INCREASE IN SUPEROXIDE DISMUTASE ACTIVITY CONCOMITANT WITH A DECREASE IN LIPID PEROXIDATION OF LIVER DURING POST PARTUM DEVELOPMENT

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1. Introduction

Superoxide dismutase (SOD) is thought to play an important role in the protection of cells against toxic effects of superoxide radicals ($O_2^{\cdot}$). As described by Fridovich [1], the enzyme is universally distributed in respiring cells and only respiring cells produce $O_2^{\cdot}$. Pederson and Aust [2] reported that the $O_2^{\cdot}$ induces lipid peroxidation of biological membranes, a reaction which is inhibited by SOD. Among microorganisms, aerobic and aerotolerant species contain the enzyme, whereas obligate anaerobic ones do not [3]. The enzyme can be induced by oxygen. There are some data indicating the induction of SOD in higher organisms [4]. Fetal calf myoblast cells are damaged when exposed to light in the presence of flavine mononucleotide plus ethylenediamine tetraacetic acid (EDTA) or xanthine plus xanthine oxidase. SOD added to the above media protected these cells against the lethal effects of $O_2^{\cdot}$ so produced [5].

From these basic ideas, it is to be expected that the enzyme activity should be lower in fetal or embryonic tissues than in adult ones.

This report describes the low level of SOD activity in homogenates of growing embryonic or new-born rat livers which occurs together with an increase in spontaneous lipid peroxidation of these homogenates under aerobic conditions.

2. Materials and methods

The livers of 20-day fetal, new-born or adult Donryu rats were used. As a comparison, the livers of embryonic chicks at various stages of development were also examined. Liver homogenates were prepared in 10 vol. 10 mM phosphate buffer (pH 7.4) and the supernatant obtained by centrifugation at 105 000 × g for 60 min. The mitochondrial fraction was obtained by modification of the method of Hogeboom-Schneider [6]. Crude SOD fractions were extracted from either the supernatant or the mitochondrial fraction with an organic solvent, by the method of Tsuchihashi [7,8]. Protein concentration was determined by Lowry's method [9]. Lipid peroxides were measured by color

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development following reaction with thiobarbituric acid (TBA) [10]. The SOD activity was measured by the method of Beauchamp and Fridovich [8], using the xanthine–xanthine oxidase system. The assay of SOD activity was carried out in 0.1 mM xanthine, 0.3 mM EDTA, 25 μM nitrobluetetrazolium (NBT) and 50 mM carbonate buffer (pH 10.2) at 37°C. A suitable amount of liver supernatant or crude SOD fraction was added to the reaction mixture. The reaction was initiated by adding 4×10^{-9} M xanthine oxidase (Sigma Chemical Co.). Under these conditions, the velocity of $A_{560\ nm}$ increase caused by NBT reduction, was about 0.01 unit/min. One unit of SOD activity was defined as the amount of the enzyme required for 50% inhibition. To measure cyanide sensitivity, 1 mM potassium cyanide was added to the reaction mixture before adding xanthine oxidase. For polyacrylamide disc-gel electrophoresis the technique of Beauchamp and Fridovich [8] was used with the flavine–NBT system under ultraviolet light.

3. Results and discussion

3.1. *Increase of lipid peroxidation in fetal rat liver homogenate*

As shown in fig.1, spontaneous lipid peroxidation in embryonic liver homogenate occurred to a greater extent than in adult liver at 37°C. These homogenates of livers from fetal rats at different stages of embryonic

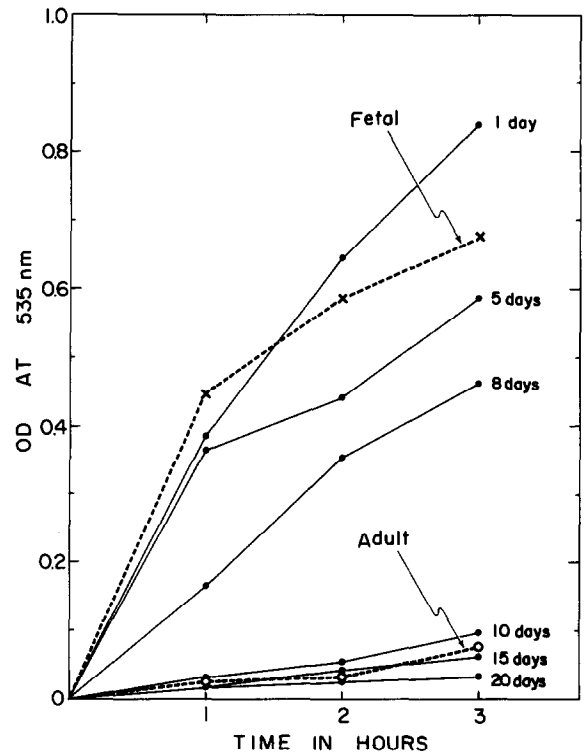

Fig.1. Lipid peroxidation of liver homogenates from fetal, new-born of various ages and adult rats. Homogenates containing approximately 4.5 mg protein were incubated in 3 ml 0.15 M KCl, 10 mM Tris–HCl buffer (pH 7.4) at 37°C. Lipid peroxide was measured by color development after reaction with TBA. New born rats were siblings.

Table 1
Superoxide dismutase activities in supernatants of homogenates of fetal, new-born and adult rat livers

Materials		Units/mg protein	Units/g tissue	%	Liver wet wt (g/rat)
Fetal	(19 days)	74.3	11 300	34.6	0.20
New born[a]	1 day	57.5	8689	25.8	0.25
	3 days	65.6	8272	25.3	0.23
	5 days	88.5	13 365	40.9	0.25
	8 days	94.7	16 667	51.9	0.36
	10 days	124.3	21 590	66.1	0.46
	15 days	114.4	22 875	70.0	1.05
	20 days	145.1	28 718	87.9	1.95
Adult	(60 days)	149.8	32 667	100.0	13.00

[a]New-born rats were siblings

development showed no significant differences in the extent of lipid peroxidation, but in homogenates of livers from new-born rats of different post partum ages; the extent of lipid peroxidation varied considerably, depending on the age. The extent of the peroxidation gradually decreased from 6–10 days post partum.

3.2. *Low activity of SOD in fetal rat liver homogenate*

The data on the increase in spontaneous lipid peroxidation in fetal rat liver homogenates suggest that SOD is either absent or present at low level of activity. SOD activity was measured in supernatants of homogenates obtained in 10 mM phosphate buffer (pH 7.4) after freezing–thawing livers from fetal (19 days gestation) and new-born of various post partum ages and adult rats.

Table 1 shows the low enzyme activity in fetal rat liver. It remained at a low level for 5 days post partum, then gradually rose to the adult level by 20 days post partum. A 2.6-fold increase in the activity of the enzyme was observed during this experimental period. A similar low level of mitochondrial SOD activity was observed at various stages of development.

3.3. *Polyacrylamide disc-gel electrophoretic analysis of SOD in fetal and adult rat livers*

Figure 2 shows the polyacrylamide disc-gel electrophoretic patterns of SOD obtained from supernatants of homogenates of livers of rats and hen. As shown in the figure, the pattern of SOD obtained with the supernatants of chick embryo and hen is identical with that of Weisiger and Fridovich [11]. Four distinct bands of SOD activity are observed. Their relative mobilities corresponded to those of cytoplasmic cyanide sensitive and mitochondrial cyanide insensitive SOD. Fetal rat liver shows only one SOD band, in front of the gel pattern of cytoplasmic SOD in chick liver, whereas adult rat liver shows an additional band located just in front of the fetal liver band..Cyanide insensitive SOD of higher molecular weight was not detected either in the supernatant from the homogenate or in the mitochondria of fetal and adult rat livers. But the enzyme may be very labile which could account for this. The mitochondrial fraction also contained cyanide sensitive SOD and the activity of the enzyme was also very low in fetal compared with adult rat liver.

From these experimental results, it is suggested that this low levels of SOD activity in fetal liver may

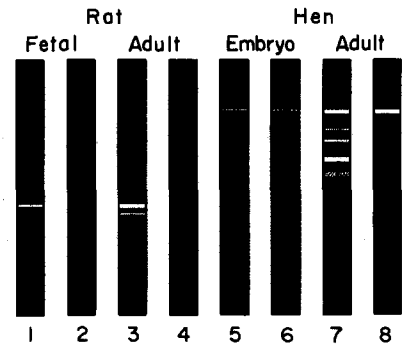

Fig.2. Polyacrylamide disc-gel electrophoresis of SOD isozymes obtained from supernatants of liver homogenates of fetal and adult rats and of chick embryo and hen. Approx. 100 μg supernatant protein were placed on each gel (7.5%). Electrophoresis was at pH 8.6. Gel 1 and 2: fetal rat; Gel 3 and 4: adult rat: Gel 5 and 6: chick embryo; Gel 7 and 8: hen. Gel 2, 4, 6 and 8: 1 mM KCN was present during development of enzyme activity staining.

be one of the factors responsible for the increased rate of spontaneous lipid peroxidation in vitro in the presence of oxygen [12]. Furthermore, the above results suggest that SOD might be induced by respiration after birth. Of course, the possibility that the decrease in lipid peroxidation is due to a decrease in the amount of liver lipid susceptible to peroxidation during the course of early development has not been excluded.

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