## Absence of Microsomal Lipid Peroxidation in Acatalasemic Mice

Christian Steffen

Pharmakologisches Institut der Universität Marburg, Lahnberge, D-3550 Marburg

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Catalase (E. C. 1.11.1.6) activity and NADPH-dependent lipid peroxidation have been measured in liver microsomes from normal and acatalasemic mice. The absence of lipid peroxidation in acatalatic microsomes is not restituted by exogenous catalase as is microsomal methanol oxidation nor is it inhibited by sodium azide, thus suggesting an additional abnormality in these mice.

Lipid peroxidation is thought to be an important factor in the toxicity of iron [1], carbon tetrachloride [2, 3] and nitrogen dioxide [4], whereas its role is controversial in alcohol-induced liver injury [5, 6] and in paraquat intoxication [7, 8]. In the present paper I wish to report that microsomal lipid peroxidation is absent in acatalasemic mice. This finding is of interest in relation to the toxicity of the above mentioned compounds.

NMRI-mice were supplied by Zentralinstitut für Versuchstierkunde, Hannover, Germany. C3H $Cs^b$-mice were progenies from the colony of Dr. Feinstein at the Argonne National Laboratory, Argonne, Illinois, and were kindly provided by Dr. Juhr, Fachbereich Veterinärmedizin, Freie Universität Berlin. Liver microsomes were prepared by differential centrifugation in 0.25 M sucrose buffered with TRIS (20 mM, pH 7.4). Microsomes were incubated in 70 mM Soerensen buffer, pH 7.4, at 37 °C. 2.5 mM glucose-6-phosphate and 0.5 U/ml glucose-6-phosphate-dehydrogenase were present. NADPH (100 $\mu$M final concentration) was added to start lipid peroxidation. Malondialdehyde (MDA) was measured by the thiobarbituric acid method as previously described [9]. Catalatic activity was determined by the increase of oxygen concentration in a partially anaerobic solution of 12 mM $H_2O_2$ in 70 mM Soerensen buffer, pH 7.4, at 37 °C after addition of microsomes by an oxygen electrode. Microsomes were diluted to give a final concentration of 1 – 5 $\mu$g protein/ml according to their catalatic activity.

Acatalasemia is not only a genetic defect of catalase in blood, but is also associated with an unstable catalase in solid tissues [10]. Catalatic activity of liver microsomes in these mice is rapidly inactivated at 37 °C, as is demonstrated in Fig. 1. After 3 min, oxygen liberation from hydrogen peroxide ceases, whereas it continues in the control experiment with microsomes from normal mice. In an attempt to evaluate the mechanism of microsomal oxygen reduction in relation to paraquat toxicity [9], we found a negligible lipid peroxidation in "acatalatic" microsomes: Lipid peroxidation, as measured by the formation of malondialdehyde, is about one per cent of that of control microsomes that are incubated in the presence of NADPH. Even after prolonged incubation, which eliminates the possibility of a lag period [11], lipid peroxidation is minimal (Fig. 2). However, the absence of lipid peroxidation cannot be attributed to the lack of catalase: Exogenous catalase, which is able to restore the ability of "acatalatic" microsomes to oxidize ethanol [12], does not increase lipid peroxidation. The presence of a soluble antioxidant factor in these microsomes is unlikely as addition of "acatalatic"

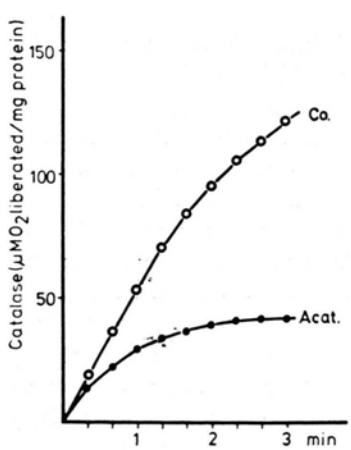


Fig. 1. Catalatic activity of liver microsomes from acatalasemic (C3H $Cs^b$) and control (NMRI) mice. Liver catalase in acatalasemic mice is rapidly inactivated at 37 °C. Catalatic activity was determined by the increase in oxygen concentration in a partially anaerobic 12 mM $H_2O_2$-solution in 70 mM Soerensen-buffer, pH 7.4, with an oxygen electrode. Microsomal protein in the test was 1–5 $\mu$g/ml, according to enzymatic activity. Values are given as $\mu$M $O_2$ liberated from hydrogen peroxide per mg microsomal protein and represent the means of registrations obtained with 4 preparations in each group.

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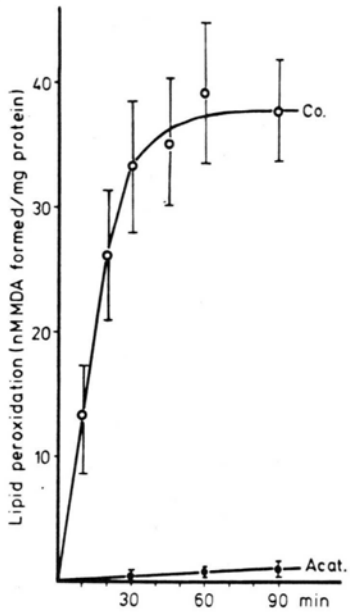


Fig. 2. Lipid peroxidation in liver microsomes from acatalasemic (C3H $Cs^b$) and control (NMRI) mice. In microsomes from acatalasemic mice, NADPH-dependent lipid peroxidation is virtually absent, even after prolonged incubation. Malondialdehyde formation was measured as previously described [9]. Microsomes (0.5 – 1 mg protein/ml) were incubated at 37 °C in 70 mM Soerensen-buffer, pH 7.4, in the presence of a NADPH-regenerating system. Lipid peroxidation was initiated by addition of NADPH (100 $\mu$M). The addition of NADPH was repeated every 30 minutes.

microsomes to control incubations did not inhibit the formation of malondialdehyde (Table I).

The finding that microsomal lipid peroxidation is absent in acatalasemic mice offers an explanation for the resistance of this mutant against a toxic effect of 3-amino-1,2,4-triazole, an inhibitor of tissue catalase [13]. This compound produces a "scaly" tail resembling the "rodent ringtail" [14] in the majority of normal C3H mice, whereas it does not exert this effect in the acatalasemic mutant [15]. As liver catalase in acatalasemic mice is abnormally labile [10], it is prone to degradation which may produce "1,3-size subunits" with peroxidase activity [16, 17]. *In vivo* application of aminotriazole leads to the development of a peroxidative activity which is greater in liver homogenates from acatalasemic than from control mice [18]. Therefore, an increased

Table I. Lipid peroxidation in liver microsomes from control (NMRI) and acatalasemic (C3H $Cs^b$) mice. Microsomes were incubated at 37 °C in 2 ml of Soerensen-buffer (pH 7.4) in the presence of a NADPH-regenerating system according to ref. [9]. Data represents means ± SD from 4 paired experiments.

| Microsomal Protein Control | Acat. | Catalase | Malondialdehyde [pM/10 min] |
|---|---|---|---|
| 0.5 mg | — | — | 596 ± 309 |
| 0.5 mg | 0.5 mg | — | 588 ± 109 |
| — | 0.5 mg | — | 4 ± 1 |
| — | 0.5 mg | 1 $\mu$g | 4 ± 1 |

peroxidation of fatty acids and a higher incidence of "scaly tail" should occur, as this symptom is associated with a deficiency of essential unsaturated fatty acids [19]. The resistance of acatalasemic mice to this toxic effect of aminotriazole can be related to the absence of lipid peroxidation *in vitro* and might indicate that a reduced sensitivity to peroxidative tissue damage might exist also *in vivo*.

From these experiments with "acatalatic" microsomes it becomes apparent that the absence of lipid peroxidation cannot be due to the lack of catalatic activity alone, as the addition of catalase does not restore the ability to form malondialdehyde. Furthermore, the inhibition of catalase by 100 $\mu$M sodium azide does not influence lipid peroxidation in control microsomes. The cause for the absence of lipid peroxidation in "acatalatic" microsomes remains to be established. Despite of a vast literature on this subject [11, 20 – 24], microsomal lipid peroxidation is still poorly understood. Therefore, it is necessary to test the effects of inhibitors and stimulators of lipid peroxidation *in vitro* as well as *in vivo*. The availability of this genetically well defined mutant [25] might prove to be a valuable tool for the study of toxic mechanisms involving lipid peroxidation as it has already been in the study of microsomal ethanol metabolism.

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