Thus, the inhibition of catalase by aminotriazole can serve to demonstrate the formation of H_2O_2 in biological systems. This method has been used to measure H_2O_2 secretion by microorganisms[8] and to detect H_2O_2 in erythrocytes in vitro[9] and in vivo[10]. We used the erythrocyte to detect formation of H_2O_2 in mice in vivo. Mice were injected with aminotriazole, which by itself (absence of H_2O_2) does not inhibit erythrocyte catalase[9,10]. On subsequent injection of 6-hydroxydopamine, erythrocyte catalase was inhibited. This showed formation of H_2O_2 from 6-OHDA in vivo.

Materials and methods. Male Swiss-Webster mice weighing 25 ± 1 g were divided into 4 groups. These received i.p. injections of 3-amino-1,2,4-triazole (Mann Research Laboratories, 1 g/kg in water; 2 injections, 1 h apart) and/or i.v. 6-hydroxydopamine (6-OHDA, hydrobromide salt, Regis Chemicals, 100 mg/kg injected within 5 min after the second dose of aminotriazole). The 6-OHDA was dissolved in dilute hydrochloric acid (pH 4.0) and stored on ice to avoid autoxidation (H_2O_2 formation).

One hour after completion of the 6-OHDA injections, 25 µl of blood was withdrawn from the tail into a heparinized micropipet. The blood was lysed in 5 ml cold water and samples were assayed for catalase activity[11]. Hemoglobin was measured with an azide-methemoglobin method[12]. Data were calculated as catalase activity per g hemoglobin. Statistical analyses were done using Student's *t*-test[13].

Results. Blood catalase activity was diminished significantly after injection of aminotriazole and 6-OHDA (Table). Neither aminotriazole alone nor 6-OHDA alone produced a significant decline in catalase.

Catalase activity in mouse blood

Group	Catalase activity (% Control ± S.D.)
Control	100 ± 12
Aminotriazole	96 ± 9[a]
Aminotriazole + 6-OHDA	72 ± 13[b]
6-OHDA	102 ± 13[a]

There were 8 animals in each group.
[a] Not significant when compared to control. [b] $p < 0.001$ when compared to control.

Discussion. Inhibition of tissue catalase in the presence of aminotriazole can serve as an indicator of intracellular H_2O_2[9,10]. The erythrocyte is a convenient detector for H_2O_2 as this cell is relatively rich in catalase and has been used previously to show H_2O_2 generation in vivo[10]. Our data (Table) showed that H_2O_2 was generated in vivo in erythrocytes of mice after i.v. injection of 6-OHDA. These results are consistent with the hypothesis that the cytotoxicity of H_2O_2 may be responsible for the degeneration of catecholaminergic neurons after the administration of 6-OHDA to experimental animals[14].

Zusammenfassung. Es gelang, die Erythrocytenkatalase der Maus durch Injektion von 6-Hydroxydopamin und in Gegenwart von 3-Amino-1,2,4-triazol zu hemmen, was auf die Wasserstoffperoxyd-Entwicklung hinweist und mit der Rolle des H_2O_2 bei der Degeneration der Nervenenden durch 6-Hydroxydopamin in Einklang steht.

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Toxicity of Pyrazole and 4-Methylpyrazole in Mice and Rats

Pyrazole effectively prevents the oxidation of ethanol both in vivo and vitro by inhibiting the liver alcohol dehydrogenases[1,2]. This discovery has opened new possibilities for studies of the pharmacology of ethanol. However, the use of pyrazole is limited due to its toxicological properties[3–5]. Some 4-substituted pyrazoles are more potent inhibitors of liver alcohol dehydrogenases than is the parent compound[6]. Of these 4-methylpyrazole appears the most promising for research and possible clinical use. The present study was performed in order to evaluate the toxicity of this compound and to compare it with the unsubstituted pyrazole.

Material and methods. The pyrazole was obtained from Fluka AG, Buchs SG, Switzerland, and the 4-methylpyrazole from the Research and Development Laboratories, AB Astra, Södertälje, Sweden. The compounds were administered as solutions in physiological saline (pH 3–5).

For the acute toxicity tests male rats of the Sprague-Dawley strain, weighing about 170 g, and male mice of the

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NMRI strain, weighing 18–22 g, were used. The test substances were given at 6 dose levels to groups of 6 rats or 10 mice by oral or intravenous route. After dosing the animals were observed for 7 days. Values for LD_{50} were calculated from the recorded mortalities by the method of LITCHFIELD and WILCOXON[7].

The subacute toxicity was studied in 2 experiments with 3-month-old Sprague-Dawley rats. The substances were given daily for 4 weeks by stomach tube at 2 dose levels (Table I). The rats were observed daily for clinical signs. At the end of the experiments, blood samples were collected for determinations of packed cell volume, hemoglobin, number of erythrocytes and leucocytes, glucose, urea-N, alanine aminotransferase (GPT), alkaline phosphatase, and serum protein. In the pyrazole study, reticulocyte and differential white cell counts were also performed. Standard analytical procedures were used, and dosed group values were compared with control group values by means of the WILCOXON[8] rank test. At termination all rats were killed and subjected to complete autopsy. An extensive microscopic examination of the tissues was carried out.

Results. The calculated LD_{50}-values expressed in mmol/kg are seen in Table II. In general, there were small differences in LD_{50}-values between rats and mice. The 24 h toxicity was considerably greater for 4-methylpyrazole than for pyrazole. It should be noticed that in animals given pyrazole there were more mortalities within 7 days than within 24 h and that this was most pronounced in mice.

Table I. Survey of the 4 weeks toxicity experiments

Compound	No. of animals per group	Dose (mmol/kg)		
		High	Low	Control
Pyrazole	10 ♂, 10 ♀	1.0	0.10	– (phys. saline)
4-Methyl-pyrazole	10 ♂	1.2–2.4[a]	0.12	– (phys. saline)

[a] The dose was increased after 3 weeks to 2.4.

The administration of 4-methylpyrazole for 4 weeks induced no toxic reaction in any dose group with regard to clinical signs, body weight gain, clinical chemistry or gross and microscopic pathology.

When pyrazole was given during the same time the most important clinical sign was a retarded body weight gain among rats in the high dose group. At autopsy the decrease in body weight gain was about 100 g for the males and about 50 g for the females. Further, in these animals significantly decreased values for packed cell volume, hemoglobin and number of erythrocytes were recorded. For instance, the mean value for packed cell volume in control male and female rats was 46.1 and 46.6, but only 42.6 and 41.0, respectively, in rats receiving the high dose.

Gross changes related to the treatment with pyrazole occurred in all rats of the high dose group and were observed in the thyroid, liver, testis and seminal vesicles. The thyroid was 2 to 3 times larger than normal. The liver was enlarged, the mean relative weight being 5.6% for treated rats and 3.9% for controls. The testes were diminished having somewhat less than the half of the normal weight. The seminal vesicles were slightly to moderately diminished.

Microscopic changes due to pyrazole were found in the thyroid, testis, prostate, seminal vesicles, bone marrow, and spleen. The change of the thyroid occurred at both dose levels but those of the other organs only at the high dose level. Within the group all rats showed the same alterations. In the thyroid there was a hyperplasia in the form of an interfollicular adenomatosis (Figure 1). The testis showed an atrophia with regressive changes of the epithelium (Figure 2). The tubules were diminished and the spermatogenous epithelium displayed vacuolization, desquamation, and necrobiotic nuclear changes. The interstitial tissue was oedematous. Atrophic changes appeared in the prostate and seminal vesicles. In the bone marrow there was a slightly to moderately reduced cellularity suggesting a depletion of cells. In both control and treated rats the staining of the spleen and bone marrow for iron gave a positive reaction indicating

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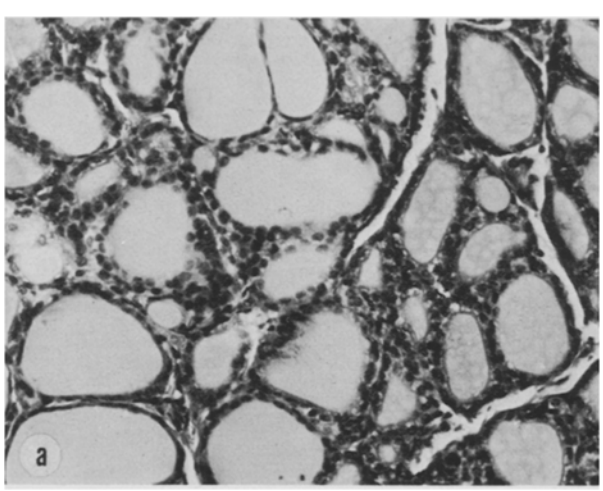

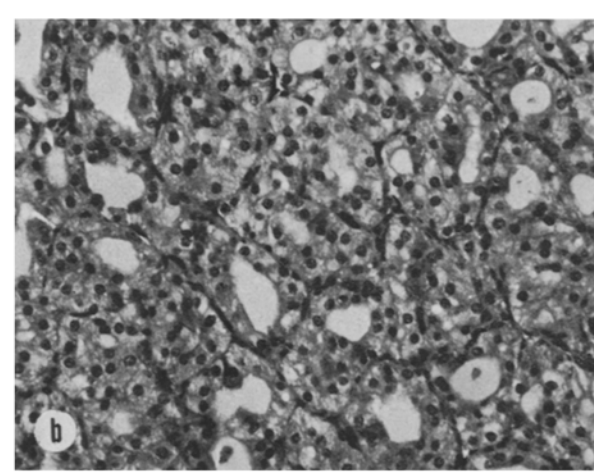

Fig. 1 a). Thyroid of a control rat. b) Thyroid of a rat after oral administration of 1.2–2.4 mmol/kg of pyrazole for 4 weeks. The thyroid consists of numerous small closely packed follicles lined by tall epithelial cells. Haematoxylin and eosin. Magnification ×180.

Table II. LD_{50}-values (mmol/kg, 95% confidence limits in parentheses), calculated on mortalities within 24 h and within 7 days

Compound	Species	24 h		7 d	
		p.o.	i.v.	p.o.	i.v.
Pyrazole	Rat	21 (20–23)	19 (18–21)	17 (14–20)	15 (12–19)
	Mouse	22 (21–24)	21 (20–23)	6.0 (3.6–9.4)	5.5[a] —
4-Methylpyrazole	Rat	7.9 (6.1–10.1)	3.8 (3.5–4.3)	6.5 (5.9–7.1)	3.8 (3.5–4.3)
	Mouse	7.8 (6.6–9.2)	3.8 (3.2–4.6)	7.8 (6.6–9.2)	3.8 (3.2–4.6)

[a] Graphical estimation.

deposits of haemosiderin. In the spleen the deposition of iron was significantly and in the bone marrow slightly denser in rats of the high dose group than in rats of the control one.

Discussion. The acute toxicity of pyrazole has previously been estimated in rats and mice after oral and intraperitoneal administration[3,4,9]. The results of the present study mainly agree with these earlier observations when comparable. In contrast to 4-methylpyrazole, pyrazole showed a greater acute toxicity after 7 days than after 1 day, especially manifested in mice. The observation indicates a delayed toxicity of pyrazole, which is in agreement with findings by LELBACH[4]. The delayed toxicity may be related to the essential differences between 4-methylpyrazole and pyrazole as indicated by the results of the subacute investigations. No lesions were found after treatment with the former compound, whereas the latter caused several changes. It was, thus, found that pyrazole induced a hyperplasia of the thyroid, a hepatomegaly, an atrophia of the testis and accessory glands, an anaemia and a depression of the bone marrow. Some of these alterations were also observed by WILSON and BOTTIGLIERI[3]. It is probable that organic lesions are involved in the mechanism of the delayed toxicity of pyrazole.

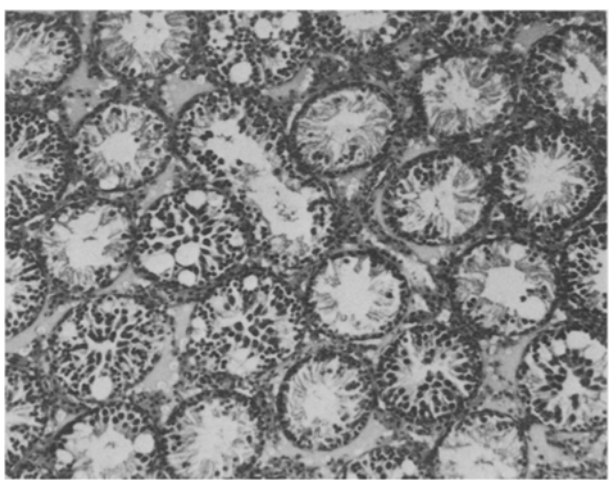

Fig. 2. Testis of a rat after oral administration of 1.2–2.4 mmol/kg pyrazole for 4 weeks. The testicular tissue shows an atrophia with degenerative changes of the seminiferous epithelium. Haematoxylin and eosin. Magnification ×75.

The present study shows that repeated oral administration of 4-methylpyrazole to rats is well tolerated in doses which inhibit the alcohol dehydrogenase efficiently[2]. This is in contrast to the unsubstituted pyrazole which in equimolar doses causes severe toxicity. Since the 4-methylpyrazole is several times more potent than pyrazole, it seems to be the compound of choice for pharmacological and metabolic studies of inhibition of ethanol metabolism.

Zusammenfassung. Subchronische Gaben von Pyrazol, nicht dagegen von 4-Methylpyrazol, führen bei Ratten und Mäusen zu toxischen Läsionen.

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Central Adrenergic Neurones and the Initiation and Development of Experimental Hypertension

Established arterial hypertension in both animals and human patients is characterized by an increased systemic flow resistance[1,2]. Mechanisms operating at the pre- and postjunctional site of the vascular neuroeffector system have been causally related to this elevated resistance. At the prejunctional site an increase of sympathetic vasoconstrictor activity (A) has been suggested[3,4]. Postjunctionally several processes have to be considered: The occurrence of humoral vasoconstrictor factors (B)[5,6], a change in the sensitivity of the individual vascular smooth muscle cells (C)[7,8] to an otherwise normal vasoconstrictor input or an increased reactivity of the blood vessels (D) due to an adaptive structural change of the vessel wall, i.e. an increase of the wall/lumen ratio[2,9]. Since D is the result of maintained high blood pressure, it seems to be of no importance for the initiation of the hypertensive state. However, A, B and C would be able to set in motion the process leading to hypertension. It may be assumed that the appearance of these factors (A, B, C) is triggered off by a hitherto unknown mechanism. A possible involvement of central adrenergic neurones in hypertension is indicated by a decrease in noradrenaline turnover in the brainstem of rats with an established DOCA/NaCl hypertension[10] and by an increase in norad-