Table II. Mitotic index of rat hepatocytes after partial hepatectomy and/or phenobarbital pre-treatment

Time after hepatectomy (h)	Mitotic index ± S.D. (%)			
	Controls (no treatment) ($n = 2$)	Phenobarbital ($n = 2$)	Partial hepatectomy alone ($n = 4$)	Phenobarbital + partial hepatectomy ($n = 4$)
16	0.017 ± 0.024	0.032 ± 0.018	0.051 ± 0.044	0.118 ± 0.083
24	0	0.018 ± 0.025	0.014 ± 0.017	1.081[a] ± 0.497
39	0	0	4.702 ± 1.127	1.749[a] ± 0.349

[a] $P < 0.01$ (vs. hepatectomy alone). Student t-test.

the total number of hepatocyte nuclei was counted. Percentage values, giving the ^{3}H-thymidine index and the mitotic index, were calculated.

The data were subjected to statistical analysis following transformation (Student's t-test and analysis of variance).

Results. The controls showed the expected minimal thymidine labelling in the hepatocyte nuclei (Table I). Phenobarbital alone produced no significant increase in labelling. Hepatectomy alone produced no obvious increase after 16 h. After 24 h there was a marked increase in thymidine incorporation, which was maintained at 39 h. Hepatectomy together with phenobarbital produced a greater increase in thymidine incorporation. At 16 h, phenobarbital pre-treatment in the hepatectomized rats was associated with an obvious and significant ($p < 0.01$) increase in the mean thymidine index. At 24 h the increased labelling in phenobarbital-hepatectomized rats was still greater than in only hepatectomized rats, although not significantly so, probably due to the large individual variation. Again at 39 h the phenobarbital pretreated rats had increased thymidine labelling compared with the hepatectomy-alone rats. The overall increase in thymidine incorporation in phenobarbital-hepatectomized rats was supported statistically using analysis of variance ($p < 0.01$).

Hepatectomy caused no increase in the mitotic index after 16 and 24 h (Table II). However, at 39 h hepatectomy produced a marked rise. Pre-treatment with phenobarbital induced a slight increase compared with hepatectomy-alone results at 16 h and a significant increase at 24 h. At 39 h, the mitotic index was significantly less in phenobarbital-treated hepatectomized rats, compared to non-treated hepatectomized animals. Analysis of variance revealed no overall significant difference between these two groups.

Comment. Our findings show that pre-operative treatment with phenobarbital of partially hepatectomized rats leads to an earlier onset of hepatocyte proliferation; in such animals, thymidine incorporation was pronounced after 16 h and mitotic activity was distinct after 24 h. In the untreated hepatectomized animals, neither was so advanced at these times. In addition, phenobarbital-pretreated rats had slightly increased thymidine incorporation after 24 and 39 h, and the analysis of variance showed a significant overall increase of labelling. There was no difference between mitotic indices in phenobarbital-pretreated and non-treated hepatectomized rats. It can be seen from Table I that maximum thymidine labelling was found 24 h after hepatectomy. The somewhat smaller values in both treated and untreated rats after 39 h indicate that thymidine uptake was on the decline at that time. Peak thymidine incorporation probably occurs between 24 and 39 h. BÜRKI et al.[12] gave rats phenobarbital immediately after partial hepatectomy. The increase of 131-iododesoxyuridine labelling and mitotic activity which they found set in a few hours later than in our study. We selected our examination times to coincide with the probable start of DNA synthesis and mitotic activity and to be near the postulated maximum DNA activity, and did not consider the time of maximum mitotic activity. Consequently we cannot be certain of the effects of phenobarbital treatment on this latter parameter. It seems probable, however, that mitotic activity is also advanced (and increased) in analogue to the advance and increase in thymidine incorporation found.

Zusammenfassung. Mit Phenobarbital behandelte Ratten zeigen nach partieller Hepatektomie in der Leber ein beschleunigtes Einsetzen der DNS-Synthese und der Mitose-Aktivität sowie eine Erhöhung der DNS-Synthese.

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In vivo Generation of Hydrogen Peroxide from 6-Hydroxydopamine

We suggested previously[1,2] that hydrogen peroxide (H_2O_2) was responsible for the degeneration of adrenergic nerve terminals caused by the injection of 6-hydroxydopamine (6-OHDA) into experimental animals[3,4]. When 6-OHDA is added to aqueous solutions at neutral pH, H_2O_2 is generated[2]. H_2O_2 is a cytotoxic agent that can oxidize sulfhydryl groups of enzymes and peroxidize structural lipids. One form of cytotoxicity that we studied was the irreversible inhibition of uptake of biogenic amines into either rat brain homogenates[1] or tissue slices[2]. The inhibition of uptake by H_2O_2 was prevented by catalase[1].

Catalase can serve as a convenient intracellular marker for H_2O_2. H_2O_2 forms a complex with catalase (complex I), which on further reaction with 3-amino-1, 2, 4-triazole leads to irreversible inhibition of catalase activity[5-7]. The reaction scheme is as follows:

$$H_2O_2 + \text{catalase} \longrightarrow (\text{Catalase}\text{-}H_2O_2)\ \text{complex I}$$

$$\text{Complex I} + \text{aminotriazole} \longrightarrow \text{irreversibly inhibited catalase.}$$

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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