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## *Originalarbeiten — Original Papers*

# Influence of Pyrazole on Ethanol Determination with the automated ADH Method

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*Summary.* It has been demonstrated that the contamination of blood samples with pyrazole will disturb automated enzymatic determinations of ethanol by methods involving the use of YADH, if the sample is not distilled. Determination of ethanol by gas liquid chromatography, the Widmark method or by the ultramicro destillation enzymatic method for ethanol analysis (AA-UMDE) according to Buijten were not disturbed by the presence of pyrazole. Contamination of samples with 4-iodo-pyrazole has not been found to disturb ethanol determinations by any method.

*Key-Words:* Enzymatic methods for ethanol—Pyrazole, Influence on.

*Zusammenfassung.* Es wurde die Kontamination der Blutprobe mit Pyrazol in wäßrigen Lösung als Salbe verarbeitet sowie als 4-Iodo-Pyrazol-Lösung appliziert überprüft. Bei der Anwendung der Hefe-ADH zur automatisierten enzymatischen Blutalkoholbestimmung ohne Destillationsverfahren treten Störungen ein. Die Anwendung der Gas-chromatographischen Methode, des Widmark-Verfahrens sowie der enzymatischen Ultramikromethode nach Buijten verursacht keine Beeinflussung durch Pyrazol-Beimischung. Die Bestimmungen des Blutalkohols mittels dieser Methoden wurden nach Kontamination mit 4-Iodo-Pyrazol nicht beeinflußt. Auf die mögliche forensische Bedeutung wird hingewiesen.

Enzymatic methods involving an oxidation of NAD, catalyzed by alcohol dehydrogenase, and the absorption of formed $NADH_2^+$ measured photometrically at a wavelength of 340 or 366 nm, belong to the most utilized methods for determination of ethanol. Various modifications have been described and the specificity of the ADH methods has been critically discussed. Also a number of other substances than ethanol react with LADH (horse liver ADH) and YADH (yeast ADH). The oxidation of a number of higher aliphatic alcohols, esters, various steroids, retinol etc., will also be catalyzed by ADH (Theorell, 1969). The interference by such substances with enzymatic methods for ethanol determination has been demonstrated (Dotzauer *et al.*, 1952; Hilgermann, 1970; Weinig *et al.*, 1963).

Theorell and Yonetani (1963) published the first experimental work on *in vitro* studies of pyrazole and analogs as inhibitors of LADH. A large number of compounds has been investigated, as published by Lester *et al.* (1968) and by Theorell *et al.* (1969). Pyrazole, 4-bromo-pyrazole, 4-iodo-pyrazole and 4-methyl-pyrazole were found as the most potent inhibitors yet known.

The inhibition of ethanol metabolism *in vivo* in experimental animals by pyrazole and analogs has also been investigated (Goldberg and Rydberg, 1969;

Reynier, 1969). Increasing doses of pyrazole were found to inhibit the elimination of ethanol by 20–90%. Also the effect of 4-iodo-pyrazole has been investigated in vivo (Rydberg, 1969).

Considerable side-effects have been noted when pyrazole was administered to rats and dogs, with signs of toxic effects on blood, liver and kidneys (Wilson and Bottiglieri, 1962). The long term administration of pyrazole is associated with disturbances in the metabolism of glutamate, succinate and $\beta$-hydroxybutyrate when incubated with isolated liver mitochondria from rats treated with pyrazole for up to 30 days (Kiessling and Rydberg, to be publ.) Lelbach (1969) also found profound disturbances in liver metabolism and morphology after the administration of pyrazole and ethanol. These side-effects exclude the experimental administration of pyrazole to human subjects. However, 4-methyl-pyrazole has been investigated with regard to acute and chronic toxicity and has been administered to human subjects, and its effect on the metabolism of ethanol has been studied (Blomstrand and Theorell, 1970).

Also yeast ADH (YADH) is to a certain extent inhibited by pyrazole (Goldberg and Rydberg, 1969; Reynier, 1969; Singlevich and Barboriak, 1970).

The aim of the present study was to elucidate the possibility of disturbing the automated enzymatic determination of ethanol in blood samples from human subjects or patients by contamination with

1) pyrazole solution,
2) pyrazole "ointment",
3) 4-iodo-pyrazole solution.

## Material and Methods

Blood samples were collected from 7 alcoholic patients, just admitted to the Alcohol Clinic, with acute alcoholic intoxication, at 12 different occasions. Triple samples were collected for analysis of ethanol by four different methods:

1. *Gas liquid chromatography.* An F & M gas chromatograph, model 609, was used, with a flame ionization detector and a column with Carbowax 20 M, 10% on Chromosorb W with n-propanol as an internal standard.

2. *The automated YADH method (*Goldberg and Rydberg 1966).

3. *The ultramicro destillation method for enzymatic ethanol analysis* ("AA-UMDE", Buijten, to be publ.).

4. *The Widmark method.*

Samples were achieved under various conditions:

a) *Control.* A finger was desinfected with a 0.1% (w/v) solution of mercury chloride, and dried with cotton. A micro lancet was used for scarification, and samples were collected.

b) Desinfection as above, but the finger was contaminated with a *solution saturated with pyrazole* (Schuchardt Ltd.) in distilled water.

c) Desinfection and administration of an "*ointment*", a gel consisting of sodium carboxymethyl cellulose 5 g., Separan NP 10, 0.5 g, *pyrazole* 5 g and purified water ad 100 g.

d) Desinfection and administration of a water solution of *4-iodo-pyrazole,* with a tenside, Brij 35, added.

The inert components of the gel and the tenside exerted no influence on the methods for ethanol determination.

## Results

### *1. Control Determinations*

In the control condition there was a good agreement between determinations of ethanol with the four various methods. The ethanol concentrations varied be-

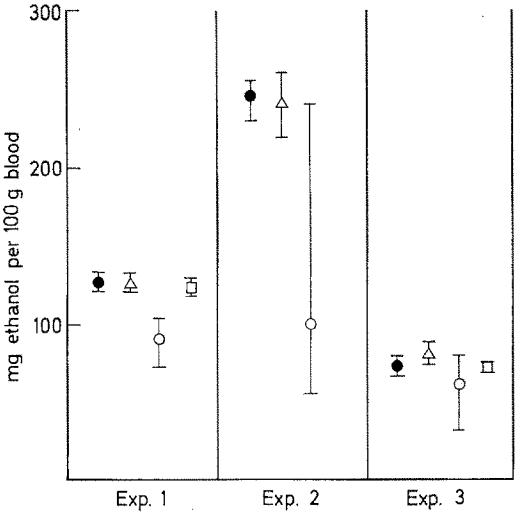


Fig. 1. Triple ethanol determinations in the presence of *pyrazole solution*, by gas liquid chromatography (●—●), the ultramicro destillation method (△—△), the automated ADH method (○—○), and by the Widmark method (□—□)

tween 48 and 301 mg/100 g blood. The S.D. in the various methods was 2 mg/100 g blood. Systematic differences between the various methods could not be observed.

*2. Contamination with Pyrazole Solution*

A comparison in three different experiments between the values obtained by the various methods when a solution of pyrazole was administered is shown in Fig. 1. In the figure the mean value and the range between the highest and the lowest value are demonstrated. Gas liquid chromatography, the Widmark method and the ultramicro destillation enzymatic method were uninfluenced by the presence of pyrazole, but the automated ADH method was significantly disturbed by the presence of pyrazole. There was a systematic tendency in the values, so that the first sample taken, when the concentration of the contaminating pyrazole was highest, the determination was most disturbed, and the apparent concentration of ethanol was lowest in the first sample. The obtained "apparent ethanol values" determined by the automated ADH method, and by gas chromatography, are given in the table.

*3. Contamination with "Pyrazole Ointment"*

An example is given in Fig. 2, when an ointment containing pyrazole first was applied to the finger from which the blood samples for ethanol analysis were taken. The same tendency can be seen, so that the ethanol analysis by the YADH method is disturbed also by this arrangement.

*4. Contamination with 4-iodo-pyrazole*

An example is given in Fig. 3. No systematic differences could be seen in the ethanol values, when the finger from which the blood sample for ethanol analysis was taken was contaminated with a solution of 4-iodo-pyrazole.

Table. *Ethanol values obtained in the presence of pyrazole solution with gas liquid chromatography (GLC-mean of three determinations) and by the automated ADH method (triple determination, single values). Mg ethanol/100 g blood*

| Exp. | GLC | ADH | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 1 | 128 | 74 | 104 | 104 |
| 2 | 249 | 56 | 88 | 242 |
| 3 | 77 | 34 | 82 | 79 |
| 4 | 100 | 87 | 97 | 103 |
| 5 | 70 | 68 | 70 | 69 |
| 6 | 48 | 46 | 46 | 49 |
| 7 | 114 | 89 | 121 | 119 |

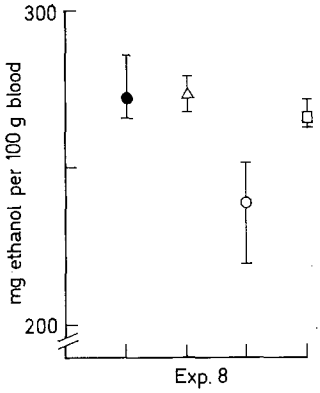


Fig. 2. Triple ethanol determinations in the presence of *pyrazole ointment*, by gas liquid chromatography (●—●), the ultramicro destillation method (△—△), the automated ADH method (○—○), and by the Widmark method (□—□)

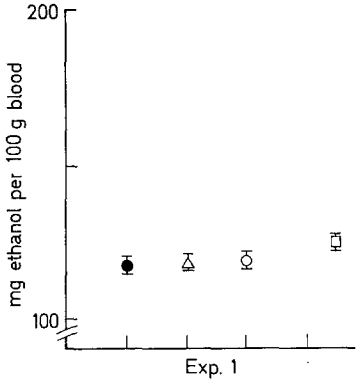


Fig. 3. Triple ethanol determinations in the presence of 4-*iodo-pyrazole*, by gas liquid chromatography (●—●), the ultramicro destillation method (△—△), the automated ADH method (○—○), and by the Widmark method (□—□)

## Discussion

The results described above confirm the in vitro findings by Reynier (1969) that though both pyrazole and 4-iodo-pyrazole are effective inhibitors of the oxidation of ethanol in the presence of LADH, only pyrazole is a potent YADH inhibitor. The practical importance of the observations described is limited, as it is not very likely that in forensic cases pyrazole would contaminate samples for enzymatic analysis of ethanol. One way to overcome the possibility of contamination is the *systematic* analysis by two independent methods, one being the Widmark method, gas chromatography or the ultramicro destillation enzymatic method (AA-UMDE). Moreover, as many analogs and derivatives of the pyrazole nucleus are utilized as drugs, other substances not yet investigated in the respect may exhibit similar properties. Also the importance of a thorough mechanical rubbing with a solution for desinfection when the sample for ethanol analysis is taken, must be stressed.

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