytochrome P-450 spectral changes induced by pyrazole and aniline.

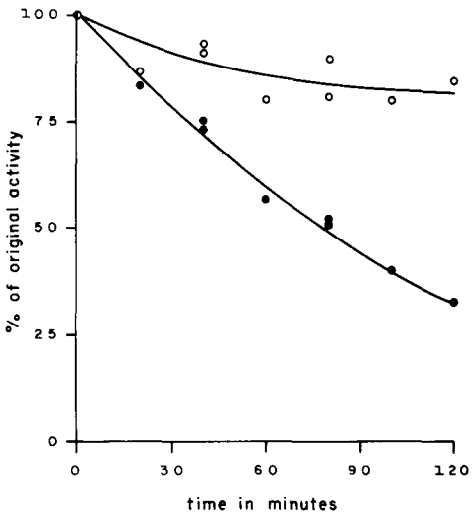

FIG. 10. Microsomal inactivation of purified catalase in the presence (solid circles) or absence (open circles) of pyrazole.

study the effect of modifications in the incubation conditions. The 58·6 per cent inactivation of catalase is partially but significantly reduced when the incubation is carried under nitrogen or a 20%–80% mixture of oxygen and carbon monoxide. The inactivation is also depressed by the presence of SKF-525A. The NADPH generating system is an absolute requirement; without it pyrazole has no effect and the pyrazole independent catalase inactivation is reduced.

DISCUSSION

Liver distribution and elimination of pyrazole. Pyrazole was found only in the soluble fraction, a distribution meaning that pyrazole either does not enter cell organelles or is washed out during the sedimentation and resuspension steps involved in the fractionation.

The elimination of pyrazole from the liver and the blood reveals widely different half-lives in these compartments. We assume that the method used detects total and not only free pyrazole, since the recovery in addition experiments with blood or liver was complete. We have also assumed that the assay detects pyrazole and not its metabolic products, which might be rapidly eliminated or lack inhibitory effect on alcohol dehydrogenase; the simple exponential nature of the two curves supports this idea.

Pyrazole is found in the same cell compartment with alcohol dehydrogenase, an enzyme it inhibits competitively for ethanol with a K_i of 2·2 μM, as we have previously determined for the rat.[5] This value is three orders of magnitude lower than the maximum concentration reached in the liver after the administration of 340 mg/kg. In the analysis made by Goldberg and Rydberg[2] of the inhibition of ethanol metabolism *in vivo* by pyrazole, the administration of a maximum dose of 600 mg/kg retards the elimination of a constant dose of ethanol from a normal 4-hr value to a

period ranging from 35 to 50 hr. Lower doses of pyrazole gave shorter elimination times. We interpret their results as also reflecting the approximate times for the elimination of pyrazole down to values close to the K_i, for the following reasons. The effect obtained is proportional to the dose employed; in fact, the reciprocal of the maximal velocities, taken from their plots of blood ethanol concentration, correlate well with the dose of pyrazole employed. Also, in the presence of pyrazole, the change in the concentration of ethanol in the blood is no longer linear with time, reflecting the competition of the drugs for the enzyme. On a semi-logarithmic plot, the apparent first-order rate constants we calculated from their data either do not change or increase with time, making unlikely the progressively higher ratio of pyrazole to ethanol that would be expected if pyrazole were eliminated at a slower rate than ethanol. The half-life of 3·45 hr we found for pyrazole in the liver permits us to calculate that 35–50 hr after the administration of 600 mg/kg the concentration in liver water would range between 16 and 0·8 μM. This value is consistent with the above results and with our data showing that pyrazole clearance from the liver is a simple first-order process.

Some values for the half-life of pyrazole in rats have been reported on the basis of indirect evidence. Lester and Benson[3] estimate the half-life to be 14 hr from the spectrophotometric measurement of pyrazole recovered in urine; this value is a maximum because metabolism of pyrazole could make it undetectable, leading to an underestimation of the velocity of elimination. Deitrich *et al.*,[16] measuring the inhibition of NADH-dependent aldehyde reduction in rat liver at various intervals after pyrazole administration, report a half-life for the inhibition of about 76 hr; however, we calculate, from their reported temporal course of inhibition and their dose–response curve, a decay of pyrazole with a half-life one-fifth as large.

The slower elimination of pyrazole from the blood as compared to the liver is difficult to interpret. Binding to albumin could account for a slower elimination. However, the apparent volume of distribution calculated from the elimination curve by extrapolation to zero time, 620 ml water per kg of body weight, is too high for a drug with an important fraction binding to albumin.

Kinetics of the changes in catalase activity in vivo. The temporal course of catalase activity after a dose of 70 mg/kg has two periods, inactivation and reactivation, separated by a point of minimum activity at 28 hr. Our previous demonstration[5] that alcohol protects catalase against the inactivation by pyrazole and our present results with purified catalase strongly suggest that catalase complex I,[17] the complex of native catalase and hydrogen peroxide, is the form inactivated in a reaction analogous to the inactivation of catalase by 3-amino-1,2,4-triazole.[9] The half-life for the inactivation of catalase *in vivo* with 3-amino-1,2,4-triazole is 8 min[7] in a process that could be rate-limited by the availability of hydrogen peroxide and consequently of complex I. With pyrazole, we found a half-life of 8 hr for the inactivation of catalase *in vivo*; this much slower process could be rate-limited at another step, probably at the production of the active pyrazole derivative discussed below. In contrast, with 3-amino-1,2,4-triazole, when the point of low activity is reached, the gradual recovery starts immediately, thus providing another argument to assume that the availability of pyrazole, or rather of its derivative, is the limiting factor in the inactivation.

The analysis of the velocity of recovery of catalase activity, as described in

Methods, reveals a half-life of 1·19 days. This value agrees with previous determinations of catalase turnover rate constants[7,18] and with all the half-life determinations made for peroxisomal proteins.* Assuming that the drug does not affect the turnover of catalase, this half-life demonstrates that the irreversibility of catalase activity is maintained *in vivo* as well as *in vitro*. We can also conclude from the rate of inactivation and the half-life of liver catalase that the reduction of catalase activity *in vivo* cannot be due to inhibition of synthesis.

Mechanism of the inactivation of catalase by pyrazole. The lack of inhibitory effect on catalase shown by pyrazole when added to liver homogenate or to purified catalase in the presence of hydrogen peroxide, together with the delayed nature of its effect *in vivo*, was interpreted as a demonstration that the inactivator of catalase is not pyrazole but another molecule.

The dialysis *in vivo* of catalase implanted in the peritoneal cavity of rats proved that a dialyzable molecule, capable of inactivating catalase in the presence of hydrogen peroxide, was present in the circulation of pyrazole-treated rats. Such a molecule could derive directly from the metabolism of pyrazole, or from another source as an indirect effect of the drug. The microsomal mixed-function oxidase system was obviously the most probable site for pyrazole metabolism, since the drug induces a type II spectral change, a modification characteristically induced by aniline and several nitrogenous bases[14] metabolized by microsomes. In fact, the incubation of pyrazole with rat liver microsomes demonstrated that added catalase is inactivated in a process requiring NADPH and O_2 and inhibited by CO and SKF-525A. However, the need for endogenous production of H_2O_2 makes it difficult to interpret the experiments in the absence of oxygen. We conclude that pyrazole is metabolized by the microsomal mixed-function oxidase system with the production of a metabolite that presumably reacts with catalase hydrogen peroxide complex I, yielding inactive catalase as a product.

Pyrazole and 3-amino-1,2,4-triazole as catalase inactivators. These two drugs have remarkably similar effects on catalase. The pattern of inhibition we describe with pyrazole inactivation in liver and kidney, and conservation of blood catalase–is the same pattern described by Heim *et al.*[19] for 3-amino-1,2,4-triazole. The lack of effect on red blood cell catalase could be attributed to a lack of catalase complex I, due to the utilization of endogenous hydrogen peroxide by glutathione peroxidase,[20] even though this possibility is controversial.[21] The mechanism of inhibition is analogous to that described by Margoliash *et al.*[9] for 3-amino-1,2,4-triazole. In both cases, the presence of hydrogen peroxide is required and the reaction is blocked by substances that act as hydrogen donors for catalase–hydrogen peroxide complex I. Moreover, both inhibitions are irreversible. Margoliash *et al*[9] established that the minimal structure required for a compound to inhibit catalase irreversibly was $N.NH.C(NH_2):R$, or one of its isomers, in which the primary amino group attached to the C atom had to be unsubstituted, and R could be S, O or NH. We do not know as yet the structure of the metabolic products of pyrazole, but it will be interesting to compare it with this minimal structure proposed for catalase inactivation.

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