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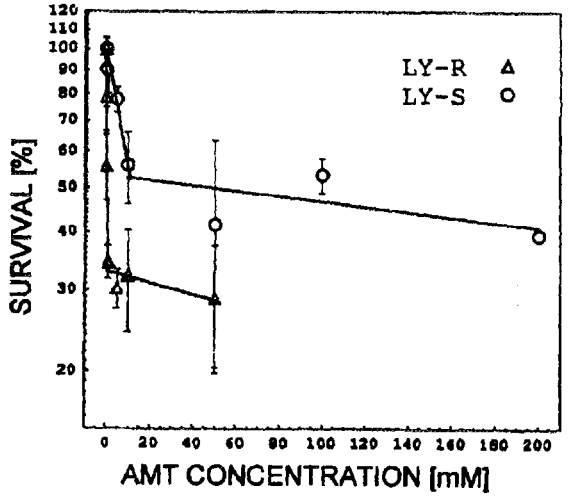

Fig.1. Sensitivity of L5178Y to AMT (3-amino-1,2,4-triazole, inhibitor of catalase). Cells were treated with AMT (1 h, 37°C). Surviving fractions were estimated by cloning and mean results shown from 3 experiments ±SD (standard deviation).

total cellular protein in LY-R cells and 26700 (±2682) - in LY-S cells (standard deviation in parantheses). Sensitivity of LY sublines to AMT was determined by cloning. As shown in Fig.1, the dose-survival curves were biphasic, with an exponential part and a plateau, probably reflecting the effect of a complete inhibition of catalase; when this was achieved, further increments in AMT concentration did not much affect the cells. The slopes of the steep exponential parts differed about tenfold for LY-R and LY-S cells (values -48.1 and -4.07, respectively), indicating a marked sensitivity difference. Also the plateau level was lower for LY-R cells (32.9% versus 53.3% for LY-S cells).

Accordingly, the decrease in catalase activity in extracts from AMT treated cells (1 h, 37°C) was greater in LY-R than in LY-S cells. Such a difference could be the result of differential AMT penetration into the intact cell or its compartments. Therefore, AMT treatment was applied in vitro, i.e. the inhibitor added directly to cell extracts for 20 min and catalase activity was estimated. Then, however, the decrease in activity percentage also was more pronounced in LY-R than in LY-S cells (Fig.2). These results showed that catalase from LY-R cells was more susceptible to AMT and that the effect measured in AMT-treated cells was not influenced by the inhibitor's accessibility inside the

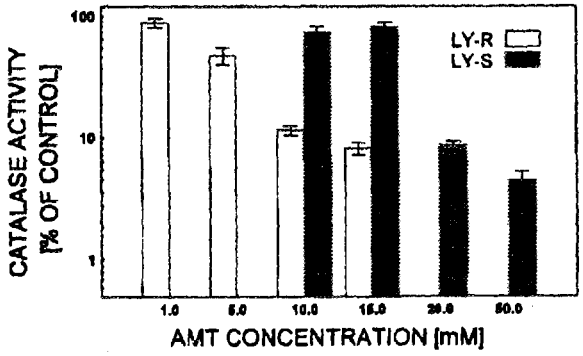

Fig.2. Catalase activity in AMT-treated LY cell homogenates. Enzymatic activity was determined in 3-4 experiments and related to the amount of protein (mg). Bars indicate SEM.

cell. The cytotoxicity of AMT alone was only roughly related to the degree of catalase inhibition; there was no statistically significant direct correlation (at $\alpha=0.05$) in the AMT concentration range corresponding to the steep decrease in survival (results not shown). Hence, the cytotoxic effect of AMT might depend on another, unspecified target, different from catalase; alternatively, lack of defense by catalase against endogenous oxidants had a more pronounced effect in LY-R cells than in LY-S cells, in agreement with the higher sensitivity of the former cells, due to higher iron content [3] and in consequence, higher danger of damage by the products of Fenton reaction.

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"PROTECTIVE ENZYMES" IN L5178Y SUBLINES AND DNA DAMAGE

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A more precise information about the role of selenium-induced glutathione peroxidase (G-Px) in the response of LY sublines to hydrogen peroxide is provided by experiments carried out with the "comet" assay. This assay (also called single cell gel electrophoresis) allows to detect DNA strand breaks in nucleoids from single cells (review in [1]). Nucleoid DNA is arranged as supercoiled loops protruding from the nuclear matrix. Damaged loops become released from the supercoiling status (1 single strand break per loop is enough to produce the effect) and - upon subsequent electrophoresis and staining - are seen in the fluorescence microscope as a "tail" of the comet, the head of which is the nucleoid core. The measure of damage is the "tail moment", i.e. the length of tail multiplied by the amount of DNA in the tail [2].

As shown in Fig., the level of DNA damage was the same in LY-R and LY-S cells after completing 1 h incubation with hydrogen peroxide, irrespective of the presence of AMT, inhibitor of catalase. Since DNA repair took place during exposure to the damaging agent, there are 2 possible interpretations of this observation: (1) catalase inhibition did not increase the damage, because reparable DNA damage was completely repaired; (2) catalase did

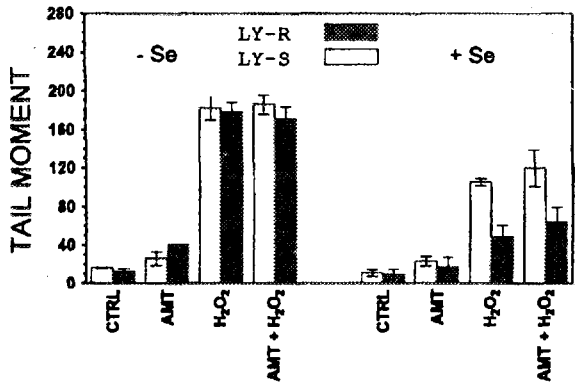

Fig. DNA damage expressed as tail moments (see text) in LY-R and LY-S cells following 1 h exposure at 37°C to hydrogen peroxide 50 μM, AMT (catalase inhibitor), and to combined simultaneous treatment with these agents. CTRL - control; AMT concentrations: LY-R cells - 5 mM, LY-S cells - 10 mM. These concentrations reduced the catalase activity by 80%.

not protect the nucleus and DNA damage was unaffected by catalase inhibition. In contrast, after induction of G-Px by selenium, DNA damage was considerably lowered. The decrease was relatively larger in LY-R than in LY-S cells, in spite of the higher increase in G-Px activity in the latter cells (LY-R cells: increase from 5.2 to 79.8 units x 10^3; LY-S cells: increase from 2.5 to 126.6 units x 10^3). This indicates a difference in the relative importance of catalase and G-Px for DNA protection in the LY sublines. It also may indicate differences in the intracellular distribution of both protective enzymes in relation to critical targets.

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THE INFLUENCE OF COPPER (II) ON RADIATION-INDUCED DAMAGES TO DNA

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Primary products of radiation-induced, direct damages to DNA can be studied by EPR spectroscopy. According to molecular ab initio calculations [1] as well as experimental evidences [2, 3] the favoured site of electron localization is thymine ($T^{\cdot-}$) and cytosine ($C^{\cdot-}$) while the holes are mainly localized on guanine ($G^{\cdot+}$). Also our results indicate that at 77 K these transients exhibit a spectrum which is superposition of all of them (Fig.1a). It is known that if the temperature is raised up above 77

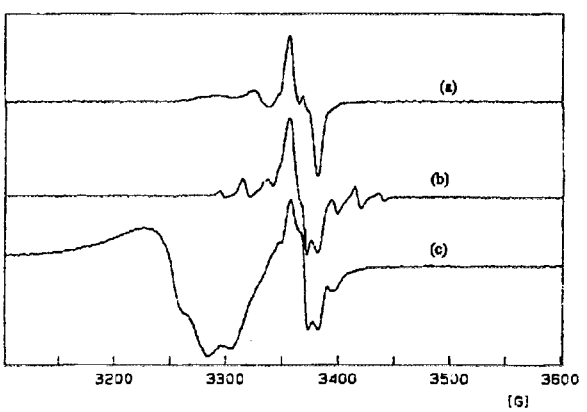

Fig.1. EPR spectra of DNA (calf thymus) 100 mg/ml, 77 K: (a) after gamma irradiation with dose 4.8 kGy; (b) after annealing, recorded at 77 K; (c) as (b) with 10 mM Cu(II).

K the radical anion of thymine $T^{\cdot-}$ reacts with a proton [2] giving the radical $TH^{\cdot}$ which could be followed by observation of very characteristic octet (Fig.1b). The addition of copper ion Cu(II) at a concentration higher than 10 mM distinctly decreases line intensities (Fig.1c). If the concentration reaches 20 mM they disappear completely.

The simplest explanation is scavenging of electrons or one electron oxidation of $T^{\cdot-}$ by Cu(II). In that case however the reduction of Cu(II)-DNA complex signal should be observed. But contrary, in our experiments, after annealing the intensity of these lines is higher. The reason could be that part of the added Cu(II) is binded to DNA in the form of a dimer structure $Cu^{2+} \cdots Cu^{2+}$, and then undetectable by EPR [4]. After one-electron reduction the dimer decomposes to Cu(II) and Cu(I) and the recorded amount of Cu(II) complexes increases almost twice. It seems that at that range of concentration the majority of all Cu(II) exsists in an aggregated, dimagnetic form. Such effect can be explained if the dimers are more favourably situated

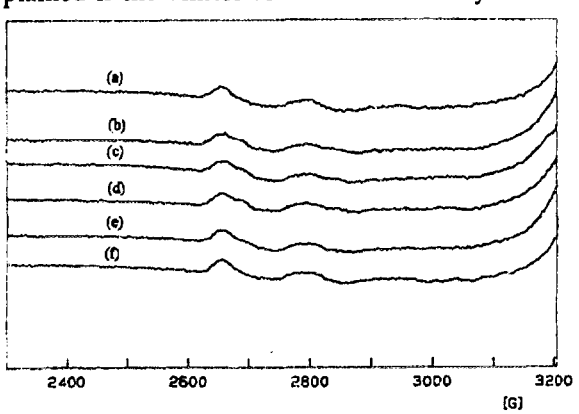

Fig.2. EPR spectra of the parallel lines of Cu(II) (2 mM) in Cu(II)-DNA complex, 77 K: (a) before irradiation; (b) after gamma irradiation with dose 4.8 kGy; (c-f) after gradual annealing, recorded at 77 K.

than the monomers (probably guanine and cytosine are involved) and then more easily accept electrons.