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Age-Related Development of Pulmonary Antioxidant Enzymes in the Rat[1] (40040)

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Two pulmonary antioxidant defense mechanisms, superoxide dismutase and the glutathione system, have been postulated to offer varying degrees of protection against oxygen (1-5), ozone (6), nitrogen dioxide (7), and paraquat (8, 9) induced lung toxicity. Oxidant-induced lung injury is generally thought to result from the cytotoxic action of the free radical superoxide anion and other highly reactive intermediates, such as hydroxyl radical, peroxy radical and hydrogen peroxide (1-10). Superoxide dismutase (SOD) is believed to diminish oxidant-induced lung injury by catalyzing the dismutation of superoxide radical (1). The enzyme catalase (CA), which can be included in the superoxide dismutase system, sequesters the hydrogen peroxide formed from the dismutation reaction (10). The glutathione system includes glutathione peroxidase (GP), which is thought to limit lipid peroxidation initiated by superoxide radical that has escaped dismutation (11). Reduced glutathione (GSH) is utilized as a cofactor in this reaction. The intracellular pool of GSH can then be replenished by glutathione reductase (GR).

It has long been recognized that newborn and neonatal animals are more tolerant to the toxic pulmonary effects of oxygen than adults (4, 12, 13). We have previously investigated the mechanism of this age-dependency of oxygen-induced lung toxicity and have observed that the relative resistance of the neonatal animal to hyperoxic lung injury is associated with an increase in the activity of their pulmonary antioxidant defense systems as compared to the adult (4). Recently Kehrer and Autor (14) have examined the activity of various rat pulmonary glutathione system enzymes as a function of age. In the present study, we have further examined the development of these pulmonary antioxidant defense systems in an expanded age range in rats including premature rats and rats between 15 and 70 days old in an attempt to gain additional understanding of the mechanism responsible for age-related susceptibility to oxygen-induced lung injury.

Materials and methods. Procedure in animals. Animals used for these studies were Sprague-Dawley albino rats of varying ages (2 days premature to 62 days old) bred and raised in the Animal Care Facility at The University of Iowa and Sprague-Dawley albino rats (70 days old) obtained from Biolab Corporation (St. Paul, MI). All rats were sacrificed by decapitation on the same day and their lungs were carefully perfused with ice-cold isotonic phosphate buffer via the pulmonary artery (4). Lungs were trimmed to remove extraparenchymal, tracheal-bronchial and vascular tissue and were homogenized for 2 min in a 1:15 (W:V) dilution with ice-cold, 0.005 M, phosphate buffer, pH 7.8, using a Sorvall Omnimixer (Ivan Sorvall Inc., Newtown, CO). Lung tissue from two to 14 premature or young animals (less than 50 days old) were pooled for purposes of analysis. Rats greater than eight days old were separated according to sex and were analyzed accordingly. Superoxide dismutase activity and GSH, deoxyribonucleic acid (DNA) and protein levels were analyzed in the lung homogenate. Glutathione reductase, GP and CA activities and protein concentration were determined in the 15,000*g* lung supernatant. Attempts were made to perform a single biochemical assay on all the samples in a random fashion on the same day.

Biochemical assays. Lung SOD activity

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was determined by inhibition of ferricytochrome *c* reduction by xanthine and xanthine oxidase (15). GR activity was determined by following NADPH disappearance (16); GP activity by the method described by Lawrence and Burk (17) using cumene hydroperoxide as substrate; and, CA activity by the method of Holmes and Masters (18). Assay of GSH level utilized the modified method of Klotsch and Bergmeyer (19) by Delucia *et al.* (20). DNA was determined using Richard's modification of the diphenylamine reaction (21) and protein concentration by the modified Lowry's assay (22).

Results. No significant differences were found in the various enzyme activities and GSH levels between male and female rats. To simplify the figure presentation, data from male and female rats of the same age group were therefore combined. Each value in the figures (1-5) represents mean ± SE of two to five individual samples for each age group. A similar pattern with age was observed in pulmonary SOD activity (Fig. 1) and GSH level (Fig. 2), whether the activity level was expressed as per mg protein or per mg DNA in lung homogenate. Pulmonary SOD activity and GSH level of 70-day-old rats were approximately 200% of that found in neonatal rats (0-21 days old) when expressed as per mg DNA and 150% of that found in neonatal rats when expressed as per mg protein.

Pulmonary GP (Fig. 3) and CA (Fig. 5) activities appeared to increase from 2 days premature to birth and then subsequently decrease to approximately the original fetal level by age 8-12 days. For SOD (Fig. 1) and GR (Fig. 4), this pattern of enzyme activity was less obvious. As with SOD, GP and GR activities showed a progressive increase with age from day 12 to 70. CA, in contrast to all the other enzymes and GSH, showed a progressive decline in activity from day 12 to 70. Activities of GP, GR and CA in 70-day-old adult rat lung were

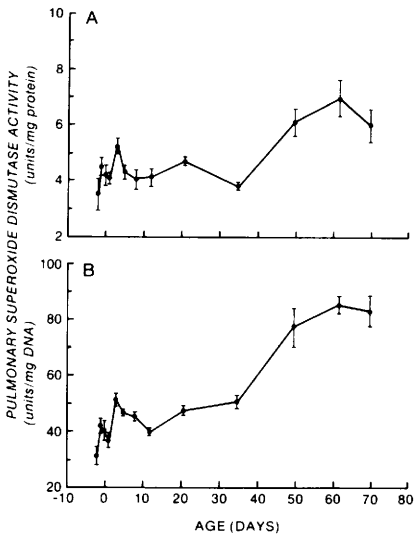

FIG. 1. Pulmonary superoxide dismutase (SOD) activity at different ages in the rat expressed as units activity per mg protein (A) and per mg DNA (B).

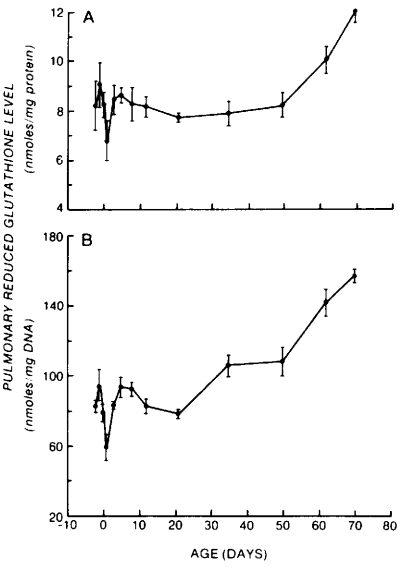

FIG. 2. Pulmonary reduced glutathione (GSH) level at different ages in the rat expressed as nmoles per mg protein (A) and per mg DNA (B).

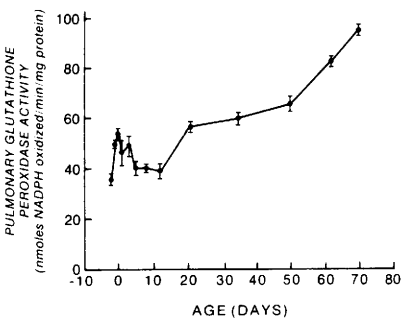

FIG. 3. Pulmonary glutathione peroxidase (GP) activity (nmoles NADPH oxidized/min/mg protein) at different ages in the rat.

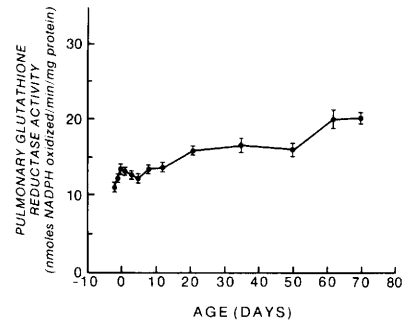

FIG. 4. Pulmonary glutathione reductase (GR) activity (nmoles NADPH oxidized/min/mg protein) at different ages in the rat.

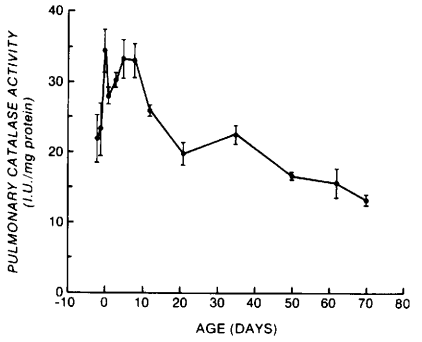

FIG. 5. Pulmonary catalase (CA) activity (I.U./mg protein) at different ages in the rat.

approximately 200%, 150% and 50%, respectively, of that found in neonatal rat lung.

Discussion. Previous studies have shown age-related changes of various pulmonary enzymes (23, 24). In the present study, the level of activity of all the pulmonary antioxidant enzymes studied (Figs. 1, 3–5) were lower at 2 days before term than at birth. This increase in activity from 2 days before term to birth may serve an important function in preparing the transition of the lung from a relatively hypoxic environment *in utero* to the much richer extrauterine oxygen environment. Fluctuations in enzyme activities and GSH levels were observed between birth and 5 days of age. The explanation for these fluctuations is not known but it could be a consequence of the multitude of environmental and physiological changes which are known to be occurring during this time.

From day 12 to 70, SOD, GP, and GR activities and GSH levels generally showed a gradual increase with age. In contrast to the other enzymes studied, pulmonary CA activity was less in the older rats compared to the younger animals (Fig. 5). Similar findings were reported by Kehrer and Autor (14) in pulmonary GR and GP activities but not with GSH levels in developing rat lung. The increase in pulmonary GSH levels with age observed in the present study may be attributed to the different analytical techniques employed in these two studies.

Young adult rats (70 days old), who have been repeatedly shown to be more susceptible to oxidant-induced lung damage (4, 12, 13) have nevertheless a 1.5- to twofold greater SOD, GP, and GR activities and GSH levels than that of neonatal animals. Human adult lung has similarly been found to have a greater SOD activity than fetal and newborn lung (25). This apparent paradox may be explained in part by our recent findings that neonatal rats, in response to high oxygen exposure, showed a rapid and significant increase in pulmonary SOD, GP, GR, CA activity, and GSH levels (4, 5, 25, 26). In these studies, adult rats, although possessing greater baseline lung activity levels of SOD, GP, GR, and GSH (Figs. 1–4), failed to respond with any increase in these enzyme activities or GSH levels in response to a toxic oxygen challenge. The lower pulmonary activity of CA in adult rats may be extremely critical and further contribute to their greater susceptibility to oxidant-induced lung damage.

On the basis of this study and previously published work (1–10, 25, 26), one could speculate that the ability of an animal to tolerate oxygen-induced lung damage is governed by a delicate balance between the generation of cytotoxic agents and the various pulmonary defense capabilities. The intracellular production of cytotoxic agents is believed to be increased during exposure to high concentrations of oxygen (27). The ability of the lung to offset this challenge would be determined not only by the total activities of pulmonary antioxidant enzymes, but also by the capability of the lung to increase its antioxidant enzymes in response to hyperoxia and the proximity of these protective systems to the site of generation of cytotoxic agents.

Summary. The age-related development

of the activity levels of the pulmonary antioxidant defense systems: superoxide dismutase, reduced glutathione, glutathione peroxidase, glutathione reductase and catalase, were examined in the rat. All the pulmonary antioxidant enzymes studied showed a lower activity at two days before term than at birth. From day 12 to 70, with the exception of catalase, these enzymes and reduced glutathione generally showed a gradual increase in activity levels with age. Pulmonary catalase activity, however, was lower in the older rats compared to the younger animals. No significant differences were found in the various pulmonary enzyme activities and glutathione levels between male and female rats.

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