Biochemical Pharmacology, Vol. 18, pp 1749–1762. Pergamon Press. 1969. Printed in Great Britain

INHIBITION OF ETHANOL METABOLISM *IN VIVO* BY ADMINISTRATION OF PYRAZOLE

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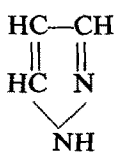
(*Received* 15 *January* 1969; *accepted* 22 *February* 1969)

Abstract—In experiments in rats, the *in vivo* effect of pyrazole on ethanol metabolism was studied. Pyrazole acts as a competitive ADH inhibitor. Ethanol at a dose of 32·6 m-mole/kg was administered. Blood samples were withdrawn at regular intervals, and ethanol analyzed by the automated ADH method and by gas chromatography. Pyrazole in doses of 0·07–8·82 m-mole/kg inhibited the elimination of ethanol by 20 to 90 per cent compared to the control experiments. In the control experiments, ethanol was eliminated in 4 hr, but, after a dose of 8·82 m-mole/kg pyrazole, elimination time was prolonged to between 35 and 50 hr. Inhibition of ethanol metabolism was confirmed by studies with ^{14}C-1-ethanol, which showed a decrease in the excretion rate after pyrazole, with a subsequent decrease in the excretion of $^{14}CO_2$. The specificity of the inhibitor is discussed.

IN INVESTIGATIONS on the interaction *in vitro* between liver alcohol dehydrogenase (LADH) prepared from horse liver, and a large number of heterocyclic compounds, Theorell and Yonetani found pyrazole ($m = 68$) to be a most potent inhibitor of LADH.[1]



The formation of an inactive ternary complex by LADH-NAD and pyrazole was demonstrated, the interaction between ethanol and pyrazole being of a competitive nature.

Tentative experiments in dogs showed pyrazole to be active as an inhibitor of ethanol metabolism *in vivo*.*

In our laboratory, as part of a study on possible means of influencing behaviour and ethanol metabolism, investigations on the metabolism of ethanol in the rat have been performed, with various doses of ethanol and with use of isotope technique.[2, 3] It was suggested to us by Theorell to study the properties of pyrazole *in vivo* in this respect.

Preliminary results, reported by Goldberg and Rydberg,[4, 5] Rydberg[6] and Theorell,[7, 8] showed the dose-dependent inhibitory effect of pyrazole on ethanol metabolism, ED_{50} in the rat being 0·29–0·43 m-mole/kg pyrazole. Also a number of

* A. Wretlind, unpublished observations.

other compounds were studied; among others 4-iodo-pyrazole was shown also *in vivo* to be as potent as pyrazole.*

Studies in rats on the effects of various pyrazole analogues and other compounds on ethanol metabolism were carried out by Lester *et al.*[9] A dose of less than 0·5 m-mole pyrazole per kg induced an inhibition in the rate of elimination of ethanol by more than 25 per cent from the control value.

With regard to the toxicity of pyrazole the lethal dose was found to depend among others on species and on time of administration (Wilson and Bottiglieri[10]). While the acute toxicity (LD_{50}) is around 11 m-mole/kg in the rat, the lethal dose after administration of pyrazole for 14 days was 2·2 m-mole/kg/day. In dogs and in man, considerable side-effects were observed after long term treatment, with doses as low as 0·15–0·30 m-mole/kg/day, causing signs of severe damage to bone marrow, hepatic and renal function. With regard to liver toxicity, tentative experiments with rat liver mitochondria after incubation with different substrates, have shown that treatment with 2·9 m-mole/kg pyrazole every third day for 20 days decreased to a certain extent the oxygen consumption as one indication of some impairment of mitchondrial function and respiration control†

The possibility that pyrazole circulating in the blood could by itself inhibit the YADH used in the enzymatic ADH method prompted us to test this hypothesis and to compare the results obtained by the enzymatic ADH technique with those by other methods, viz. the Widmark method and gas chromatography.

The aim of the present study was (1) to ascertain the possible influence of pyrazole on different methods for determination of ethanol; (2) to investigate the interaction between pyrazole and ethanol metabolism *in vivo* in rats, with special reference to (a) changes in the rate of elimination of ethanol from the blood, and (b) the relationship between degree of inhibition of ethanol metabolism and dose of pyrazole, and (3) to elucidate the possible influence by pyrazole on the excretion of $^{14}CO_2$ from labelled ethanol.

MATERIAL AND METHODS

Material

Thirty-five male rats of the Sprague–Dawley strain, weighing 265–280 g, and for comparison seventeen male rats of the Wistar strain.

Chemicals

Ethanol in a 12% w/v solution, in distilled water.

Pyrazole (synthetized by Dr. B. Sjöberg, Astra Ltd., or obtained from Schuchardt Ltd.), in a 2% w/v solution in distilled water.

^{14}C-1-*ethanol* (Radiochemical Centre, Amersham, UK) was used, with a specific activity of 5·6 mCi/mM.

Methods

Ethanol was injected intraperitoneally (i.p.), in either a constant dose of 32·6 m-mole/kg (1·5 g/kg), or in doses varying from 10·9 to 65·2 m-mole/kg.

Pyrazole was injected i.p. either 15 min *before* or 120 minutes *after* the administration of ethanol (1 m-mole/kg = 68 mg/kg).

* U. Rydberg, to be published.
† K.-H. Kiessling and U. Rydberg, to be published.

In the isotope experiments, *^{14}C-1-ethanol* was administered in a dose of 4μCi to each animal. This activity was diluted with an ethanol solution of 12% (w/v) in an amount required to obtain the desired dose of ethanol for each animal.

Ethanol sampling and analysis. Duplicate samples for ethanol analysis were withdrawn from the tip of the tail, at 30 min and then at 60 min intervals for 7 hr, then at longer intervals, after administration of ethanol until the blood ethanol concentration reached zero, for a total of 5–50 hr.

Ethanol was analyzed by (1) *gas chromatography.* An F & M gas chromatograph, model 609, was used, with a flame ionization detector and a column either with Carbowax 20 M, 10% on Chromosorb W[11], or with Triton X-305, 10% on Chromosorb W.* An internal standard of *n*-propanol was utilized (Curry *et al.*[12]). In duplicate samples, the experimental error was $\pm$3 mg per 100 ml blood.

(2) *The Widmark method*[13] was used for comparison in some experiments, the samples being withdrawn into Widmark capillaries. The experimental error was $\pm$ 3 mg per 100 ml blood.

(3) *The enzymatic ADH method* (Bonnichsen[14]), adapted to an automated procedure with the use of a Technicon Auto Analyzer (Goldberg and Rydberg[15]), used for comparison with other techniques, the experiment error 2·4 mg ethanol per 100 ml blood.

In the *isotope* experiments, the radioactivity of the expired $^{14}CO_2$ was measured according to Tolbert,[16, 17] in an ionization chamber of 200 ml volume, connected to a sensitive electrometer (Frieseke & Hoepfner), with a high input resistance, 10^{11} ohms, and continuously recorded on an Enograph G recorder (Rohde & Schwarz, Type ZSG). The system has an accuracy in zero line for 24 hr of $\pm$1%. For further details, see Rydberg and Skerfving.[3]

Evaluation. From the blood ethanol curves, the following variables were calculated:

Time in minutes till blood ethanol equalled zero, defined as the x-intercept of the rectilinear regression line;

Rate of elimination in mg/kg/min, defined as dose eliminated per time interval.

The excretion rate of $^{14}CO_2$ is here not reported as per cent of total amount per time interval, but in the relative means of "*mV*", versus time.

RESULTS

I. *Effect of pyrazole on methods for ethanol determination*

(*a*) In vitro *studies.* In one series of experiments, the possible influence of pyrazole on the enzymatic properties of yeast alcohol dehydrogenase (YADH) was studied. (Fig. 1). In two different sets, 3 ml phosphate buffer pH 8·8, 85 μg NAD, 40 μl YADH (50 mg/ml) and 40 μl ethanol solution of various concentrations (52, 103, 198 and 300 mg ethanol per 100 ml solution), were incubated at room temperature. In one of the sets, 0·2 mg pyrazole was added to each cuvette. The specific absorption at 340 nm was recorded after 60 min.

It was found that the addition of pyrazole depressed the specific absorption observed, the degree of inhibition in the concentrations used corresponding to 80–87 per cent.

This technique might be adapted as one means of determining in an indirect way the amount of pyrazole present in samples of various kinds.

* J. Buijten, L. Goldberg and U. Rydberg, unpublished data.

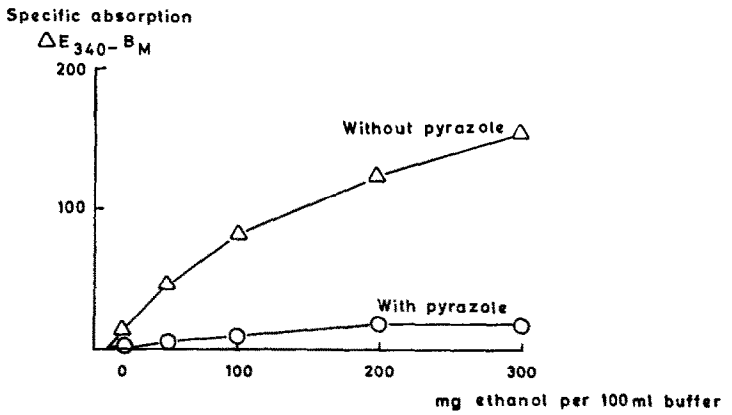


FIG. 1. Specific absorption at 340 nm after incubation of YADH and NAD, with and without pyrazole, and with ethanol.

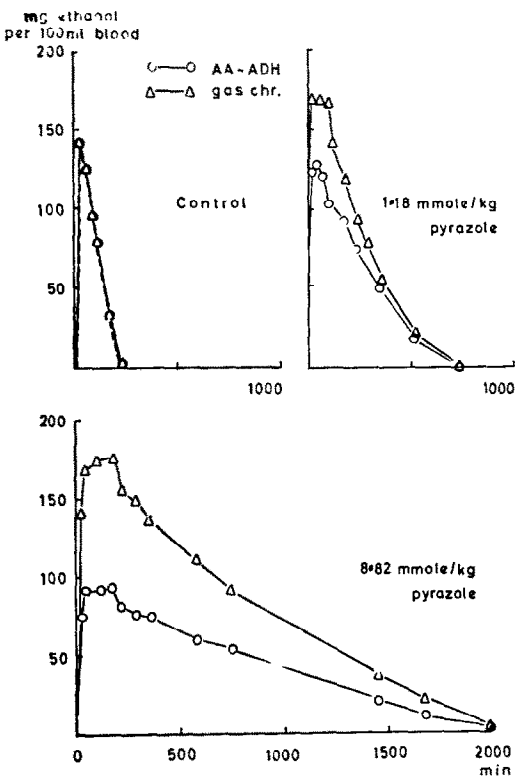


FIG. 2. Ethanol determinations by gas chromatography and by the ADH method. (a) control experiment (b) with 1·18 or (c) with 8·82 m-mole/kg pyrazole.

(b) In vivo *studies.* To test the *in vitro* findings under *in vivo* conditions, the possible inhibition of YADH by pyrazole was studied by comparison of ethanol concentrations in blood samples from rats given ethanol and pyrazole, ethanol determined by three independent methods: (1) the alcohol dehydrogenase method, utilizing YADH (2) the Widmark method (3) gas chromatography.

In the control experiments when only ethanol was given, the results obtained by the various methods were in good agreement, and no systematic differences could be found between the values obtained by the three various methods (Fig. 2a). In Fig. 2 only the determination with the ADH method and with gas chromatography are shown.

When pyrazole in doses of 0·07–8.82 m-mole/kg was given 15 min before the ethanol, already after a dose of pyrazole of 0·07 m-mole/kg, the blood ethanol concentration when determined by the YADH method was systematically lower than when determined by gas chromatography. With higher doses of pyrazole, the differences between the two methods of determination increased (Fig. 2b and 2c).

One explanation may be that the pyrazole circulating in the blood inhibits part of the YADH used for determination of ethanol, causing less free ADH to be available, but not causing any change, when determining ethanol by gas chromatography or by the Widmark method.

In order to elucidate whether the degree of reduction of the blood ethanol curve when determined by the YADH method is the same during the whole course or changes with time, the ratio between the ethanol concentration determined by the ADH method and by gas chromatography was calculated during the whole course of the experiments. Within the same experiment, the ratio kept constant, indicating the relation pyrazole : ethanol to be unchanged. With increasing doses of pyrazole the ratio between blood ethanol values when determined by the ADH method and gas chromatography decreased; with a pyrazole dose of 8·82 m-mole/kg the ratio was on an average 0·55 (Fig. 3).

This indicates that when the enzymatic ADH method for determination of ethanol is used in the presence of pyrazole, the possibility of pyrazole causing a depression of the ethanol values must be taken into account.

II. *Effects of pyrazole on ethanol metabolism*

1. *Control experiments*

A typical experiment is shown in Fig. 4a, when 32·6 m-mole/kg ethanol was given intraperitoneally. Blood ethanol concentration was followed for 5 hr. The rectilinear decline of the curve is evident. The mean time until blood ethanol reached zero was 240 min. The mean rate of elimination was 0·64 mg per 100 ml per min.

2. *Pyrazole administered after ethanol*

A typical finding is illustrated in Fig. 4b, showing the effect of administering pyrazole in a dose of 0·30 m-mole/kg 120 min *after* the administration of 32·6 m-mole/kg ethanol. Within a few minutes after the administration of pyrazole, the rate of elimination of ethanol declined, to 0·31 mg per 100 ml per min. The inhibitory effect of pyrazole on the metabolism of ethanol *in vivo* is clearly seen, the duration of ethanol in the blood being prolonged. Moreover, the normal rectilinear curve shows a suggestive exponential course; this is more evident with the higher doses.

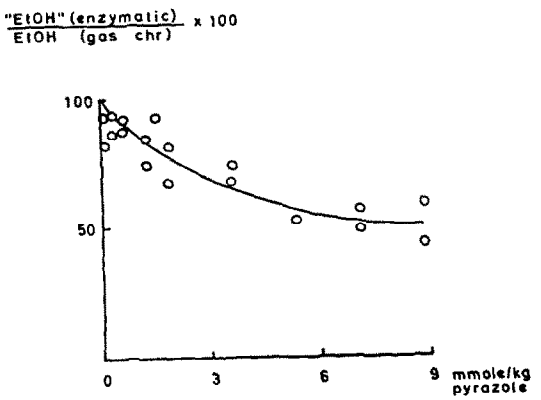


FIG. 3. Reduction in per cent of "enzymatically determined ethanol" from "gas chromatographically determined ethanol", in rats after 32·6 m-mole/kg ethanol and various doses of pyrazole.

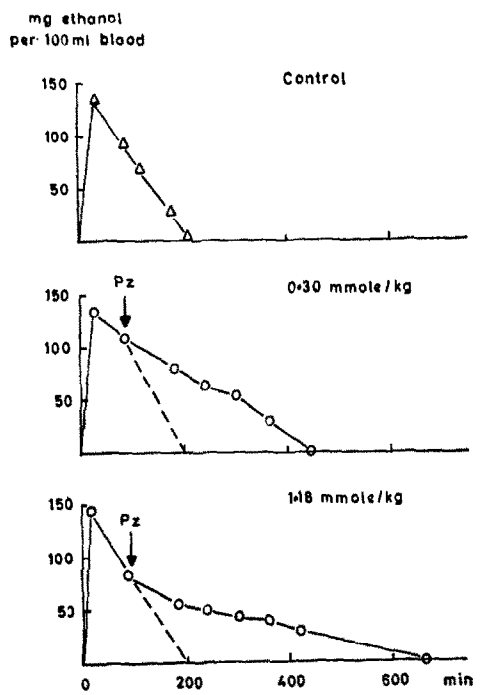


FIG. 4. Ethanol values after 32·6 m-mole/kg ethanol in rats. (a) control; (b) 0·30 m-mole/kg pyrazole 120 min *after* the ethanol; (c) 1·18 m-mole/kg pyrazole 120 min after the ethanol.

In Fig. 4c the effect of a higher dose of pyrazole, 1·18 m-mole/kg is illustrated. The rate of elimination of ethanol is in this experiment only 0·13 mg ethanol per 100 ml blood per min.

Degree of inhibition. The degree of inhibition of the ethanol metabolism brought about by the pyrazole was in these cases calculated from the change in rate of disappearance of ethanol from the blood (β, Widmark), according to the formula:

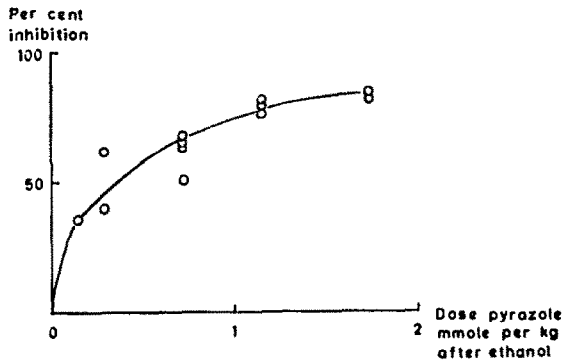


FIG. 5. Inhibition of ethanol elimination after various doses of pyrazole administered *after* ethanol.

$$\text{Degree of inhibition} = 100 \cdot \frac{^{\beta}\text{control} - ^{\beta}\text{pyrazole}}{^{\beta}\text{control}} \quad (1)$$

Relation between degree of inhibition and dose. The doses studied of 0·07–1·76 m-mole/kg pyrazole administered 100–120 min after the ethanol caused a degree of inhibition of 35–83 per cent (Fig. 5). A 50 per cent inhibition (ED_{50}) was obtained with a dose of 0·29 m-mole/kg pyrazole.

3. *Effects of pyrazole administered before ethanol*

The long-lasting effects of pyrazole observed suggested the possibility that pyrazole might also exert an inhibitory effect when administered before the ethanol. Therefore pyrazole in doses of 0·07–8·82 m-mole/kg was administered 15 min *before* a dose of 32·6 m-mole/kg ethanol. Typical examples are given in Fig. 6. It is seen that the

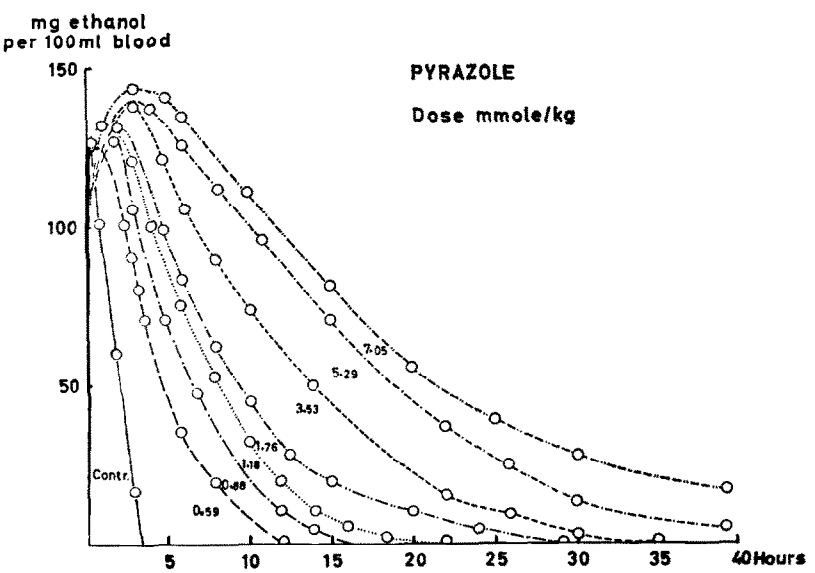


FIG. 6. Ethanol values after 32·6 m-mole/kg ethanol in rats, (a) control; (b) 0·59–7·05 m-mole/kg pyrazole given 15 min *before* the ethanol.

administration of pyrazole before the ethanol brought about a change in the course of the blood ethanol concentration, leading to a less steep and longer-lasting decline of the curve representing the disappearance phase of the ethanol. Already 0·20 m-mole/kg brought about a clear change, ethanol being present for 600 min against 235 min in the controls. With increased doses of pyrazole the ethanol curve is prolonged. With a dose of 7·05 m-mole/kg, the time for elimination was very prolonged, from *ca* 4 hr in the control experiments to 35–40 hr at an average. In some experiments with these high doses, ethanol was present for over 72 hr.

Also in this kind of experiments, the type of the disappearance curve changed from linearity to a curvilinear course, resembling an exponential curve, with an initially rapid, later only slowly declining course (Fig. 6). Moreover, a change in the absorptive part of the blood ethanol curve was evident. The sharp peak of absorption observed in the control experiments was changed into a prolonged absorption period (Fig. 6).

Rate of elimination. In the control experiments, the time until ethanol reached zero was 235 min. The rate of elimination was 45 mg ethanol per kg per min. With the highest doses of pyrazole, up to 8·82 m-mole/kg, the time for elimination was prolonged up to 2100 min and the rate of elimination decreased to 0·8 mg/kg per min.

Degree of inhibition. In the experiments when pyrazole was given *before* ethanol, the degree of inhibition was calculated from the change in time (in min) until the ethanol disappeared from the blood, according to the formula:

$$\text{Degree of inhibition} = 100 \times \frac{\text{minutes}_{\text{pyrazole}} - \text{minutes}_{\text{control}}}{\text{minutes}_{\text{pyrazole}}} \qquad (2)$$

Relationship between dose of pyrazole and degree of inhibition. With increasing doses of pyrazole, the degree of inhibition rose. As examples may be mentioned that a dose of 0·07 m-mole/kg brought about an inhibition of 21 per cent, and 7·05 m-mole/kg of 89 per cent (Fig. 7). A 50 per cent inhibition (ED_{50}) was obtained with a dose of 0·30 m-mole/kg pyrazole, thus well in accordance with the experiments when pyrazole was administered after pyrazole (see above).

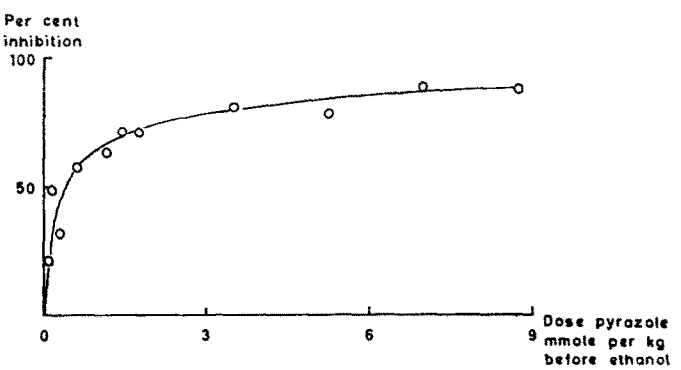


FIG. 7. Degree of inhibition of ethanol elimination after 0·07–8·82 m-mole/kg pyrazole administered *before* ethanol.

4. *Relation of dose of pyrazole to dose of ethanol*

The results given using a constant dose of ethanol and varying doses of pyrazole illustrate the competition between ethanol and pyrazole (Fig. 6), as was found *in vitro* by Theorell and Yonetani.[1] In order further to elucidate the competitive nature of the interaction both the doses of ethanol and of pyrazole were varied, pyrazole always administered 15 min before the ethanol. As seen in Table 1 it can be derived that at all levels of pyrazole used, an increase in the ethanol dose caused a decrease in the degree of inhibition, thus confirming the competitive nature of the inhibition caused by pyrazole.

5. *Calculated degree of inhibition utilizing different methods for ethanol determination*

In a series of experiments when various doses of pyrazole were administered before the ethanol, samples were analyzed according to both the YADH method and by gas chromatography (Fig. 2). Though the absolute values were different when calculated by the two different methods, the degree of inhibition when calculated by formula (2) above, turned out to be the same whether based on true ethanol values, determined by gas chromatography, or by "reduced" ethanol values obtained by the enzymatic YADH method.

6. *Strain differences in susceptibility to pyrazole on ethanol metabolism*

In tentative experiments with another strain of rats, the Wistar strain, a slightly higher susceptibility for pyrazole was observed. Thus in this strain a certain dose of pyrazole brought about a higher degree of inhibition of ethanol metabolism than in the Sprague–Dawley strain.

7. *Change in behaviour by ethanol and pyrazole*

The dose of ethanol given, 32·6 m-mole/kg, caused a certain degree of immobilization, ataxia for some time, and complete recovery within 1–2 hr. Small doses of pyrazole, 0·07–0·30 m-mole/kg, caused no essential change in behaviour of the rats, when given with the ethanol, as compared to controls with ethanol only. Larger doses of pyrazole, 1·20–8·82 m-mole/kg, caused an increase in the behavioral changes brought about by the ethanol alone. The addition of pyrazole thus not only brought about a prolongation of the presence of ethanol, but also a higher degree of impairment. With the highest doses the animals were immobilized with loss of righting reflexes for many hours.

These results may point to the possibility of the interaction of pyrazole and ethanol to influence central nervous mechanisms. The nature of this interaction has not yet been elucidated.

III. *Inhibition of* ^{14}C-1-*ethanol metabolism* in vivo *by pyrazole*

1. *Control*

In a control series, 32·6 m-mole/kg ethanol and 4 μCi ^{14}C-1-ethanol were administered. A typical example is seen in Fig. 8. The blood ethanol curve followed the same principal course as in the series described above (Figs. 2, 4), with ethanol concentration equalling zero at about 245 min.

The excretion of $^{14}CO_2$ showed three stages:

(1) An *exponential rising part*, parallel to the absorption phase in the blood ethanol

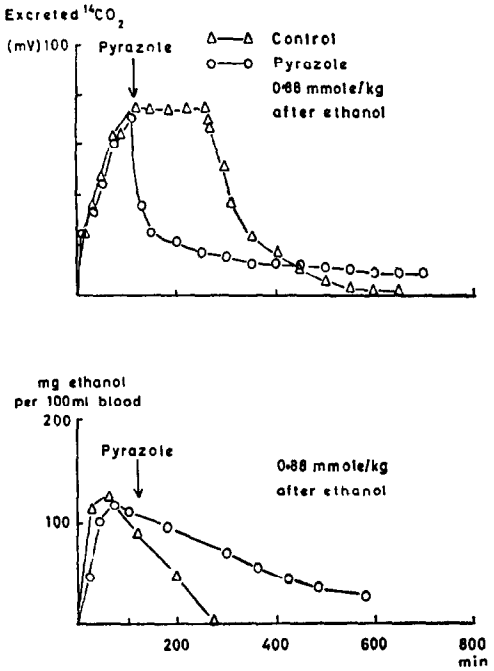


FIG. 8. Blood ethanol values and excretion of $^{14}CO_2$ in a control experiment and with 0·88 m-mole/kg pyrazole 120 min *after* ethanol.

curve, the end of this part essentially coinciding in time with the peak of blood ethanol, or occurring somewhat later.

(2) A *horizontal part* coinciding with the declining part of the blood ethanol curve, lasting until the blood ethanol had reached a level where the elimination system is no more saturated or at a concentration of 5–10 mg ethanol per 100 ml blood, the horizontal part indicating a constant rate of metabolism of ethanol.

(3) An *exponential falling part*, lasting for many hours after ethanol left the blood, indicating that part of the ^{14}C from the ethanol is incorporated into other substances, the turnover of these being inferior to that of ethanol.

2. *^{14}C-1-ethanol metabolism and pyrazole given after ethanol*

The effect of 0·88 m-mole/kg pyrazole, given 120 min after the administration of 32·6 m-mole/kg ethanol, is given in Fig. 8.

The blood ethanol curve changed its slope and followed a less steep decline, i.e. a delay in the breakdown of ethanol occurred. Using formula (1) above, the degree of inhibition was calculated to be 80 per cent.

This inhibition is corroborated by the course of the $^{14}CO_2$ excretion. Already 5–10 min after the administration of pyrazole the $^{14}CO_2$ curve falls steeply to a new, lower level, the reduction in level was 66–74 per cent of the "pre-pyrazole" level, or inhibition of the same magnitude as that seen in the slope of the blood ethanol curve. The $^{14}CO_2$ curve then showed a very slow, gradual decline, lasting for a longer time than after ethanol only.

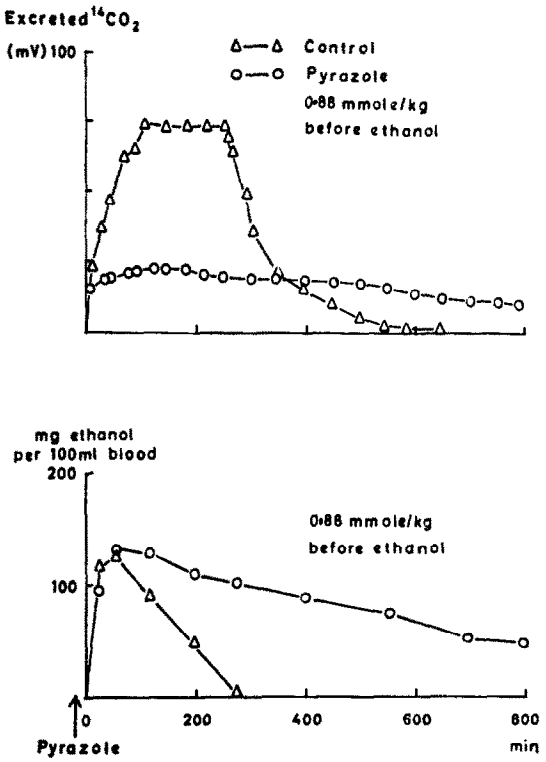


FIG. 9. Blood ethanol values and excretion of $^{14}CO_2$ in a control experiment and with 0·88 m-mole/kg pyrazole 15 min *before* ethanol.

TABLE 1. RELATION BETWEEN DEGREE OF INHIBITION BY PYRAZOLE OF ETHANOL METABOLISM *IN VIVO*, DOSE OF PYRAZOLE AND DOSE OF ETHANOL

Pyrazole m-mole/kg	Ethanol m-mole/kg	Degree of inhibition per cent
0·44	16·3	70
	32·6	48
0·59	10·9	77
	16·3	72
	21·7	60
	32·6	58
	43·4	64
	65·2	35
0·88	16·3	74
	32·6	57
1·76	16·3	74
	32·6	57
5·29	32·6	77
	65·2	71

3. *Metabolic effects of pyrazole administered before* ^{14}C-1-*ethanol*

One control experiment with 32·6 m-mole/kg ethanol and 4 μCi ^{14}C-1-ethanol is illustrated in Fig. 9, and one experiment when 0·88 m-mole/kg pyrazole was given 15 min before the ethanol.

The blood ethanol curve showed a slower decline after pyrazole than in the control situation, confirming the inhibition of the ethanol metabolism by the administered pyrazole.

The $^{14}CO_2$ curve after pyrazole showed the same principal elements as after ethanol only, viz. an exponential rising part, a horizontal part and an exponential part, but was quantitatively different. The horizontal part was depressed by 69 per cent, and lay on a lower level than as compared to the control with ethanol only. Further the horizontal level lasted for a considerably longer time than in the control situation. Finally $^{14}CO_2$ was excreted for a considerably longer time after ethanol had left the blood than after ethanol only.

DISCUSSION

The experiments described have clearly demonstrated that pyrazole already in small doses, down to 0·07 m-mole/kg, inhibits ethanol metabolism *in vivo*, both by (a) reducing the rate of decline of ethanol from the blood, leading to a delay of ethanol leaving the blood, and by (b) reducing the rate of excretion of $^{14}CO_2$ after administration of ^{14}C-labelled ethanol, as demonstrated by the depression of the horizontal part of the $^{14}CO_2$ curve, and by prolonging both the horizontal and the post-ethanol declining parts (Figs. 8 and 9).

The essential mechanism of action *in vivo* is most probably the same as that acting *in vitro*, viz. an inhibition of the ADH enzyme, as demonstrated by Theorell and Yonetani.[1]

The interaction of pyrazole and ethanol on the ADH molecule is competitive, which is supported by the *in vivo* findings: a dose–response relationship between degree of inhibition and dose of pyrazole. This dose–response relationship is demonstrated both with a constant dose of ethanol and varying doses of pyrazole, and with varying doses of ethanol and a constant dose of pyrazole. In some tentative experiments, when after an initial dose of ethanol a dose of pyrazole was given, the inhibition of ethanol elimination as described earlier was brought about. Later a second dose of ethanol was administered, this second dose again causing an increase in the rate of elimination of ethanol, thus confirming the competitive nature of the chemical reaction.

The change in the course of the declining part of the blood ethanol curve, from a rectilinear to an exponential course with the concavity upwards, can be interpreted as one indication of the long-lasting effect of pyrazole. Assuming that a constant ratio between pyrazole and ethanol leads to a constant degree of inhibition, three different situations may occur:

(1) a reduced concentration with time of pyrazole *in vivo* in relation to the ethanol concentration would lead to a declining blood alcohol curve first declining at a slow rate, while the pyrazole concentration still was high, and then increasing in rate, when the pyrazole concentration would fall and bring about a lower degree of inhibition. This condition would thus lead to a convex blood ethanol curve with the convexity upwards.

(2) a reduction in pyrazole concentration parallel to the reduction in blood ethanol would keep the degree of inhibition constant and lead to a more or less rectilinear fall of the blood ethanol.
(3) a fall in blood ethanol but the pyrazole concentration only slowly changing or not at all, would lead to a relative increase in the degree of inhibition and an increasing reduction in the rate of fall of the blood level, i.e. an exponential curve with the concavity upwards.

The actual curves found thus agree with condition (3), viz. one interpretation is that the concentration of pyrazole decreases more slowly than that of ethanol.

Another fact pointing in the same direction is the experiments with high doses of pyrazole (5·29–8·82 m-mole/kg), where the effects lasted for 2–3 days.

A third fact is that when the course of the blood ethanol curve is determined by the ADH method and by gas chromatography, the percentual reduction of the ethanol concentration as determined by the YADH method in comparison to the ethanol value obtained by gas chromatography was not changed during the course of an experiment, even when testing for many hours.

In this study it was found that the degree of inhibition *in vivo* of ethanol metabolism, brought about by the administration of pyrazole, was the same when calculated from the change in rate of ethanol disappearance, whether a method involving YADH was used, or techniques not influenced by the simultaneous presence of pyrazole in the sample, as the Widmark method or gas chromatography.

It has, however, been demonstrated that in a system where pyrazole is present, ethanol cannot be accurately determined by a method employing YADH, as even small quantities of pyrazole partially inhibit the YADH used for the ethanol determination. With the Widmark method, employing dichromate for reduction, or with gas chromatography, no interference was seen.

Acknowledgements—The problem was brought to our attention by Professor Hugo Theorell, the Nobel Institute for Biochemistry, Karolinska Institutet, Stockholm, who also supplied us with pyrazole, synthetized by Dr. Bernt Sjöberg, Astra Ltd., Södertälje, Sweden.

We are grateful to Professor Theorell and to Dr. M. Reynier, Toulon, France, for valuable discussion on the problem. The skilful technical assistance of Mr. J. Buijten, Mrs. A. Hultén and Mrs. A. Neuman is also acknowledged.

The costs of the study have been defrayed by grants from the Swedish Medical Research Council and the Swedish Institute for Research in Malt Beverages.

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