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Superoxide Dismutase in Bovine Fetal Ductus Arteriosus, Thoracic Aorta, and Pulmonary and Umbilical Arteries (40277)

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Soon after birth the lumens of the ductus arteriosus and the umbilical artery are obliterated. Some researchers have suggested that oxygen toxicity, resulting from the increased arterial oxygen tension occurring after birth and the development of an "active hypersensitivity" reaction to oxygen prior to birth, is the cause of widespread intracellular and extracellular destruction noted in the subintimal regions of the media (muscular layer), as well as the intimal layer itself in the ductus arteriosus (1, 2). This is similar to the explanation offered for the closure of premature infants' retinal arteries and consequent retrolental fibroplasia and blindness from exposure to excessively high oxygen levels in the hyperbaric chamber (1, 3). Other workers (4–6) have presented histological and other evidence that the ductus arteriosus of guinea pig, rabbit, rat, and mouse fetuses allowed to breathe showed widespread intracellular and matrix destruction in the histological regions previously mentioned; such changes were not noted in fetuses frozen without permitting respiration.

A suitable explanation for the temporal relationship between increasing arterial oxygen tensions and cellular degeneration could certainly be superoxide radicals, hydroperoxides, and hydroxyl radicals, all powerful oxidants which are destructive in biological systems of cells existing in aerobic conditions (3, 7, 8). It has been demonstrated that rats exposed to gradually increasing levels of O_2 in their environment had significantly greater levels of SOD in lung tissue and survived for significantly longer periods of time after exposure to toxic levels of O_2 than did control rats (7, 8). We postulated that an overproduction of hydroxyl radicals could result after an increase in oxygen tension in tissues such as the ductus arteriosus and umbilical artery, if such tissues possessed lower levels of SOD, as compared with such permanent tissues as the pulmonary artery and thoracic aorta. Superoxide dismutase catalyzes the dismutation of two molecules of the superoxide anion forming one molecule each of oxygen and hydrogen peroxide, while the superoxide anion and hydrogen peroxide can react to form the hydroxyl radical:

(a) $2O_2^- + 2H^+ \xleftarrow[SOD]{} O_2 + H_2O_2$
· (Superoxide Dismutase Reaction)

(b) $O_2^- + H_2O_2 \rightarrow HO^- + HO\cdot + O_2$ (Haber-Weiss Reaction)

SOD functions to remove one of the reactants of the Haber-Weiss reaction, and this enzyme has been extensively studied by McCord and Fridovich (3), as well as by others.

In this study we chose to examine the levels of SOD in several tissues of bovine fetuses, using an enzymic activity assay and a radial immunodiffusion assay. There were two groups of two tissues used for comparative purposes; the two fetal blood vessels which obliterate after birth, the ductus arteriosus and umbilical artery, and the two blood vessels which do not obliterate after birth, the pulmonary artery and the thoracic aorta. Reported herein are the results of this study which we feel are in support of our hypothesis.

Materials and methods. A local meat packing firm allowed us access to fetal calves approximately forty minutes following the killing of the mother. Gestational ages of the calves were estimated using such criteria as crown-rump length, body hair patterns, and the presence of erupted incisor teeth (9). Eighty percent of the calves in this study were "full-term" by the criteria mentioned. The thoracic cavity was then entered and an *en bloc* excision of the heart, great vessels, and the entire length of the thoracic aorta was performed; additionally, a small segment of

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umbilical artery was obtained from the umbilical cord. The great vessels were then identified, dissected free, excised, washed three times in 0.15 *M* NaCl, immediately frozen on dry ice and stored for 2 weeks at −30°. The vessels were then thawed, washed again three times in 0.15 *M* NaCl, homogenized in 4 vol of 0.15 *M* NaCl with a Tenbroeck glass homogenizer, and centrifuged at 100,000*g* for 75 min at 2°. The supernatant was then used for analysis of SOD activity. If there is significant blood in the prepared tissue, this will contribute to the total SOD activity of the sample as erythrocytes do possess significant levels of SOD. Whole blood was obtained from four fetal calves in the study. The erythrocytes were lysed with an equal volume of deionized water and the solution then restored to 0.15 *M* NaCl. The lysed erythrocyte mixture was then centrifuged and the supernatant was diluted with 0.15 *M* NaCl to obtain solutions with hemoglobin concentrations in ranges comparable to the tissue supernatants from the blood vessel preparations. Hemoglobin levels of the lysed erythrocyte and blood vessel supernatants were then measured at 24.7 cm^{-1} using a Cary 118C spectrophotometer. SOD activity of the lysed erythrocyte supernatants was measured.

SOD activity was measured using the xanthine oxidase–cytochrome *c* assay of McCord and Fridovich (10). Bovine erythrocyte SOD was purified to electrophoretic homogeneity using the method of McCord and Fridovich (10). This preparation was used to prepare antibodies in rabbits. Immune rabbit γ-globulins were isolated as previously described, and these were used to determine the levels of SOD using a radial immunodiffusion assay (11). Bovine xanthine oxidase was purified to homogeneity from raw cream (12). Protein concentrations were determined by the method of Lowry *et al.* (13).

Tris·HCl and base and cytochrome c were obtained from the Sigma Chemical Company. Agar was obtained from Difco Company. All other chemicals were reagent grade quality.

Results and discussion. The results of enzymic and immunochemical assays for SOD in four tissues from thirteen bovine fetuses are presented in Table I. In all individual animals the levels of SOD determined in the four tissues indicated that the ductus arteriosus and umbilical artery were always lower than the pulmonary artery and thoracic aorta although a comparison between animals did not always follow this pattern.

The data were compared using the "t" test of significance and the results of such comparisons are shown in Table II. As can be seen, in nearly all comparisons the levels of SOD in the ductus arteriosus and umbilical artery were statistically significantly lower than those found in the pulmonary artery and thoracic aorta. The level of SOD in the ductus arteriosus and the umbilical artery were not statistically significantly different from each other. Likewise, the levels of SOD in the pulmonary artery and thoracic aorta were not statistically significantly different from each other.

Erythrocytes do contribute to the SOD activity of tissue extracts although this contribution is negligible if it is possible to wash the tissues relatively free from blood (7). In this study the hemoglobin in the tissue supernatants was in the range of 1–2 × 10^{-6} *M*.

TABLE I. BOVINE FETAL SUPEROXIDE DISMUTASE.

Tissue	Activity enzyme units[a] mg protein	Radial immunodiffusion μg[b] mg protein
Ductus Arteriosus	2.32 ± 0.33[c]	77.8 ± 5.8[c]
Umbilical Artery	1.97 ± 0.16	80.2 ± 7.8
Pulmonary Artery	3.64 ± 0.32	94.7 ± 6.7
Thoracic Aorta	3.45 ± 0.31	113.9 ± 8.3

[a] Determined with xanthine oxidase–cytochrome *c* assay, expressed per mg cytosolic protein.

[b] Expressed as μg superoxide dismutase per mg cytosolic protein.

[c] Values are expressed as the mean ± SE for thirteen samples run in duplicate.

TABLE II. "t" TEST OF SIGNIFICANCE.[a]

Paired tissues	Enzyme assay	RID
DA-PA	0.001	0.01
DA-TA	0.05	0.01
UA-PA	0.001	0.05
UA-TA	0.001	0.02
DA-UA	0.4	0.7
PA-TA	0.7	0.2

[a] The data of Table I were analyzed by pairing the indicated tissues. The confidence levels are indicated for the two types of assay, enzymic and immunochemical (RID, radial immunodiffusion).

The SOD activity of the lysed erythrocyte supernatants in this hemoglobin concentration range was negligible (less than one percent). Additional evidence to discount the contribution of erythrocytes in this study is noted in that the hemoglobin concentrations varied randomly in the tissue samples and did not correlate with the differences between the SOD activity of the blood vessel preparations.

Undoubtedly the etiology of ductus arteriosus closure is multifactorial, and it is not possible here to elaborate the numerous mechanisms proposed (14–16). It is helpful to view ductus arteriosus closure as both a physiological and anatomical event; that is to say, the ductus arteriosus responds to varying oxygen tensions and hemodynamic changes by changing its lumen size *in situ*, and it undergoes obliterative fibrotic changes to ultimately become the ligamentum arteriosum in the usual case. The *in vitro* responsiveness of this vessel to varying oxygen tensions has been consistently reported in the literature. The role of prostaglandins in the closure of the ductus arteriosus is also of current interest (17–19).

This study suggests that a deficiency of SOD could contribute to the degenerative cellular changes presumed to occur as part of the obliterative process in the bovine ductus arteriosus and umbilical arteries. Further studies need to be conducted to determine if the rise in arterial oxygen tension at parturition is sufficient to create the oxidant stress this study is proposing. In addition we are not capable of ascertaining the distribution of SOD across the wall of the tissues we have examined, which might be of importance in the degeneration of the ductus arteriosus and umbilical artery. Perhaps in the future a histological stain for SOD of sufficient sensitivity will be developed and can be used to answer such questions.

The levels of SOD seen in the ductus arteriosus and in the umbilical artery are 54–67% (activity assay) and 68–84% (RID assay) of the levels found in the pulmonary artery and in the thoracic aorta. Michelson *et al.* (20) have suggested that "levels of less than 50% of the normal mean for superoxide dismutase are more or less lethal due to the increased toxicity of uncontrolled superoxide." This contention was based on a survey of SOD activities in a cross section of the human population in France, comparing normal and abnormal populations. Extremely low levels of SOD correlated in several cases with associated physical and mental problems. The ability of a newborn to handle an increased flux of superoxide, consequent to exposure to increased oxygen tensions, may reflect the absolute and quantitative amounts of SOD present in particular tissues. Those with high levels of SOD will survive, and those with low levels of SOD will degenerate.

Summary. Soon after birth the lumens of the ductus arteriosus (DA) and umbilical artery (UA) are obliterated. It has been suggested that oxygen toxicity, resulting from an increased oxygen tension, is the cause of this destruction with superoxide radicals and hydroxyl radicals being implicated as mediators. A deficiency of superoxide dismutase (SOD) in these tissues was hypothesized as being responsible for an increase in the levels of superoxide and hydroxyl radicals. SOD levels were determined enzymatically and immunochemically in four tissues obtained from thirteen bovine fetuses. SOD levels in the DA and UA were found by both assays to be statistically significantly lower than that found in such permanent vessels as the pulmonary artery and thoracic aorta. These data are in support of the hypothesis that a lower level of SOD in the ductus arteriosus and umbilical artery may contribute to the rapid deterioration of these tissues upon exposure to greatly increased oxygen tensions.

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