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GROWTH-RELATED CHANGES IN TUMOR SUPEROXIDE DISMUTASE CONTENT

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The decrease of cytosolic and mitochondrial superoxide dismutase (SOD) activity is considered, with a few exceptions, a general feature of experimental tumors (see ref. 1 for review). Data have been provided also on the loss of the copper-zinc enzyme in human tumors (2). This condition may have at least three kinds of implications: i) an increased susceptibility of tumors to γ-therapy; ii) a reduced protection of transformed cells to O_2^--induced lipid peroxidation and iii) an enhancement of the effectiveness of certain chemical carcinogens, such as polycyclic aromatic hydrocarbons, whose metabolic derivatives (e.g. benzpyrenediols) seem to involve O_2^- radicals in the damage to DNA (3). In the light of these considerations it appeared relevant to measure in the present study the content of cytosolic SOD and the effect of its diminution on intracellular membranes of tumors with different degrees of growth rate and differentiation. Some data will also be presented on the enzyme content of a tumor derived from an actively proliferating tissue, i.e. rat thymus, with the purpose of assessing whether the lowered SOD content is a peculiar property of transformed cells or simply of rapidly dividing cells.

Table I reports the values of SOD content extracted from minimal- (Morris hepatoma 44) and maximal-deviation (Morris 3924A

TABLE 1. Content of cytosolic SOD in rat liver, hepatomas and Ehrlich ascites cells

	SOD (μg/g wet weight)
Rat liver	*218.9 ± 9.8 (6)*
H 44	*54.3 ± 6.9 (4)*
H 3924A	*11.7 ± 1.0 (4)*
H Novikoff	*11.0 (2)*
Ehrlich Lettré	*13.3 (2)*

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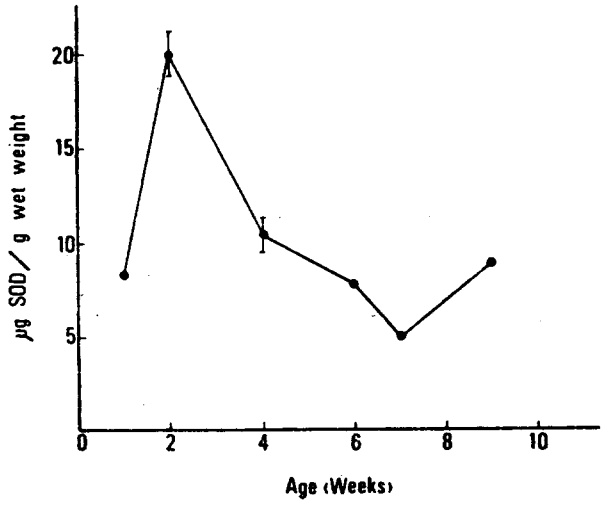

FIGURE 1. Age-related changes of SOD in F344 inbred rat thymus.

and Novikoff hepatomas; Ehrlich Lettré adenocarcinoma) tumors, in comparison to that of rat liver. The enzyme is maximally lowered in the three poorly differentiated and fast-growing tumors, whereas is present at higher values (about 25% of the control) in the hepatoma 44. Fig. 1 shows the behavior of SOD in rat thymus as a function of age. The enzyme reaches its maximum value at about two weeks after birth and decreases gradually to the initial values within 6 to 9 weeks. A thymic lymphoma, induced by i.p. injection of Gross leukemia virus at birth, has a SOD content of 12.8 ± 0.61 (6) µg/g wet weight, that is about 35% lower than the maximum value in normal thymus. These results suggest i) a dependence of SOD content in tumors on growth rate and ii) a specificity of the enzyme decline even for tumors generated from rapidly dividing cell systems. Fig. 2 shows that the rate of malonaldehyde production in microsomal membranes exposed to exogenous lipoperoxidative agents, such as the O_2^- radical generating system xanthine-xanthine oxidase (A) or ascorbate (B), decreases progressively with the increasing growth rate of the tumors. The

TABLE II. Lipid Content and Fatty Acid Composition of Rat Liver and Hepatoma Microsomes

	Rat liver	*H44*	*H3924A*
Lipid-protein	*0.66*	*0.56*	*0.32*
Polyenes/monoenes	*1.5*	*1.0*	*0.6*
Double-bond index	*82*	*60*	*50*

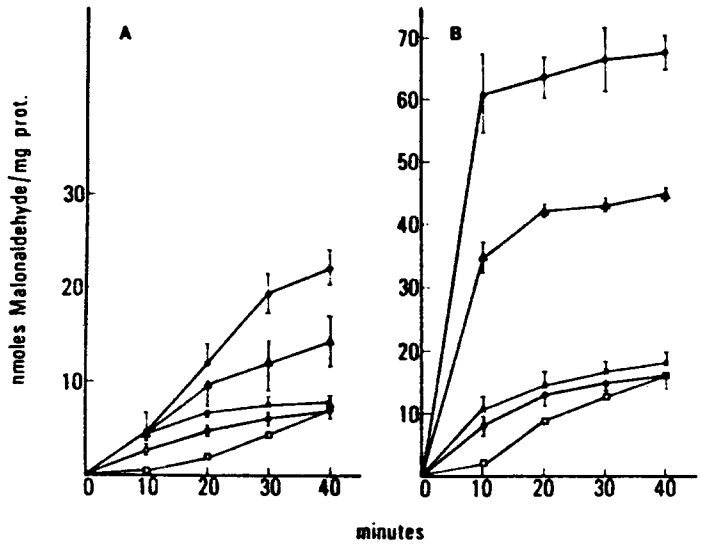

FIGURE 2. Lipid peroxidation induced by O_2^- radicals (A) and ascorbate (B) of microsomes derived from rat liver ( ), Morris hepatoma 44 ( ) and 3924A (o), Novikoff hepatoma ( ) and Ehrlich (X) ascites cells. Means ± SE (3-5 experiments).

decrease in the vulnerability of microsomal membranes and mitochondria (not shown) to peroxidative agents may be ascribed to changes in the intrinsic features of the lipid bilayer which seem to occur in a fashion related to the growth ability of the transformed tissue. Such evidence is given by the changes in the lipid content and fatty acid composition as well as in the structure of the tumor microsomal membranes. As shown in Table II, the lipid to protein ratio and the degree of fatty acid unsaturation decrease gradually from normal to hepatoma 44 and 3924A microsomes. The differences in lipid composition are reflected also by a difference in some physical properties of the bilayer. Table III gives the parameters obtained from ESR spectra of microsomes labeled with 5-nitroxide stearate and 16-nitroxide stearate, which report the order and the freedom of motion of the hydrocarbon chains in the vicinity of the lipid polar head groups and in the lipid-core, respectively. The gradual increase of the order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$ is indicative of a more orderly structure of the tumor membranes. Moreover, while the fluidity of the membrane region close to the surface does not seem to be affected, the core of the membrane appears to be less fluid in tumors.

In conclusion it can be proposed that the loss of the protective effect of SOD against O_2^--induced lipid peroxidation in tumors may be a factor responsible for the alterations of the subcellular membrane components. In light of the view that lipid peroxides

TABLE III. ESR Parameters of Doxyl Derivatives in Microsomes from Rat Liver and Morris Hepatomas

	5-NS				16-NS
	$\langle P_2 \rangle$	$\langle P_{22} \rangle$	$\langle P_4 \rangle$	τ_o(nsec)	τ_o(nsec)
Rat liver	0.60	-0.11	0.10	4.0 ± 0.2	1.4
H 44	0.61	-0.247	0.10	4.0 ± 0.2	1.7
H 3924A	0.67	-0.06	0.20	4.0 ± 0.2	2.1

inhibit cell division (4), the limited amount of peroxides produced by membranes already damaged would result inadequate to control the mitotic activity of tumors in a proportion directly related to the magnitude of SOD content loss.

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