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Developmental Characteristics of Pulmonary Superoxide Dismutase: Relationship to Idiopathic Respiratory Distress Syndrome

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Extract

Pulmonary superoxide dismutase (SOD) activity was determined for various groups of human fetuses, infants, and adults. Enzyme activity was found to increase with age from a low of 17 ± 1 units/mg DNA in fetal lung to 49 ± 6 units/mg DNA in infant lung and finally to 110.2 ± 14.8 units/mg DNA in adult lung ($P < 0.05$). No difference in lung SOD activity was demonstrated between normal infants and those with idiopathic respiratory distress/hyaline membrane disease (IRDS/HMD). No significant differences in SOD activity were found among all the samples of infant blood. Adult blood samples, however, contained significantly greater SOD activity both in terms of heme concentration and volume of whole blood ($P < 0.05$). SOD activity in lung tissue from both rats and rabbits was also found to increase with age from a low value in fetal animals to a maximum activity in adults ($P < 0.05$). Exposure of New Zealand White rabbits, prematurely delivered by caesarian section, to 80% oxygen for 24 hr resulted in a 42% increase in lung SOD activity. Similarly, 7-day-old Sprague-Dawley rats exposed to 85% oxygen for 24 hr showed a 43% increase in pulmonary SOD activity. No increase in pulmonary SOD was observed when adult rats were exposed to 85% oxygen for 24 hr. The effect of hyperoxia on SOD activity in excised lung was investigated. Rat lung, incubated in either heparinized whole blood or in plasma and exposed to 100% oxygen, showed a 30% increase in SOD activity after 2 hr. This capacity of lung tissue to respond to hyperoxia *in vitro* with increased SOD activity was age dependent. The maximum increase in SOD activity was seen with lungs from 10–12-day-old rats. The oxygen-stimulated increase in lung SOD activity disappeared at about 19–20 days of age.

Speculation

Superoxide dismutase which catalyzes the dismutation of the oxygen free radical may well be a primary lung protectant against the depredations of environmental oxygen. SOD appears to be a maturationally important enzyme since the activity of this enzyme increases with development in both lung tissue and blood of animals and humans. The premature infant may be compromised when exposed to the relative hyperoxia of the extrauterine environment by a reduced complement of the enzyme or a reduced ability to increase pulmonary SOD activity in response to hyperoxia. Lung damage resulting from deficient endogenous protection may be a factor in the clinical picture of IRDS/HMD. Treatment of the immature lung with high concentrations of oxygen may further compromise a lung already deficient in SOD protection. Because prolonged exposure to hyperoxia precedes diagnosis of bronchopulmonary dysplasia, an SOD deficiency may also be important in the etiology of this condition.

Superoxide dismutase is an enzyme found ubiquitously in oxygen-metabolizing cells (18). This enzyme, existing in the cytosol in the Cu/Zn-containing form and in the mitochondria in the Mn-containing form, catalyzes the reaction: $2O^-_2 - 2H^+ \rightarrow H_2O_2 + O_2$ and apparently functions to remove from the cell the highly reactive free radical superoxide anion (O^-_2). This superoxide anion is generated in the presence of oxygen in association with a number of important biochemical reactions and processes, among which are the action of xanthine oxidase (12, 17), spontaneous oxidation of reduced flavoproteins (20) and catacholamines (19), and phagocytosis (3). Its production can be shown, in some cases, to be enhanced by hyperoxia (8). It is considered to be inimical to the normal functioning of the cell because of its suspected role in promoting lipid peroxidation (1) and provoking denaturation of certain enzymes (14). Fridovich and others (9, 23) have proposed that the oxygen free radical, which can act effectively as either an oxidizing or reducing species, is a likely cytotoxic agent responsible for the manifestations of oxygen toxicity. SOD would, therefore, serve a vital protective function for aerobic organisms. The protective action of superoxide dismutase against the lethality of oxgen has been clearly demonstrated by McCord *et al.* (16) using *Escherichia coli* mutants which were both O_2 intolerant and SOD deficient. Crapo and Tierney (5) have demonstrated increased SOD activity in response to long term exposure of rats to hyperoxic conditions.

Because infants are subjected to an abrupt increase in oxygen tension at the time of birth and because SOD has been proposed as the primary endogenous protectant against oxygen toxicity, an investigation of SOD activity in the neonatal human lung was undertaken. Of particular interest was the determination of enzyme activity in the lungs of premature infants who are the group most frequently associated with IRDS/HMD and who may, therefore, receive oxygen therapy during the course of clinical care. SOD activity was determined in blood samples as well, since enzyme activity in circulating blood could be important in pulmonary protective effects. A critical tissue level of SOD might be required in the newborn in order for the relative hyperoxia of extrauterine life to be tolerated, thus providing a possible identity for the oxygen protective agent first proposed by Gerschman (9) and later by Shanklin (24). As is apparently the case in the surfactant system, the premature infant may be severely compromised by a decreased activity of an enzyme or enzyme system that performs an essential role in the maintenance of normal pulmonary function. The goal of the study reported here was to determine and compare SOD activity in blood and lung tissue in humans and

animals of various ages, including infants with IRDS/HMD, and to determine the response of pulmonary SOD to hyperoxia.

METHODS

HUMAN STUDIES

Fetal lung tissue was obtained from aborted fetuses 18-20 weeks old. Adult lung tissue was obtained from postmortem material (less than 12 hr after death) from patients aged 43-70 years who died from causes other than those involving the lung. All infants included in this study who were suffering from IRDS/HMD or bronchopulmonary dysplasia (BPD) were treated in the Pediatric Intensive Care Unit of the University of Iowa Medical Center. This project was reviewed and approved by the University of Iowa Committee on Research Involving Human Beings. IRDS/HMD was diagnosed in prematurely born infants according to the following criteria. (*1*) Clinical and laboratory findings included progressive respiratory distress observed shortly after birth, hypoxia. and acidosis. (*2*) Roentgenographic findings indicated prominent air bronchogram pattern and a diffuse granular appearance of the lungs. (*3*) Histologically, lungs of infants with IRDS/HMD showed gross atelectasis with eosinophilic membranes lining terminal bronchioles and alveolar ducts. The diagnosis of bronchopulmonary dysplasia depended upon the observation of chronic respiratory distress and hypoxia with a history of prolonged exposure to hyperoxia (more than 150 hr at 80-100% oxygen) and roentgenographic evidence including diffuse infiltrations, spherical radiolucent areas, and radiodense strands. Those infants who were designated normal for the purposes of the study of pulmonary SOD died from causes not involving the lung. Histologic examination of tissue from these infants revealed normal lung patterns.

Fresh lung was washed in cold 0.15 M KCl, placed in 0.01 M potassium phosphate buffer, pH 7.8, and homogenized (1:5, w/v) in a Waring Blendor for 30 sec with the temperature maintained at 4°. This preparation was further homogenized in a Potter-Elvehjem glass homogenizer, strained through a double layer of cheesecloth, and pressed thoroughly. The homogenate thus obtained was analyzed for SOD activity by the standard determination of its inhibition of ferricytochrome *c* reduction by xanthine and xanthine oxidase (18). One unit of enzyme activity was determined according to the definition established by McCord and Fridovich (18). Homogenates of lung tissue treated in the described manner contained less than 5% of the total SOD activity from blood contamination. Erythrocyte SOD activity was assayed in the chloroform ethanol extract of clotted whole blood (18).

DNA was determined according to the method of Richards (22). Heme determinations were conducted either by the carbon monoxide (7) or the cyanide methods (15). Statistical analysis of the data utilized the two-tailed Student's *t*-test for the comparison of experimental groups ($P < 0.05$ for rejection of the null hypothesis).

ANIMAL STUDIES

All rats used in these studies were the Sprague-Dawley albino variety, bred and raised at the University of Iowa. Mature female rats were exposed to a 12-hr breeding period. The onset of pregnancy was timed from the midpoint of this 12-hr period. Prematurely delivered rats were obtained by caesarian section of pregnant animals after ether anesthesia.

For studies in which lung tissue was exposed to oxygen *in vitro* animals were killed by decapitation and the lungs removed immediately. Tissue was washed in 0.005 M potassium phosphate buffer, pH 7.8, blotted dry, and minced with a razor blade to a tissue size of 1-2 mm^3. Lung tissue was obtained from several litters of animals and was pooled for each experiment. Incubations were carried out either in rat blood or plasma obtained by centrifugation of heparinized blood at 5,000 $\times$ *g* for 10 min or in 0.005 M potassium phosphate buffer, pH 7.4. After incubation in either air or 100% oxygen, the lung and incubation medium were diluted 1:3 with 0.005 M potassium phosphate buffer, pH 7.8, and homogenized for 2.5 min in a Sorvall Omnimixer at 5°. SOD activity of the homogenized lung tissue was determined by the method described above.

For experiments in which rats were exposed *in vivo* to hyperoxia, 7-day-old Sprague-Dawley rats and dams were maintained in a controlled atmosphere chamber at a concentration of 85% oxygen monitored with a Beckman model OM-11 gas analyzer. The temperature of the chamber was 24-26°, vapor pressure was less than 10 mm Hg, and the carbon dioxide concentration was kept below 0.6%. Carbon dioxide concentration was monitored with a Beckman model LB-2 gas analyzer. After the exposure period, animals were decapitated and the lungs either perfused or removed immediately and washed in cold 0.005 M potassium phosphate buffer, pH 7.8. Lungs were then homogenized in the same buffer (1:8, w/v) and analyzed for SOD activity as described previously.

For those studies in which neonatal rabbits were exposed to hyperoxia, pregnant New Zealand White rabbits were anesthetized with diazepam and ether and the pups delivered by caesarean section after 28 days of gestation. The pups were placed in humidified chambers at 37° with the oxygen content maintained at 80%. The flow rate of the air-oxygen mixture was 3 liters/min. Because the rabbit pups were separated from the mother during this procedure, the animals were fed with a standard infant formula (Similac) via nasogastric tube. Pups exposed to oxygen or room air were killed at the end of a 24-hr exposure period. The excised lungs were analyzed for SOD activity as described above.

RESULTS

Total SOD activity in human lung tissue is listed in Table 1. Because of variations in the condition of lung samples, particularly with regard to edematous change, and in order to allow comparison among the tissue samples (6), enzymatic activity was expressed as units per mg of DNA, as well as units per g of lung tissue. Pulmonary SOD activity (expressed in terms of milligrams of

Table 1. *Superoxide dismutase activity in human lung tissue*

Group	Sample no.	Units/g lung[1]	Units/mg DNA[1]
Fetus	6	109 ± 11	17 ± 1[2]
IRDS/HMD[3] infant	3	152 ± 11	37 ± 4
Normal infant (18 hr-2 mo)	4	152 ± 32	49 ± 6
Adult[4] (43-70 years)	5	182 ± 19	110 ± 15[2]

[1] Reported as mean unit of enzyme activity ± SEM.
[2] Statistically different from other groups ($P < 0.05$).
[3] Idiopathic respiratory distress syndrome/hyaline membrane disease.
[4] Died from causes unrelated to the lung.

Table 2. *Oxygen exposure of idiopathic respiratory distress syndrome/hyaline membrane disease infants*

Patient	Birth weight, g	Gestational age,[1] weeks	Survival time, hr	Oxygen therapy, hr		
				30-50%	50-75%	75-100%
1	1,040	26-28	19	2	5.5	11.5
2	1,300	32	96	45	17	22
3	1,910	32	22	8	11	3

[1] Estimate based on history, physical, and neurologic criteria.

DNA) increased significantly ($P < 0.05$) from fetal to infant lung to adult lung. Table 2 documents the course of oxygen therapy for the IRDS/HMD infants included in Table 1.

Table 3 summarizes SOD activity in blood from several age groups; infants with IRDS/HMD, premature infants of approximately the same age but without IRDS/HMD, normal full term infants, normal infants varying in age from 44 to 142 days, infants aged 44–142 days receiving treatment for BPD, and normal adults. The results are expressed as units per ml of whole blood and units per μmol of heme to correct for the variation in hemoglobin concentration. Adult SOD values were significantly greater ($P < 0.05$) than those for all the other groups. There was no significant difference in SOD activity between premature infant blood and IRDS/HMD blood or BPD infant blood and their age controls. The same was true for SOD activity in blood of normal premature infants when compared with normal term infants. A slight but nonsignificant increase in blood SOD activity was apparent (in terms of micromoles of heme) in the 44–142-day-old infant group when compared with the premature and normal term group.

Table 3. *Superoxide dismutase activity in blood*[1]

Group	Sample no.	Units/ml whole blood	Units/μmol heme
IRDS/HMD	5	135 ± 20	53 ± 8
Premature (non-IRDS/HMD)	5	147 ± 21	56 ± 1
Normal (term)	8	163 ± 14	60 ± 5
Normal (44–142 days)	8	143 ± 3	79 ± 1
Bronchopulmonary dysplasia	3	113 ± 8	78 ± 5
Adult	10	213 ± 4[2]	93 ± 3[2]

[1] IRDS/HMD: idiopathic respiratory distress syndrome/hyaline membrane disease.

[2] Statistically different compared with all other groups ($P < 0.05$). Values represent mean ± SEM.

Table 4. *Fetal to adult changes in lung superoxide dismutase activity*[1]

Species	Fetus,[2] units/g	Adult, units/g	Increase, %
Rabbit	70 ± 3	344 ± 20	390
	(n = 21)	(n = 8)	
Rat	563 ± 61	1144 ± 165	103
	(n = 26)	(n = 15)	
	(70 ± 6)[3]	(279 ± 59)[3]	(294)
Human	109 ± 11	182 ± 19	67
	(n = 6)	(n = 5)	
	(17 ± 1)[3]	(110 ± 15)[3]	(547)

[1] Values represent mean ± SEM (n = number of samples). The percentage of increase for each species from fetus to adult was significantly different ($P < 0.05$).

[2] Gestational ages are 28 (31) days for rabbits, 19–20 (21) days for rats, and 18–20 (40) weeks for humans.

[3] Units per mg of DNA.

Because the data illustrated in Tables 1 and 3 suggested that increased SOD activity may be an integral part of normal development, a study of pulmonary SOD activity as a function of age was undertaken with rats and rabbits. Table 4 shows that, as with human lung, SOD activity increased significantly ($P < 0.05$) between late fetal and adult stages in the rat and rabbit lung. Data obtained from several groups of rats between the fetal and adult extremes indicate a steady rise in SOD activity with maturation in this species.

Although it appears that the basal pulmonary SOD activity among normal premature, IRDS/HMD, and normal infants did not differ significantly, it was necessary to ask what effect prolonged exposure to oxygen would have on SOD activity in IRDS/HMD infant lung since such infants are treated clinically with oxygen therapy. Appropriate control values for such experiments are not possible because treatment of normal infants with oxygen therapy would be required. An animal model was sought, therefore. Since pulmonary SOD in both rats and rabbits was demonstrated to be an enzyme whose activity depends upon age, as described in the previous section, it was concluded that these animals provided suitable models for the study of the effects of hyperoxic exposure upon the activity of the enzyme.

Crapo and Tierney (5) showed that exposure of adult rats to 85% oxygen resulted in a 42% increase in lung SOD activity after a period of 7 days. Table 5 shows a 42% increase in lung SOD activity after only 24 hr of exposure to 80% oxygen of premature New Zealand White rabbit pups. Similarly, young rats responded to hyperoxia with a significant increase in lung SOD after only 24 hr of exposure to 85% oxygen (Table 5). No significant increase in lung SOD activity was observed in adult rats exposed to the same conditions of hyperoxia as the 7-day-old rats.

In order to determine whether the oxygen effect occurred initially in the lung or whether the observed increase in enzyme activity occurred secondarily to a primary event elsewhere, the effect of hyperoxia on isolated lung tissue was investigated. Figure 1 shows the results of a typical *in vitro* experiment using rat tissue. Enzyme activity increased about 30% in this system after 2 hr of incubation in 100% O_2. This increase in SOD activity was unaffected when analyzed in the presence of 5×10^{-5} M CN^-. Similar incubation of lung tissue in room air did not stimulate an increase in SOD activity. Incubation of lung tissue in buffer alone did not result in an oxygen dependent increase in enzyme activity, although incubation in plasma effectively replaced the requirement for whole blood. Identification of the factor or factors in plasma necessary for the observed effect are under investigation.

Figure 2 illustrates typical results obtained after *in vitro*

Table 5. *Total pulmonary superoxide dismutase activity after exposure of young rats, premature rabbits, and adult rats to 85% O_2 for 24 hr*[1]

	Rabbits (premature)		Rats (7 day old)		Rats (adults)	
	Control[2] (n = 8)	24 hr 80% O_2 (n = 6)	Control[2] (n = 3)[3]	24 hr 85% O_2 (n = 3)[3]	Control[2] (n = 6)	24 hr 85% O_2 (n = 3)
Lung weight (g)	1.4	1.4	0.4	0.3	1.4	1.4
SOD (units/g)	90 ± 4	128 ± 19	537 ± 16	767 ± 49	1,069 ± 30	1,097 ± 44
% Change		+42[4]		+43[4]		+3 (NS)

[1] Data reported as mean units of enzyme activity ± SEM. SOD: superoxide dismutase; NS: not significant.

[2] Control animals were exposed to room air.

[3] Each number represents pooled tissue from eight individual animals.

[4] $P < 0.05$.

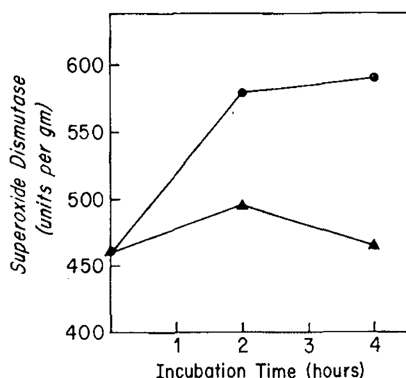

Fig. 1. Superoxide dismutase activity in minced lung tissue of 12-day-old rats after incubation in air (▲——▲) and in 100% oxygen (●——●). Tissue was incubated in adult rat blood plasma. Superoxide dismutase activity is expressed as units per g of lung tissue. Each curve represents data obtained from pooled tissue of 13 animals.

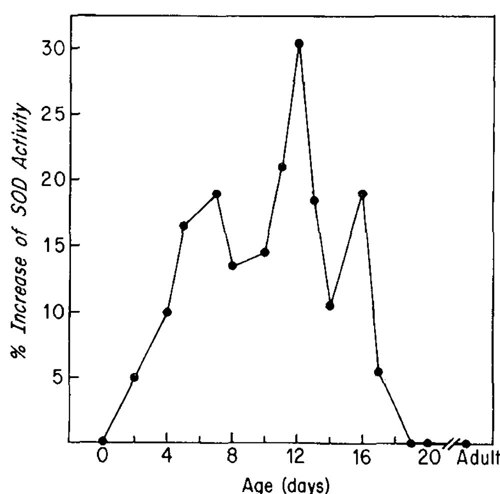

Fig. 2. Percentage of change in activity of pulmonary superoxide dismutase (*SOD*) as a function of rat age after incubation of minced lung tissue in adult rat blood plasma at 37° and under an atmosphere of 100% oxygen. Results indicate the percentage of change in SOD activity after 4 hr of incubation compared with lung tissue treated identically but incubated under an atmosphere of air. When lung tissue from all age groups was incubated in air, SOD activity was virtually unchanged after 5 hr of incubation (Fig. 1). Each point represents pooled tissue from 8–10 animals.

incubation of pooled lung tissue in high oxygen. The percentage of increase in lung SOD activity as a function of the age of the rat between 1 day and 22 days is shown. A striking correlation between capacity for increased SOD activity and age can be observed. This capacity began to rise just after birth, reached a peak at approximately 10–12 days, and then decreased until the capacity for the oxygen-stimulated increase in enzyme activity disappeared at about 19–20 days of age.

DISCUSSION

In both the humans and the laboratory animals studied, pulmonary SOD activity was significantly higher in adults than in newborns and fetuses. These results support the proposal that SOD is a developmentally important enzyme in the animal and human lung and possibly in human blood. As with the enzymes of the surfactant system (9), SOD may play an essential role in normal pulmonary function with the result that an enzyme deficiency in the immature animal could be highly detrimental. Interestingly, SOD activity has recently been found to increase in human placenta with increased placental age (26).

McCord *et al.* (16) and Crapo and Tierney (5) showed that SOD is an enzyme which will respond to hyperoxia with an increase in specific activity. Although Fridovich demonstrated this effect in prokaryotes, Crapo showed a similar effect in higher animals, *i.e.*, that exposure of rats to hyperoxia resulted in higher lung SOD activity. Crapo also showed that increased tolerance to hyperoxia paralleled this increased SOD activity and that species in which lung SOD activity did not increase after oxygen exposure failed to develop tolerance to hyperoxia. Our data suggests that the animal lung is most responsive to hyperoxic stimulation of SOD activity during the first days of life. The difference in the hyperoxic exposure time necessary to provoke this response *i.e.*, 24 hr for neonatal animals in this study, compared with 7 days for adult animals, as reported by Crapo, is dramatic. Other investigators have demonstrated that immature animals of various species are more tolerant to high oxygen exposure than are older animals (13, 21). This information plus the data on neonatal rat and rabbit pulmonary response to hyperoxia reported here, suggests that lungs of young animals may be protected against the toxic effects of chronic hyperoxic exposure via a rapid increase in activity of the protective enzyme SOD.

The age-dependent increase of SOD activity after hyperoxic exposure can be compared with the results of Kaufman *et al.* (10) who demonstrated an age-dependent proliferation pattern of the type II alveolar cell in the postnatal rat lung. Both phenomena show a peak activity at 7–12 days after birth. The coincidence of these two curves may mean that the oxygen-dependent increase in SOD activity occurs in the type II pneumocyte. The type II cell, the synthetic source for pulmonary surfactant (11), not only shows an increased proliferation rate but also retains a greater viability in hyperoxia than do other pulmonary cells such as the type I pneumocyte and the capillary endothelial cell (27). There are a number of possible ways to explain the rapid increase in pulmonary SOD activity after hyperoxic exposure in neonatal animals. (*1*) Hyperoxia may favor one cell type in the growing lung which could be either the major or only source of SOD. (*2*) Oxygen could either enhance the synthesis, inhibit the degradation, or activate a proenzyme form of SOD. (*3*) The effect of oxygen-provoked mediating influences upon the rate of synthesis and/or the rate of degradation of pulmonary SOD could be related to age.

The proposal that SOD plays an important role in the detoxification of oxygen (16) and the observation that the histopathology of oxygen toxicity and IRDS/HMD are similar in certain respects (4, 25), provided the rationale for the studies of SOD enzyme activities in infants with IRDS/HMD and BPD previously exposed to hyperoxia for prolonged periods during the first weeks of life. Considering that SOD appears to be an enzyme which shows increased activity during maturation and that young animals respond to hyperoxic exposure with a significant increase in pulmonary SOD activity, it might have been expected that lung SOD activity in infants with IRDS/HMD would have proved to be higher than in normal infants since all of the former group were treated with high concentrations of oxygen. This was not, in fact, what was observed. It was not possible, as explained previously, to obtain data on the effect of oxygen exposure upon pulmonary SOD activity in normal or non IRDS/HMD infants. Utilizing the results obtained from both *in vitro* and *in vivo* exposure of animal lungs to hyperoxia to predict human lung response to hyperoxia leads to the speculation that an inability to increase the activity of this putative protective enzyme after hyperoxic challenge may be a defect partially explaining the pathophysiology of IRDS/HMD and/or BPD. In the case of IRDS/HMD infants, an acute response involving compromised pulmonary function could result when SOD-deficient lungs are stressed with oxygen treatment. Furthermore, because lungs are well perfused with blood, the activity of SOD in erythrocytes could be critical during hyperoxia, as well. As in the lung, SOD activity in blood increases upon development, albeit more slowly. Successful adjustment to hyperoxia may demand maximum erythrocyte SOD activity as well as pulmonary activity. BPD, generally believed to reflect toxic pulmonary changes secondary to chronic oxygen exposure (2), may represent a more chronic continuum of such a deficiency in pulmonary protection against oxygen.

SUMMARY

Superoxide dismutase activity was determined in blood and lung tissue from adults and various infant groups. Enzyme activity in human fetuses and infants was significantly lower than in adults. Similarly, SOD activity was related to age in rats and rabbits with much lower activity found in late fetal animals compared with the adult animals. No significant differences in enzyme activity, however, were seen between (*1*) infants with IRDS/HMD and their premature peers without IRDS/HMD and between (*2*) premature infants and full term infants.

Experiments in which animal lung tissue was exposed either to 80–85% oxygen *in vivo* or 100% oxygen *in vitro* demonstrated an enhancement of pulmonary SOD activity in response to this hyperoxic challenge. In the rat, the ability to increase pulmonary SOD activity under hyperoxic conditions was age related, with the maximum effects occurring during the first 18 days of life.

These data suggest that SOD is an enzyme for which increasing activity associated with maturation may be obligatory to newborn lung for the provision of protection against the relative hyperoxia of extrauterine life.

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Amino acid metabolism
glycogen
gluconeogenesis
liver
metabolic development
phosphoenolpyruvate carboxykinase activity
starvation

Utilization of Dietary Amino Acids for Energy Production in Neonatal Rat Liver

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Extract

The 3-day-old rat has a high basal level of phosphoenolpyruvate carboxykinase (PEPCK), the activity of which is not increased upon starvation. The lower basal activity of the enzyme in 19-day-old rat liver can, however, be stimulated by starvation. Serum glucose levels increased from 3 days to 19 days of age, with a decrease to adult levels. Liver glycogen concentration increased from 3 days to 19 days of age, with no additional increase observed at 3 months. There was a decrease with age in the specific activity of liver glycogen (from [^{14}C]alanine and [^{14}C]leucine). In fed rats given [^{14}C]alanine, $^{14}CO_2$ expiration tended to decrease with age. The $^{14}CO_2$ produc-