Interaction between Ascorbate and Light-Exposed Riboflavin Induces Lung Remodeling

Jean-Claude Lavoie, Thérèse Rouleau, and Philippe Chessex

Pediatrics Department, Sainte-Justine Hospital, University of Montreal, Montreal, Quebec, Canada (J.C.L., T.R.); and Division of Neonatology, Children’s and Women’s Health Centre of British Columbia, Vancouver, British Columbia, Canada (P.C.)

Received April 29, 2004; accepted July 13, 2004

ABSTRACT

Light-exposed parenteral multivitamins induce in lungs peroxide-like oxidant responses as well as the initiation of fibrosis. We hypothesized that peroxides generated in light-exposed total parenteral nutrition (TPN) affect lung remodeling. The objective was to assess the specific roles of peroxides, multivitamin preparation (MVP), and light exposure on lung remodeling during TPN. Three-day-old guinea pigs fitted with an indwelling catheter were assigned to the following intravenous regimens: TPN or MVP ± photoprotection, H$_2$O$_2$ ± glutathione, MVP ± metabisulfite, or ascorbic acid ± riboflavin. Fed animals served as controls. After 4 days, lungs were sampled to determine alveolarization (intercepts), β-actin mRNA (protection assay), and apoptosis (terminal deoxynucleotidyl transferase dUTP nick-end labeling). Data were analyzed by analysis of variance. The infusion of light-exposed multivitamins induced a 20% lower ($p < 0.01$) alveolarization index than fed controls, and 3-fold higher ($p < 0.01$) apoptotic events. This was prevented by photoprotecting TPN. The effect of multivitamins on the alveolarization index was reproduced ($p < 0.05$) by infusion of light-exposed riboflavin in the presence of vitamin C. The alveolarization index correlated ($r^2 = 0.35; p < 0.05$) with β-actin mRNA, suggesting alveolar disruption. Antiperoxides conferred no protection against decreased alveolarization. Lung remodeling induced by exposure of TPN to ambient light is not due to a direct effect of infused peroxides but rather to an interaction between vitamin C and peroxides generated by the exposure of riboflavin to light. It is speculated that this interaction may play a role in the development of chronic lung disease of premature infants who receive TPN and have immature antioxidant defenses.

Total parenteral nutrition (TPN) is an integral part of the care of subjects with intestinal failure and those who cannot be fed by mouth. This technique of intravenous nutritional support, which provides essential nutrients, has contributed to save lives (Gunn et al., 1978); however, it has also been associated with numerous complications. Light exposure of TPN induces a loss of antioxidant vitamins (Silvers et al., 2001), the generation of by-products of lipid peroxidation (Helbock et al., 1993), as well as peroxides (Lavoie et al., 1997). Hydrogen peroxide is the major peroxide generated in TPN solutions (Lavoie et al., 1997); organic peroxides are also produced by peroxidation of lipid emulsions and multivitamins (Neuzil et al., 1995; Lavoie et al., 2004). Light-exposed riboflavin serves as a catalyst in a reaction between oxygen and electron donors such as amino acids, lipids, and ascorbate, leading to the generation of peroxides (Laborie et al., 1998).

Parenteral multivitamin supplementation induces both oxidant and antioxidant responses (Chessex et al., 2001). Although multivitamins possess antiradical properties as measured by their protective effect on hepatic isoprostane levels (Chessex et al., 2002), light-exposed MVPs induce peroxide-like oxidant responses in lungs (Lavoie et al., 2000) and steatosis in liver (Chessex et al., 2002) of guinea pig pups. We have demonstrated that parenteral multivitamins produce effects on biochemical markers of oxidation that are similar to those induced by peroxides (Lavoie et al., 2000; Chessex et al., 2001). The addition of glutathione disulfide (GSSG) to the TPN solution protects against the initiation of lung fibrosis (Lavoie et al., 2002), further supporting the general concept that under specific conditions, TPN can induce an oxidative stress. Overall, this indicates that multivitamin solutions do not have antiperoxide properties.

In guinea pig pups, kept in room air, we found that the infusion of photoxposed TPN induced in lungs the transcription of the gene encoding for procollagen, one of the initial steps in fibrosis (Lavoie et al., 2002). This was prevented by photoprotection and was reproduced by infusion of hydrogen peroxide. The increase of procollagen mRNA was attributed

ABBREVIATIONS: TPN, total parenteral nutrition; MVP, multivitamin preparation; GSSG, glutathione disulfide; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; (−)light, photoprotected; (+)light, exposed to ambient light; BPD, broncho-pulmonary dysplasia.
to the infusion of peroxides coupled with a drop in lung glutathione content. These conditions are associated with the formation of by-products of peroxidation such as hydroxynonenal, which stimulates the transcription factor AP-1 (Camandola et al., 1997). Because AP-1 can induce the transcription of genes encoding both procollagen (Maatta et al., 1993) and collagenases (metalloproteases) (Chakraborti et al., 2003), we hypothesized that the infusion of photoexposed TPN induces lung remodeling characterized by modifications of markers of lung growth, histological indexes of alveolarization, and apoptosis. The objective of the present study was to assess the specific roles of peroxides, multivitamins, and light exposure in lung remodeling during TPN.

Materials and Methods

Animal Model

Three-day-old Hartley guinea pig pups (Charles River Canada, Montreal, QC, Canada) received, via an indwelling catheter, continuous intravenous nutrient infusions. This animal species has been characterized in studies of oxygen-induced lung injury (Kelly et al., 1991) and in studies on the effects of oxidant loads given with parenteral nutrition on markers of oxidation (Chessex et al., 1999, 2001; Lavoie et al., 2000). As described previously, intravenous regimens were prepared from a base solution (5% dextrose + 0.45% NaCl + 1 U/ml heparin, at 260 ml/kg/day) (Chessex et al., 1999, 2001; Lavoie et al., 2000, 2002). Animals were on 12-12 h light/dark cycle, and solutions were changed daily 2 h after the beginning of the light cycle. After 4 days of continuous infusion, animals were anesthetized. Through an incision in the trachea, the left lung was filled with 10% formalin (in phosphate-buffered saline) at a pressure of 10 cm water to completely fill the lung. The lung was then excised and stored in the same solution, embedded, and prepared for determination of the alveolarization index by histology, and apoptosis, which can be induced by peroxides (Schafer and Buettner 2001), by the TUNEL technique adapted for histological preparation. Growth in total lung tissue was estimated by determining β-actin mRNA in an aliquot from the right lung. A 4-day period of infusion was chosen based on our previous experience with this animal model (Chessex et al., 1999, 2001; Lavoie et al., 2000) and on the fact that survival was low in conditions of high oxidative stress lasting more than 4 days (Chessex et al., 1999). The protocols were carried out in accordance with the Canadian Council of Animal Care guidelines.

Protocols

The effect of duration of the protocols on the alveolarization index was determined in two groups of animals who received ad libitum oral feeding (High Fiber Guinea Pig Diet 2041; Harlan, Indianapolis, IN) after they were weaned off mother’s milk at 3 days of age, when received from Charles River. In the first group, lungs were sampled on day 3 when experiments started. In the second group, lungs were sampled on day 7 of life. The alveolarization index was compared between groups to determine the effect of the duration of the protocols (4 days) on lung growth.

The effect of light-exposed parenteral solutions on alveolarization was studied separately from that of nutrients in four groups of guinea pig pups (MVP ± light and TPN ± light).

Animals in the MVP group received the base solution + 1% Multi-12 pediatric (MVP) (Sabex, Boucherville, QC, Canada) at 260 ml/kg/day.

Animals in the TPN group received two solutions (a and b) infused in a “piggy-backed” set-up, mixed close to the infusion site: base solution + 5.3 g/kg/day amino acids (Travasol; Baxter, McGaw Park, IL) + 2% TPN at 130 ml/kg/day (a) and base solution + 5.3 g/kg/day amino acids + 7.6 g/kg/day lipids (Intralipid 20%; Pharmacia Upjohn, Baie d’Urfe, QC, Canada) at 130 ml/kg/day (b) for a final concentration (at 260 ml/kg/day) of 1% MVP + 5.3 g/kg/day amino acids + 3.8 g/kg/d lipids.

Animals in both groups were separated between those receiving solutions photoprotected [(-)light] or exposed to ambient light [(+)light].

Ambient light corresponded to 32-foot candles in the laboratory where the solutions were prepared and 16-foot candles in the animal facility. Photoprotection was achieved by preparing the solutions in darkness and covering the infusion set with opaque material and using amber intravenous tubing (Laborie et al., 1999). An index of apoptotic events was also quantified in these animals.

To separate the effect of photoexposure of MVP from H2O2 on alveolarization, four further groups of guinea pig pups were infused with 1% MVP in the base solution. Two groups were infused with peroxides: (+)light or (−)light + 200 μM H2O2, whereas two other groups were infused with solutions without peroxides: (−)light or (+)light + 300 μM sodium metabisulfite. Sodium metabisulfite was added 3 h after the MVP solution was exposed to light to quench the peroxides formed (Lavoie et al., 1994), whereas 200 μM H2O2 was added to correspond with the mean amount of peroxides generated in 1% MVP unprotected from light (Table 1).

To understand the mechanism by which light exposure of MVP induces its effect, we tested the role of riboflavin in the presence or absence of vitamin C or multivitamins on the alveolarization index. Six groups of guinea pig pups received the base solution enriched or not with riboflavin in the presence or absence of vitamin C or 1% MVP-R (MVP solution devoid of riboflavin, graciously provided by Sabex). Riboflavin and vitamin C were added in amounts corresponding to the levels reported in 1% MVP (15 μM and 0.9 mM, respectively). All these solutions remained unproctected from light.

To control for the peroxide variable generated in the solution containing riboflavin and ascorbic acid exposed to light (Table 1), 200 μM H2O2 was added to the solution containing only vitamin C, and a further mixture of riboflavin and ascorbic acid was prepared in a solution containing 300 μM sodium metabisulfite.

To determine whether there is a direct effect of infused peroxides, two series of experiments were performed: four groups of guinea pig pups received the base solution enriched with 0, 200, 500, and 800 μM H2O2; in a further group, we tested if confining 10 μM of GSSH with 500 μM H2O2 prevented any effect of peroxides (500 μM H2O2) on the alveolarization index. GSSH has been associated with a 150% increase in lung glutathione (Lavoie et al., 2002). The 10 μM concentration of GSSH was used with the aim of obtaining a normal plasma concentration (Hill et al., 1985). There was no interaction between GSSH and peroxides in infused solutions because no difference in peroxide concentrations was noted between solutions con-

<table>
<thead>
<tr>
<th>Peroxides</th>
<th>Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base solution</td>
<td>5 ± 5</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Ascorbic acid + 200 μM H2O2</td>
<td>309 ± 7</td>
</tr>
<tr>
<td>Riboflavin + ascorbic acid</td>
<td>246 ± 15</td>
</tr>
<tr>
<td>Riboflavin + ascorbic acid + metabisulfite</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>MVP without riboflavin</td>
<td>134 ± 2</td>
</tr>
<tr>
<td>MVP (−)light</td>
<td>100 ± 9</td>
</tr>
<tr>
<td>MVP (−)light + 200 μM H2O2</td>
<td>251 ± 6</td>
</tr>
<tr>
<td>MVP (−)light</td>
<td>263 ± 12</td>
</tr>
<tr>
<td>MVP (−)light + metabisulfite</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>TPN (−)light</td>
<td>99 ± 3</td>
</tr>
<tr>
<td>TPN (−)light</td>
<td>283 ± 34</td>
</tr>
<tr>
<td>200 μM H2O2</td>
<td>208 ± 14</td>
</tr>
<tr>
<td>500 μM H2O2</td>
<td>529 ± 5</td>
</tr>
<tr>
<td>800 μM H2O2</td>
<td>783 ± 1</td>
</tr>
<tr>
<td>10 μM GSSH + 500 μM H2O2</td>
<td>544 ± 10</td>
</tr>
</tbody>
</table>
taining or not GSSG, whereas the concentration of glutathione in solution did not differ from the added 10 \( \mu M \) GSSG.

**Analytical Measurements**

** Peroxides.** Peroxides were measured by quantification of the complex ferric-orange xylene (Lavoie et al., 2000; Chessex et al., 2001). This simple method, based on a Fenton-like reaction with peroxides to generate ferric ion, detects different peroxides such as \( \text{H}_2\text{O}_2 \), cumin- and tert-butyloxyl-hydroperoxides (Lavoie et al., 1994). The determination of global peroxide content in parenteral solutions measured with this chemical method has been previously validated using an enzymatic assay (Laborie et al., 2000). Briefly, samples were centrifuged for one min at 5000g. Twenty microliters of supernatant was diluted in 1000 \( \mu L \) of a freshly prepared solution containing 90 \( \mu M \) orange xylene, 0.1 \( N\ \text{H}_2\text{SO}_4 \), 225 \( \mu M \) \( \text{FeCl}_3 \), and 3.6 mM butylated hydroxytoluene in methanol. After 30 min of incubation, the solution was centrifuged for 1 min at 5000g, and the absorbance of the supernatant read at 560 nm was compared with a standard tert-butyloxyl-hydroperoxide curve. Peroxide concentrations measured in infused solutions are reported in Table 1.

**Alveolarization Index.** An index of the alveolar count was quantified by determining, at a standardized magnification (\( \times 200 \)) on a low-powered photomicrograph, the number of intercepts between a standardized straight line (1.3 mm) and histological structures (Fig. 1). Results were derived from the mean of four separate 0.85-mm\(^2\) section areas free of blood vessels and were presented as an absolute number per millimeter. This alveolarization index based on tissue structure density was used to compare the alveolar development between animal groups. The method is similar to that used by others estimating the alveolar development by a radial alveolar count (Emery and Mithal, 1960; Cooney and Thurlbeck, 1982). The number of intercepts with a straight line or the internal surface area is directly proportional to the number of intercepts with a linear chord (Jouvenel et al., 2003).

**\( \beta \)-Actin mRNA.** \( \beta \)-actin mRNA was used as an index of total lung tissue in animals receiving TPN \( \pm \) light. After isolation of lung RNA by chloroform/isoamyl (24:1) extraction (Lavoie et al., 2002), mRNA of \( \beta \)-actin was determined by an RNA protection assay using a \( ^{32} \text{P} \)-labeled cRNA probes for rat \( \beta \)-actin (Promega, Madison, WI). For the ribonuclease protection assay, 10 \( \mu g \) of total RNA was hybridized according to a published protocol (Lavoie et al., 2002).

**Apoptosis.** The presence of apoptotic events in lung tissue was estimated by the TUNEL assay (Ansari et al., 1993; Li et al., 2003) adapted for histological preparations according to Roche Diagnostics (Indianapolis, IN). The end-labeling DNA with fluorescein-dUTP was detected by antibody against fluorescein and a second antibody conjugated to peroxidase. The quantification of apoptotic cells was estimated by counting the brown coloration of apoptotic nuclei on blue color from live cell nuclei (Image-Pro 4.1 software; Media Cybernetics, Inc., Silver Spring, MD).

**Statistical Analysis.** Data were treated by factorial analysis of variance after validation of homoscedasticity using Bartlett’s \( \chi^2 \) test. All groups were compared orthogonally. Linear regression analysis was performed between \( \beta \)-actin mRNA and alveolarization index. The level of significance was set at \( p < 0.05 \).

**Results**

The initial body weights (mean \( \pm \) S.E.M., 111 \( \pm \) 2 g, \( n = 123 \)) and relative right lung weight (0.65 \( \pm \) 0.01 g/100g body weight, \( n = 123 \)) did not differ statistically between animal groups. Only one death was recorded during the experiments, and the data were excluded because this animal received 800 \( \mu M \) \( \text{H}_2\text{O}_2 \).

A lower number of intercepts was associated (Fig. 2) with the infusion of solutions containing no amino acids and lipids \( (F_{1,18} = 5.7, p < 0.05) \) and with solutions exposed to ambient light \( (F_{1,18} = 18.9, p < 0.01) \). There was no interaction \( (F_{1,18} < 0.1) \) between these two parameters. In animals receiving solutions exposed to ambient light, the number of intercepts were 18\% (TPN solution) and 23\% (MVP solution) lower than in the fed control group (33 \( \pm \) 1, \( n = 6 \)). The numbers of intercepts observed in the lungs from 3-day-old guinea pig pups (32 \( \pm \) 2, \( n = 8 \)) as well as 7-day-old fed animals (33 \( \pm \) 1, \( n = 6 \)), and those receiving photoprotected solutions (33 \( \pm \) 1, \( n = 13 \)) were similar.

These findings suggest that the lower alveolarization index represents tissue loss rather than a maturational delay in lung septation. This is supported by the correlation found between \( \beta \)-actin mRNA and alveolarization index \( (r^2 = 0.35, n = 11, p < 0.05) \) (Fig. 3). The presence of peroxide in the infused solutions suggested a potential involvement of apoptosis because reactive oxygen species are directly involved in the activation of caspase-3, an irreversible step toward apoptosis (Shimoke et al., 2003). Results presented in Fig. 4 demonstrate that lungs of animals receiving parenteral solutions exposed to ambient light presented higher apoptotic activity.

Fig. 1. Lung histology. Photomicrograph comparing lung sections (\( \times 200 \)) from animals infused with MVP exposed \([+\)light\]) or not \([-\)light\]) to ambient light.

Fig. 2. Alveolarization index as a function of parenteral nutrition and light exposure. Guinea pig pups were infused with MVP \( = \) base solution \( (+) \) multivitamin or TPN = MVP + amino acids + lipids, exposed \([+\)light\]) or not \([-\)light\]) to ambient light. The factorial analysis shows a significant \( (+, p < 0.05) \) difference between TPN and MVP and a significant \( (++, p < 0.01) \) effect of photexposure without interaction between parameters. Dotted line represents fed control animal (33 \( \pm \) 1, \( n = 6 \)). Results are expressed as mean \( \pm \) S.E.M. (\( \mu \)).
events \( F_{1,15} = 26.7, p < 0.01 \) independently of the infusion of amino acids and lipids \( F_{1,15} < 0.1 \).

The effect of MVP on the alveolarization index suggested a process involving peroxides and/or light exposure. As shown in Fig. 5, \( H_2O_2 \) had a significant \( F_{1,12} > 5.6, p < 0.05 \) effect only at concentrations greater than 500 \( \mu \)M. The alveolar disruption observed in animals receiving 500 \( \mu \)M \( H_2O_2 \) (alveolar index, 26 \( \pm \) 1; \( n = 4 \)) was not prevented by the coinfusion of glutathione with peroxides (26 \( \pm \) 1, \( n = 5 \)). Fig. 6 shows that the alveolarization index was significantly lower \( F_{1,19} = 10.04, p < 0.01 \) in animals receiving light-exposed solutions. At lower concentrations of \( H_2O_2 \) (200–250 \( \mu \)M), no effect of peroxides was detected \( F_{1,19} = 1.02 \), and there was no interaction \( F_{1,19} = 0.55 \). These data led us to test for the role of photosensitive riboflavin previously shown as a reactive substrate (Laborie et al., 1998).

The statistical analysis of data presented in Fig. 7 revealed a significant \( F_{1,33} = 5.84, p < 0.05 \) interaction between riboflavin and other vitamins. A lower index of alveolarization was found in animals infused with solutions containing vitamin C \( F_{1,33} = 4.95, p < 0.05 \) or MVP \( F_{1,33} = 12.57, p < 0.01 \) in the presence of riboflavin. Animals receiving solutions containing ascorbic acid, riboflavin, and metabisulfite, added at the same time, presented a similar alveolarization index \( 30 \pm 1, n = 4 \) than the solution without riboflavin.

**Discussion**

The main finding reported in this study is that the TPN solution is associated with a lower alveolarization index in newborn guinea pigs. The clinical impact is potentially high since lower alveolarization, combined with the previous report on the involvement of TPN in the initiation of lung fibrosis (Lavoie et al., 2002), suggest the presence of lung remodeling. The results indicate that the interaction among
light exposure, peroxide contamination, and the multivitamin preparation is needed to induce the lung remodeling. The light-induced drop in the alveolarization index points toward a process involving either delay in lung growth or loss of lung tissue. Since the fed animals displayed no change in the alveolarization index over 4 days, and since the alveolar count was lower at 7 days in animals receiving light-exposed TPN compared with the 3-day-old fed pups, the results from Fig. 2 are interpreted as representing a loss of lung tissue. This is confirmed by the significant correlation shown in Fig. 3, and the increase in apoptotic events observed in animals infused with light-exposed TPN and MVP (Fig. 4). The high apoptotic events (30% of cells) contrast with normal alveolar development in which about 10% of cells die by apoptosis (Luyet et al., 2000) to thin septa between alveoli (Bruce et al., 1999; Luyet et al., 2000), allowing for a better gas exchange. The loss of alveoli (about 20% in 4 days) coupled with a high number of apoptotic events suggests alveolar disruption. Because the TUNEL assay used here to determine the presence of DNA damage is not specific to apoptosis (Ansari et al., 1993), further investigations are required to clarify the mechanisms leading to the observed tissue loss and to compare baseline apoptotic events in enterally fed animals with those receiving various intravenous regimens.

The effects of light-exposed TPN on alveolarization could be linked to peroxides, which are known to induce apoptosis, and/or to components of the multivitamins modified by light (Figs. 6 and 7). Previously, we demonstrated in the same animal model that, similarly to H$_2$O$_2$, the infusion of solutions containing MVP induced the transcription of the gene encoding for procollagen type I (Dauger et al., 2003) toward a process involving either delay in lung growth or loss of lung tissue. Because H$_2$O$_2$ can initiate apoptosis, H$_2$O$_2$ generated by photoexposure of MVP (Lavoie et al., 1997; Laborie et al., 1998) appears to be a potential source of oxidant causing alveolar disruption. The low alveolarization index observed with the infusion of a solution of 500 and 800 μM H$_2$O$_2$ (Fig. 5) confirms this concept. However, these active peroxide concentrations were higher than those measured in TPN solutions (Table 1).

On the other hand, the fact that solutions inducing a loss in lung tissue [MVP(+)-light, TPN(+)-light, and riboflavin + ascorbic acid] had peroxide concentrations (Table 1) similar to those solutions that did not affect the alveolar count [MVP(-)-light + 200 μM H$_2$O$_2$ and ascorbic acid + 200 μM H$_2$O$_2$] excludes a direct effect of hydrogen peroxide. This is supported by the data showing that the antiperoxides glutathione and metabisulfite (Fig. 6) conferred no protection against decreased alveolarization. The low alveolarization index found in animals receiving MVP(+)-light enriched with the antiperoxide metabisulfite (Fig. 6) is the clearest demonstration that light exposure of multivitamins rather than peroxide generation accounts for the alveolar disruption.

The light-induced oxidation of MVP (Laborie et al., 1998; Chessex et al., 2001) could generate active agents (Lavoie et al., 2004) or cause the loss of an essential element for lung development such as vitamin A (Allwood and Martin, 2000) and the antioxidative vitamin C. Indeed, degradation by photolysis is the main cause for the loss of vitamins A (Allwood and Martin, 2000) and C (Lavoie et al., 2004) in TPN solutions. The loss of these vitamins is an unlikely explanation because the infusion of the base solution without vitamins led to a significantly higher alveolar count than MVP (Fig. 7). The fact that the infusion of MVP(+)-light, generating 203 ± 12 μM peroxides, induced a low alveolarization index and that the infusion of 208 ± 14 μM H$_2$O$_2$ did not (Fig. 5) points toward a separate effect from a toxic compound formed in MVP solution exposed to ambient light. A mixture of vitamin C, riboflavin, and ambient light generates various breakdown products of vitamin C. Dehydroascorbate and 2,3-diketogulonic acid are classic by-products of oxidation of ascorbic acid (Lavoie et al., 2004). However, in the presence of H$_2$O$_2$, other breakdown products of vitamin C are generated that have peroxide-like properties (Lavoie et al., 2004). To test the implication of these by-products, animals were infused with different solutions from which riboflavin was isolated (Fig. 7) to modulate their production. Metabisulfite, which quenches peroxides (Lavoie et al., 1994), did not prevent impairment in the alveolarization index when this antiperoxide compound was added 3 h after exposure of MVP to ambient light. This would leave sufficient time for the formation of vitamin C by-products, which occurs in less than 3 h (Lavoie et al., 2004). However, the concomitant addition of metabisulfite prevented the formation of vitamin C by-products (Lavoie et al., 2004) and the impairment in the alveolarization index observed in the group receiving ascorbic acid + riboflavin. Because photoexcited riboflavin generates reactive radicals (Kim et al., 1993), it might also have been involved in this process, but we have documented that light-exposed riboflavin alone has no demonstrable effect on the alveolarization index (Fig. 7). Overall, these results support the speculation that the causal agent is a by-product of vitamin C generated by light exposure.

The pertinence of the findings of this study relate to very low birth weight infants who have immature antioxidant defenses and are unable to quench peroxides infused with...
Lung Remodeling by Light-Exposed Multivitamins


Address correspondence to: Dr. Jean-Claude Lavoie, Research Centre, Sainte-Justine Hospital, 3175 Côte Ste-Catherine, Montreal, QC, Canada H3T 1C5. E-mail: jclavoie@justine.umontreal.ca