

Increased Activity of the Respiratory Burst in Cord Blood Neutrophils: Kinetics of the NADPH Oxidase Enzyme System in Subcellular Fractions

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ABSTRACT. Previous studies with neutrophils from newborn infants compared to neutrophils from healthy adults have documented increased respiratory burst activity including enhanced superoxide anion (O_2^-) production, nitroblue tetrazoleum dye reduction, and hexose monophosphate shunt activity. To investigate the biochemical basis for these observations, we examined oxidative metabolism in membrane-rich fractions of neutrophils. Neutrophils from cord blood of vaginally delivered term infants or healthy adults were disrupted by nitrogen cavitation and subcellular fractions collected on discontinuous sucrose density gradients. Subcellular fractions of newborn neutrophils separated in a fashion identical with samples from healthy adults. Activity of alkaline phosphatase, a plasma membrane marker, was increased 4- to 5-fold in disrupted cells free from nuclei (postnuclear supernatant) as well as plasma membrane fractions from newborn samples compared to those from healthy adults. Content of lactoferrin, a specific granule marker, was decreased in postnuclear supernatants but equivalent in specific granule fractions of newborn cells compared to those from adults. No differences were noted in myeloperoxidase content of postnuclear supernatants or any other subcellular fraction. Plasma membrane fractions from phorbol myristate acetate-stimulated cord blood neutrophils made significantly more O_2^- than samples from adults (newborn 32.9 ± 8.1 nmol O_2^- /min/mg protein mean \pm SEM, $n = 3$ versus adult 10.8 ± 4.2 , $n = 3$; $p < 0.05$). Plasma membrane-rich fractions were also collected by the technique of differential centrifugation and kinetic parameters of the NADPH-dependent oxidase enzyme(s) were measured for vaginally delivered newborn and adult samples. The K_m^{app} for NADPH for newborn fractions was significantly increased compared with adult samples (newborn 66 ± 10 μ M, $n = 6$ versus adult 30 ± 6 , $n = 6$; $p < 0.025$) but not to the extent that would be associated with abnormal cell function. In contrast, V_{max} of newborn membrane-rich fractions was 1.7 times that of adult samples (newborn 30.6 ± 27 nmol O_2^- /min/mg protein, $n = 6$ versus adult 18.0 ± 4.0 , $n = 6$; $p < 0.025$). Plasma membrane-rich fractions from term infants delivered by cesarean section without labor had a K_m^{app} of 67 ± 20 μ M, $n = 5$ which was different from adult samples ($p < 0.05$) and a V_{max} for O_2^- production of 14.8 ± 4.5 which was less than that measured from samples from vaginally delivered infants ($p < 0.01$). The K_m^{app} results

imply a qualitative difference in the fetal oxidase enzyme system. In addition, the increased O_2^- production and V_{max} demonstrated in samples from vaginally delivered infants as compared to infants delivered by cesarean section or healthy adults suggests an effect of parturition on the fetal neutrophil which is similar to the effect of certain compounds such as lipopolysaccharide. The increased catalytic activity of the NADPH oxidase enzyme system in neutrophils from newborns may reflect "priming" during parturition. (*Pediatr Res* 21: 205-210, 1987)

Abbreviations

O_2^- , superoxide anion
PMA, phorbol myristate acetate
 \cdot OH, hydroxyl radical
KRPD, Krebs Ringer phosphate buffer
psi, pounds per square inch
MPO, myeloperoxidase

Initiation of the respiratory burst is an important component of the microbicidal activity of the neutrophil and is crucial to the body's defense against infection. Diminished microbicidal activity by neutrophils from newborn infants, particularly stressed neonates, has been documented (1-3). Our previous studies demonstrated that neutrophils from newborn infants generate relatively less \cdot OH, a particularly toxic oxygen metabolite important to the oxygen-dependent microbicidal activity of the neutrophil (4). The decreased production of \cdot OH was explained by diminished content of lactoferrin in newborn neutrophils (5). This protein is found in the specific granules of neutrophils and, when saturated with iron, enhances the production of \cdot OH (6). While \cdot OH production in newborn neutrophils is decreased, activity of the respiratory burst is increased in normal infants and may be decreased in stressed infants (4, 5, 7-10, 11). Understanding the biochemical basis for these differences in the respiratory burst is important in defining the microbicidal defect in newborn cells and the reasons for the newborn's risk for infection. In the following study, we evaluated the production of O_2^- and the kinetic parameters of the oxidase enzyme system in membrane-rich subcellular fractions of neutrophils from newborn infants.

MATERIALS AND METHODS

Reagents. The following reagents were purchased commercially: superoxide dismutase, cytochrome *c*, *Micrococcus lysodeiketicus*, *o*-dianisidine, Triton X-100, and E-toxate No. 210

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(Sigma Chemical Company, St. Louis, MO); PMA (Consolidated Midland Corporation, Brewster, NY); and IgG fraction of rabbit anti-human lactoferrin (U.S. Biochemical Corporation, Cleveland, OH). Endotoxin-free reagents were obtained as follows: NaCl (14.6%), dextrose (50%) (Abbott Laboratories, Chicago, IL); KCl (15%), $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ (50%), CaCl_2 (10%) (Invernex, Chagrin Falls, OH); EDTA, sodium cyanide, and sodium mono and dibasic phosphate (J. T. Baker, Phillipsburg, NJ); and Macrodex 6% in normal saline (Pharmacia Laboratories, Piscataway, NJ). Stock solutions were made to the appropriate concentrations from these reagents with water determined to be free of endotoxin by the Limulus lysate assay (Sigma Chemical Company). All buffers and solutions used during isolation, stimulation, and disruption of neutrophils were determined to be endotoxin free by this method.

Isolation of neutrophils. Cord blood was obtained from placentas immediately after delivery and anticoagulated with 5–10 U/ml heparin. For control studies, blood samples were taken from healthy adults in the same way. Informed consent was obtained in accordance with the requirements of the Human Subjects Committee of the University of Colorado Health Sciences Center. Infants delivered vaginally were products of full-term, uncomplicated pregnancies, labors, and deliveries; and no general anesthesia or intravenous analgesia administered to the mothers. As a separate control, samples were processed from placentas of five infants delivered by cesarean section. All of these infants were products of uncomplicated term pregnancies. None of the mothers was in labor prior to or during cesarean section and all deliveries were performed under spinal anesthesia. No perinatal complications occurred in any of the babies studied and all had Apgar score at 1 and 5 min of ≥ 8 .

Separation of neutrophils in endotoxin-free reagents were performed by a modification of previous described techniques (5). Whole blood was placed in plastic conical tubes and centrifuged at $600 \times g$ for 10 min. After removing the plasma, a volume 2.5 times the original whole blood volume of Macrodex diluted 1.5:1 with normal saline was added to the cells. The mixture was inverted several times and allowed to sediment at room temperature for 1 h. The supernatant was removed, centrifuged at $600 \times g$ for 10 min and the red cells were lysed by hypotonic lysis. After washing three times with KRPD with glucose, pH 7.34, the cells were resuspended to 5×10^7 cells/ml. Neutrophils obtained with this technique were 85–95% pure and >99% viable as determined by exclusion of trypan blue.

Stimulation of neutrophils. Neutrophils in KRPD were preincubated at 37°C for 4 min in the presence of 1.0 mM KCN, and then were stimulated by the addition of 100 ng/ml PMA. After incubation for 3 min at 37°C , an equal volume of cold disruption buffer (same as KRPD except calcium was deleted and MgSO_4 was present at 2.5 mM) was added to the cells and the mixture was centrifuged at $800 \times g$ for 8 min. The pelleted cells were then resuspended in disruption buffer.

Disruption of neutrophils. Neutrophils in disruption buffer were placed in a small Falcon tube with a magnetic stir bar and subjected to nitrogen cavitation in a Parr cell disruption bomb (4635, Parr Instrument Company, Moline, IL) as previously described by Klempner *et al.* (12). The exact conditions were somewhat different than originally described. After flushing for 5 min with nitrogen gas, the exhaust port of the bomb was closed, allowing the pressure to increase to the desired level. This pressure was maintained for 20 min at 4°C . The exhaust port was then opened and the cavitate collected dropwise from the port into an equal volume of buffer containing 0.34 M sucrose (wt/wt) and 2.5 mM EDTA. In a series of experiments to determine the optimal pressure for disruption, breakage of granules was monitored by measuring lysozyme (13) in the supernatant following centrifugation of the cavitate at $27,000 \times g$; cell disruption was determined by cell counts before and after cavitation; and breakage of nuclei was documented by comparing hemocytometer cell counts before disruption with nuclei counts after disruption.

At pressures greater than 250 psi, there was evidence of significant breakage of granules (30–40% of total lysozyme activity in the $27,000 \times g$ supernatant). The extent of cell breakage was dependent on cavitation pressure, 50% cell disruption at 75–150 psi, 80% at 200–300 psi, and >90% at pressures >300 psi. Significant nuclear disruption was not seen at pressures <300 psi. A pressure of 225–250 psi minimized the nuclear and granule breakage and maximized the cell disruption. In all subsequent experiments, disruption was carried out under these conditions and the cavitate was centrifuged at $800 \times g$ for 10 min to remove nuclear debris and unbroken cells, yielding the “postnuclear supernatant.”

Separation of subcellular fractions. Separation of subcellular fractions from resting and PMA-stimulated cells disrupted by nitrogen cavitation was performed by two different techniques. In the first technique, discontinuous sucrose density gradients were used. The postnuclear supernatant of the cavitate was layered over a gradient consisting of 1.5–2.0 ml layers of 18, 28, 33, and 42% sucrose (w/w) on a 2.0 ml cushion of 50% sucrose. These gradients were centrifuged at $100,000 \times g$ for 2 h in a Beckman L5-50 ultracentrifuge equipped with swinging bucket rotor (SW-41), and fractions were removed by hand or with a gradient unloader. Alternatively, the postnuclear supernatant was transferred to Corex tubes and centrifuged in a Beckman J-21B high-speed centrifuge with a JA-20 rotor at $9500 \times g$ for 15 min. The pellet was resuspended in 0.34 M sucrose and stored at -70°C for further studies. The supernatant was centrifuged at $32,000 \times g$ for 45 min, and the resultant pellet was resuspended in 0.34 M sucrose and was also stored at 70°C .

Enzyme assays and superoxide anion production. The activities of MPO and alkaline phosphatase were measured by the oxidation of *o*-dianisidine and the reaction with *p*-nitrophenyl phosphate respectively (5, 13). Lactoferrin was quantitated by rocket immunoelectrophoresis (5), and total protein was measured by the method of Lowry *et al.* (14). Production of O_2^- was measured as superoxide dismutase-inhibitable reduction of ferricytochrome c in a dual beam recording spectrophotometer (Cary 118) as previously described (15). The reaction mixture contained 0.08 mM ferricytochrome c, 25 μl of membrane-rich fraction, and the appropriate concentration of NADPH in a total volume of 300 μl . The reference cuvette contained all of the above plus 10 μg of superoxide dismutase. The exact concentration of NADPH was determined by measurement of the absorbance of 340 nm and calculation using the millimolar extinction coefficient ($6.2 \text{ mM}^{-1} \text{ cm}^{-1}$) (16). Quantitation was carried out using the extinction coefficient $2.1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ for reduced versus oxidized cytochrome c and the results were expressed as nmol O_2^- produced/mg protein/minute. In kinetic studies of the oxidase enzyme(s), superoxide anion production at concentrations of NADPH ranging from 0.01 to 0.25 mM was measured, and the data analyzed using Lineweaver-Burke plots. The kinetic parameters were calculated using the statistical methods of Wilkinson (17).

RESULTS

Separation of subcellular fractions. The results of separation of subcellular fractions from unstimulated neutrophils on discontinuous sucrose density gradients are summarized in Table 1. The plasma membrane marker, alkaline phosphatase, was found in highest specific activity in the 18 and 28% bands accounting for a 19- to 29-fold increase in specific activity over that found in the postnuclear supernatant. Of interest is the increased activity of alkaline phosphatase demonstrated in neutrophils (newborn $2.7 \pm 0.3 \text{ mU}/10^7$ cells, mean \pm SEM, $n = 4$; adult 0.7 ± 0.6 , $n = 8$) and in all subcellular fractions of cord blood neutrophils from vaginally delivered infants. The highest content of the specific granule marker, lactoferrin, was seen in the 33 and 38% fractions. No differences between adult and cord blood fractions were seen except for a significantly decreased amount of lacto-

Table 1. Separation of subcellular fractions from unstimulated neutrophils on discontinuous sucrose density gradients*

Fraction	Specific activity of marker proteins					
	Alkaline phosphatase†		Lactoferrin†		Myeloperoxidase†	
	Adult	Cord	Adult	Cord	Adult	Cord
Postnuclear Supernatant	3.3 ± 0.6‡	20.3 ± 4.0§	56.0 ± 5.1	19.6 ± 4.0	4.9 ± 2.1	8.8 ± 4.8
18%	57.1 ± 28.4	342.4 ± 62.7§	19.5 ± 7.1	42.7 ± 3.7	10.4 ± 3.5	39.6 ± 14.0
28% (membrane)	89.7 ± 25.9	460.7 ± 46.3	49.9 ± 6.0	62.7 ± 15.3	47.2 ± 14.0	39.3 ± 5.9
33% (specific)	7.6 ± 5.1	95.2 ± 4.2	289.6 ± 29.5	278.3 ± 77.2	46.6 ± 6.0	73.2 ± 19.4
38%	3.1 ± 0.5	29.5 ± 4.1	201.6 ± 65.3	132.6 ± 5.1	124.6 ± 39.0	190.3 ± 24.2
42% (azurophilic)	3.6 ± 2.9	23.9 ± 6.8	58.3 ± 18.7	60.1 ± 15.0	114.2 ± 22.4	126.4 ± 44.5

* Isolation and disruption of neutrophils and separation of subcellular fractions on discontinuous sucrose density gradients as described in "Materials and Methods."

† Assays for marker proteins measured by reaction with *p*-nitrophenyl phosphate (alkaline phosphatase) rocket immunoelectrophoresis (lactoferrin), and oxidation of *o*-dianisidine. Results are specific activity, expressed as $\Delta A/\text{mg protein} \times 10^{-3}$, for alkaline phosphatase and myeloperoxidase or ratio of lactoferrin to total protein ($\mu\text{g}/\text{mg protein}$).

‡ Numbers represent mean \pm SEM of gradients performed on three cord blood and adult control samples.

§ Significant difference between cord blood and adult samples, $p < 0.01$ by unpaired *t* test.

|| Significant difference between cord blood and adult samples, $p < 0.005$ by unpaired *t* test.

ferrin in the postnuclear supernatant of cord cells ($p < 0.005$). The highest amount of MPO activity was found in the 38 and 42% bands, and no differences between adult and newborn samples were noted. Insignificant amounts of protein markers were found in the 50% cushion (data not shown). In accordance with the location of the marker proteins, the membrane appeared to be located in the 28% band, the specific granules in the 33% band, and the azurophilic granules in the 42% band. Although there were quantitative differences in marker proteins, subcellular fractions from cord blood neutrophils appeared to separate on the discontinuous gradients in a fashion qualitatively identical to fractions from neutrophils of adult controls.

O₂⁻ production by membrane fractions. In subsequent experiments, cells were stimulated with PMA; subcellular fractions were prepared on density gradients; and the membranes (28% band) were collected. O₂⁻ production was measured in the presence of 0.1 mM NADPH. Membranes from cord blood neutrophils of vaginally delivered infants produced three times as much O₂⁻ as membranes from control neutrophils (adult 10.8 ± 4.2 nmol O₂⁻/min/mg protein, mean \pm SEM, $n = 3$; newborn 32.9 ± 8.1 , $n = 3$; $p < 0.05$ by unpaired *t* test).

Because of the sucrose gradient procedure produced insufficient quantity of membrane fraction, kinetic analysis of NADPH oxidase was performed with membrane-rich fractions prepared by differential centrifugation. Membrane fractions prepared by this technique have previously been shown to contain at least 75% of the NADPH-dependent oxidase activity (18). Rates of O₂⁻ production by membrane fractions prepared by differential centrifugation were measured at concentrations of NADPH between 0.01 and 0.25 mM for six adult and six vaginally delivered infant specimens. Plots of velocity versus substrate concentration revealed a hyperbolic saturation curve for all samples. Figure 1 presents double reciprocal plots for O₂⁻ production versus substrate (NADPH) concentration for representative sample neutrophil membrane fraction from adult control and cord blood. Data from all newborn samples analyzed in this way exhibited the same pattern, decreased slopes, and y-intercepts compared to adult samples. The kinetic parameters for oxidase activity for these samples are summarized in Table 2. The membrane fraction of cells from vaginally delivered babies exhibited a significantly increased K_m^{app} for NADPH compared to adult samples ($p < 0.025$). However, this was not out of the range of normal values published by other investigators (19–21). The V_{max} for cord blood samples was 1.7 times greater than values for adult cells ($p < 0.025$). Also included in Table 2 is the calculated rate of O₂⁻ production by subcellular fractions based on measured kinetic parameters and an estimated intracellular concentration of NADPH of 90 μM . This estimation of NADPH concentration

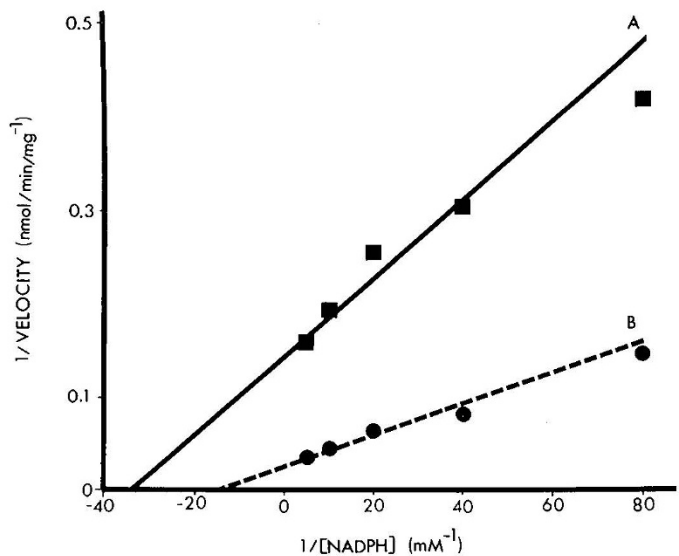


Fig. 1. NADPH-dependent O₂⁻ production by membrane-rich fractions harvested by discontinuous centrifugation. Data presented as plot of O₂⁻ production versus substrate concentration by double reciprocal Lineweaver-Burk analysis. A, membrane fraction from neutrophils of adult controls; B, cord blood.

in neutrophils was made with data from previous studies documenting content of NADPH to be 2.4 nmol/10⁸ cells (22) and estimates of average neutrophil size. At physiologic concentrations of NADPH, the rate of O₂⁻ production was calculated to be 30% greater in newborn compared to adult membrane samples, a value which is comparable to results reported in whole cells (5).

To determine the role of labor in the differences in kinetic parameters of the oxidase enzyme system, blood samples from placentas of five infants delivered by cesarean section under spinal anesthesia without preceding labor were obtained and membrane-rich fractions were prepared by differential centrifugation. As with adult and vaginally delivered term infants, membrane-rich fractions from babies delivered by cesarean section demonstrated a hyperbolic curve when O₂⁻ production was plotted against substrate concentration. The kinetic parameters for this group of babies were K_m^{app} , $67 \pm 20 \mu\text{M}$, $n = 5$, and V_{max} 14.8 ± 4.5 nmol/min/mg protein. The K_m^{app} for babies delivered by cesarean section was identical to that of vaginally delivered infants but higher than that from adults ($p < 0.05$). V_{max} for the

Table 2. Kinetic parameters for O_2^- generation by membrane fractions of neutrophils*

Membrane source	K_m^{app} (μ M NADPH)	V_{max} (nmol O_2^- /min/mg protein)	Calculated velocity† (nmol O_2^- /min/mg)
Adult	30 \pm 6‡	18.0 \pm 4.0	13.5
Cord	66 \pm 10§	30.6 \pm 2.7§	17.6

* Membrane-rich fractions of PMA-stimulated neutrophils harvested after disruption by nitrogen cavitation and separation by differential centrifugation as described in "Materials and methods."

† Velocity calculated using concentrations of intracellular NADPH of 90 μ M (see "Results"), mean values for K_m^{app} and V_{max} above, and the hyperbolic equation:

$$v = V_{max} \cdot [NADPH] / K_m + [NADPH]$$

‡ Numbers represent mean \pm SEM of results from six separate cord blood and adult samples.

§ Values for cord blood samples significantly different from adult samples, $p < 0.025$ by unpaired t test.

cesarean section infants was identical to adults but less than vaginally delivered infants ($p < 0.01$).

DISCUSSION

In recent years, many studies have addressed the adequacy of phagocyte function in the newborn infant. Defective neutrophil microbicidal activity has been documented especially at high bacteria-to-neutrophil ratios (1–3). Associated with the killing defect are abnormalities in oxidative metabolism including increased nitroblue tetrazolium dye reduction, increased stimulated hexose monophosphate shunt activity, and enhanced production of O_2^- (4, 7, 8, 10). In addition, the rate of O_2^- production by cord blood neutrophils was increased compared to neutrophils from adults (11). While the respiratory burst is quantitatively increased in newborn neutrophils, chemiluminescence, a phenomenon that reflects interaction of a variety of free radicals with cellular excitable substrates (18), is decreased (23, 24). We have shown that despite normal-to-increased production of O_2^- , generation of $\cdot OH$ is relatively decreased in cord blood neutrophils (4, 5). This diminished $\cdot OH$ production was associated with normal degranulation of specific and azurophilic granules but diminished content of lactoferrin in neutrophils from newborns compared to adult controls. Lactoferrin is an iron-binding protein which has been shown to increase production of $\cdot OH$ in whole cells as well as cell-free systems (6). Although the diminished content of lactoferrin could account for the decreased $\cdot OH$ generation by neutrophils from newborns, the reason for the increased O_2^- production and associated measurements of the initiation of the respiratory burst is not well understood. These abnormalities in the respiratory burst appear to be limited to neutrophils since there are no great differences in production of $\cdot OH$ and/or O_2^- by monocytes or monocyte-derived macrophages from cord blood compared to those from healthy adults (25). In the current study we attempted to examine the kinetics of the oxidase enzyme system to further delineate the basis for the increased O_2^- production in neutrophils from newborn infants.

In our initial experiments, cells were isolated with endotoxin-free reagents to avoid additional priming of the oxidase demonstrated by Guthrie *et al.* (26). Membrane-rich fractions were separated from other subcellular components by centrifugation on discontinuous sucrose density gradients. Subcellular fractions from unstimulated cord blood neutrophils from vaginally delivered term infants separated in a manner identical to subcellular fractions of neutrophils from adult blood. However, two differences documented in the analysis of newborn and adult samples merit further discussion. First, the 4- to 5-fold increase in alkaline phosphatase activity measured in the postnuclear supernatants as well as the various subcellular fractions from neonatal samples confirms previous histochemical studies (27). The reason for difference in alkaline phosphatase activity in cord blood neutrophils is not known. We have recently demonstrated that during

purification of alkaline phosphatase from neutrophils by butanol extraction and concanavalin A affinity chromatography, activity of this enzyme in cord blood samples remains 3- to 4-fold increased compared to the enzyme isolated from adult cells at every step of the purification (28). The increased alkaline phosphatase activity was documented for samples obtained from infants delivered vaginally or those delivered by cesarean section without labor, indicating that changes during parturition were not responsible for the differences noted. In addition, there were no differences in temperature stability at 56 and 65° C for all sources of the enzyme. Furthermore, enzyme from both newborn and adult neutrophils exhibited a pattern of inhibition with standard inhibitors identical to liver/bone/kidney alkaline phosphatase and distinct from intestinal or placental alkaline phosphatase. These results taken together with those from the density gradients performed in this study suggest that the increase in alkaline phosphatase in newborn neutrophils may be related to increased specific activity of the enzyme rather than increased content of the enzyme in neutrophils. Determination as to whether this represents a fetal enzyme or stimulation of alkaline phosphatase during pregnancy awaits further evaluation.

Second, the decrease in content of lactoferrin in postnuclear supernatants of neutrophils from newborns confirms our previous studies in whole cells (5). The question still remains, however, whether this decrease in content is a result of decreased numbers of specific granules because of degranulation or a decreased content of lactoferrin within the granules themselves because of diminished production of the protein. In our previous study, we showed that there was a small decrease in specific granules in cord blood neutrophils by electron microscopy. However, this could not completely account for a 50% decrease in cellular content of lactoferrin. The results of the current study demonstrating equivalent content of lactoferrin per mg total protein of the specific granule fraction of newborn neutrophils compared to adult samples suggest that specific granule fractions from neonatal cells contain normal amounts of lactoferrin and support degranulation of specific granules rather than diminished production of the protein as the cause for diminished whole cell levels of lactoferrin.

Production of O_2^- by membrane fraction of PMA-stimulated neutrophils from vaginally delivered newborns was increased, a finding that is consistent with measurements of O_2^- production in whole cells. Because of the limitations in the amount of cord blood that could be obtained from placentas and hence, neutrophils, limited quantities of membrane could be harvested; O_2^- production could be performed at only one concentration of NADPH. With a second technique employing differential centrifugation for isolating membrane-rich fractions, sufficient quantities of membrane were recovered to complete the kinetic analysis of O_2^- production (18). Examination of newborn neutrophil membrane-rich fragments demonstrated differences in both K_m^{app} as well as V_{max} compared to samples from healthy adult controls. The K_m values for cord cells of vaginally delivered term

infants were higher than for adult samples suggesting the possibility of a qualitative difference in the fetal oxidase enzyme(s). However, the actual values for both adult and newborn were within the range published by other investigators for normal neutrophils (15, 16, 18–21, 26, 29, 30) and were not at a level which would be associated with abnormal cell function (29, 30). Values for V_{\max} in membrane-rich fractions from newborn neutrophils was nearly twice that for the samples from normal adults. A high V_{\max} for O₂⁻ production could explain the increased O₂⁻ production in whole neutrophils (4, 5, 10).

Evaluation of kinetic parameters of the oxidase enzyme system in membrane fractions from infants delivered by cesarean section without labor was completed to help define the effects of parturition on fetal neutrophils. The K_m^{app} for NADPH of fractions from these infants was significantly higher than adult controls, further confirming a qualitative difference in the fetal oxidase enzyme system. Furthermore, V_{\max} of samples from cesarean delivered infants was similar to adult samples and lower than that measured for vaginally delivered infants. This suggests that the increased activity in the respiratory burst in neutrophils from newborns is related to changes that occur during parturition.

Several implications of these data warrant further consideration. First, since K_m^{app} for the oxidase in newborn cells was increased, the question arises as to whether there is sufficient NADPH in the cell to saturate the oxidase in newborns. Should NADPH be at levels less than the K_m^{app} for the newborn, the newborn oxidase would run more slowly than adult oxidase. Based on previous measurements (see "Results"), the concentration of NADPH in the cell is estimated to be 90 μM , a concentration sufficient to saturate both newborn and adult oxidase. With an estimate of the concentration of NADPH and measurement of V_{\max} , calculation of the velocity of the oxidase from vaginally delivered babies and adult controls can be completed. Results of these calculations for our experimental data were presented in Table 2; the oxidase enzyme system in newborn cells from vaginally delivered infants should generate O₂⁻ at a rate 30% greater than adult cells. This is consistent with measurement of O₂⁻ generation in intact neutrophils from newborns (4, 5). While a higher K_m for NADPH in newborn samples implies a fetal form of the oxidase enzyme(s), the mechanism by which V_{\max} is increased in vaginally delivered infants remains to be defined.

Recent studies link granule constituents with the respiratory burst. Evidence for the role of MPO in terminating the respiratory burst has been presented (31). Furthermore, activation of the respiratory burst may require translocation of cytochrome b and other constituents from specific granules to plasma membrane (32). The data presented herein as well as in our previous study showed that neutrophils from term vaginally delivered infants have equivalent activity of MPO compared to cells from healthy adults, eliminating altered MPO activity as a reason for increased O₂⁻ production. However, we have demonstrated decreased numbers of specific granules and their contents in neutrophils from newborn infants (5). The importance of specific granule components to the respiratory burst, our previous studies demonstrating loss of specific granules and/or their contents, and our current measurement of higher V_{\max} of the oxidase enzyme system in term, vaginally delivered infants compared to infants delivered by cesarean section without labor and healthy adults suggest that degranulation of neutrophils during parturition may result in assembly of the components of the respiratory burst and priming of the oxidase enzyme(s). This effect would account for the increased O₂⁻ production in whole neutrophils from newborns and would appear similar to the action of endotoxin on the respiratory burst of neutrophils *in vitro* demonstrated by Guthrie *et al.* (26). Further investigations are necessary to explore the respiratory burst in newborn neutrophils and to define those characteristics which are related to a fetal form of the oxidase enzyme system. However, the current studies suggest

that the increased activity of the NADPH oxidase enzyme system in neutrophils from newborn infants reflects "priming" during parturition.

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