TISSUE RESPONSES TO HYPEROXIA

Biochemistry and Pathology

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All tissues of the body, in the long term, require oxygen to sustain cellular integrity and effective organ function. At molecular level, oxygen is utilized primarily for the oxidation of substrates, and the associated production of energy used for ionic transport, contraction and biosynthesis. The four-electron reduction of dioxygen to yield energy during aerobic metabolism necessarily progresses through toxic free-radical intermediates (superoxide anion, hydrogen peroxide and hydroxyl radical). Although normally prevented from release to the components of the cell by the arrangement of the cytochromes, each intermediate can cause cellular damage, either directly or by the initiation of radical chain reactions. In addition, there are other sources of free radicals within cells: for example, during phagocytosis, the xanthine-xanthine oxidase system, or from the conversion of haemoglobin to methaemoglobin (Carrell, Winterbourne and Rachmilewitz, 1975). Hence, life within an oxygen-containing environment, whilst conferring advantages in terms of energy production, is not without its hazards. Red blood cells and cells of the lung (and possibly arterial endothelium), in particular, are unique within the body as they are exposed to the greatest oxygen tensions.

To combat the harmful effects of oxygen-derived free radicals, aerobic organisms have evolved elaborate protective mechanisms. These include enzymes (superoxide dismutase, catalase and glutathione peroxidase), thiol-containing compounds (reduced glutathione) as well as other antioxidants (e.g. vitamins A, C and E).

Although it is widely accepted that structural and functional changes occur in the lungs of animals exposed to increased oxygen tensions, there is as yet no conclusive evidence that these are caused by oxygen-derived free radical species. However, it has been shown that, at increased P0₂, there is enhanced production of superoxide anion and hydrogen peroxide in lung mitochondria and

SUMMARY

An animal model was established to study the toxic effects of hyperoxia and the consequent changes in intracellular antioxidant status. Superoxide dismutase, catalase and glutathione peroxidase activities were measured in erythrocytes, liver and lung, in addition to cellular glutathione concentrations and its associated metabolism. Overt cellular damage was assessed biochemically by measurement of lipid peroxidation, hydrogen peroxide-induced haemolysis and osmotic fragility. Pathological changes were assessed by light and electron microscopy. Up to 11 days exposure of rats to 80% oxygen was not lethal, but resulted in overt cellular damage to red blood cells (haemoglobin concentration decreased from 13.8±1.4 (SD) g dl⁻¹ to 12.4±0.5 g dl⁻¹; hydrogen peroxide-induced haemolysis increased from 7.7±1.6% to 75.1±13.5% after 11 days of hyperoxia) and to cells of lung (4-fold increase in lipid peroxidation) as well as a biochemical adaptation to the increased concentration of oxygen metabolites (superoxide dismutase increased 3-fold, catalase 5-fold and glutathione peroxidase 2-fold). It is suggested that red cell hydrogen peroxide-induced haemolysis and reduced glutathione concentration may be useful indicators of oxidant stress in the clinical situation.
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microsomes (Turrens et al., 1982; Turrens, Freeman and Crapo, 1982) and it seems very likely that these and other species are the mediators of oxygen toxicity.

It is not possible to measure the concentrations of oxygen-derived free radical species in vivo. However, it is possible to measure the activities of the enzymes responsible for the detoxification of these radicals (superoxide dismutase, catalase and glutathione peroxidase). The activities of such enzymes will reflect changing substrate concentrations. In addition, there are several markers of oxidant damage which can be determined (products of peroxidation and hydrogen peroxide-induced haemolysis) which provide evidence of increases in the concentrations of free radicals.

Various workers, cited in the discussion below, have reported the response to increases in \( P_{O_2} \) of one or other of these indices of oxidant damage in single organs or cell cultures, but there has been no integrated study of the response of the full range of antioxidant enzymes, together with a number of markers of oxidant damage in several organs, in the intact animal exposed to hyperoxia. Such a study would provide evidence of increased free radical concentrations in vivo, as well as suggesting markers of oxidant damage to be used in investigations in man. We have, therefore, examined the changes in lung, liver and red blood cells in the rat exposed to 80% oxygen for up to 11 days. The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and the concentration of the antioxidant reduced glutathione (GSH), as well as the enzymic pathway responsible for its regeneration, were measured. In addition, the markers of injury, hydrogen peroxide haemolysis, osmotic fragility and lipid peroxidation were examined, along with histological changes in the lungs of rats exposed to oxygen.

MATERIALS AND METHODS

Young adult male Wistar rats (weight 175–225 g) reared under specific pathogen-free conditions were placed in a cage within a Perspex enclosure. Gas mixtures were delivered to the enclosure at a flow rate selected to provide four to five total enclosure washouts per hour, so that adequate removal of carbon dioxide could be achieved (carbon dioxide content less than 1%). Gases used were either a mixture of 80% oxygen and 20% nitrogen (British Oxygen Special Gases Division) or air. We selected 80% oxygen as the greatest concentration compatible with survival in this species. Oxygen and carbon dioxide tensions were assessed by gas sampling from the enclosure after 24 h using a glass syringe and Corning 175 blood-gas analyser.

Animals were kept continuously in this environment for 0 (n = 22), 3 (n = 9), 5 (n = 12), or 11 (n = 6) days, except for short periods each day (10 min maximum) to permit cleaning and administration of fresh food and water. A cycle of 12 h light and 12 h darkness was adhered to. Feed (CRM pelleted diet (Labsure, Cristopher Hill Group)) and water were allowed ad libitum. A control group of animals (n = 22), which were exposed to air in the Perspex enclosure for 5 days, was included.

Following predetermined periods of exposure, rats were removed from the exposure chamber and anaesthetized with chloroform. An incision was made in the anterior abdominal wall, the aorta cannulated and 6 ml of blood taken in a heparinized syringe and collected into a tube containing EDTA as anticoagulant. The chest was then opened, the pulmonary artery cannulated and the left atrium drained. Blood was then washed from the pulmonary circulation with 5 ml of isotonic saline. The heart, lungs and trachea were removed. A sample of liver (approximately 0.5 g) was taken for examination. Lung was dissected free of heart, major vessels and fatty tissue, blotted dry and weighed.

Tissue preparation and histology

Blood. With the exceptions outlined below, all assays were performed on saline-washed red cells. Haemolysates for enzyme assays were prepared in mercaptoethanol EDTA stabilizing solution (Beutler, 1975). Whole unwashed blood was used for the estimation of the concentration of reduced glutathione and the determination of osmotic fragility and hydrogen peroxide haemolysis.

Lung and Liver. The left main bronchus was ligated and the left lung removed distal to the ligature. The right lung was inflated to 5 cm H\(_2\)O pressure with 10% neutral buffered formalin (pH 7.0) and maintained at this pressure for 5 min; the tissue was then placed in neutral buffered formalin to await histological examination. The left lung and liver sample were each placed in 10 ml of 50-mmol litre\(^{-1}\) phosphate buffer (pH 7.5) and homogenized using an ultrasonic homogenizer. Care was taken to ensure that samples did not
overheat, by surrounding them with ice and using three 30-s pulses. The homogenates were cleared of cell debris by centrifugation at 400 rev min\(^{-1}\) for 10 min and biochemical assays carried out as described below.

**Histological examination** was performed with the treatment schedule not being disclosed until after reporting. After fixation in neutral buffered formalin solution, samples for light microscopy were prepared using the standard 18-h paraffin embedding process, with sections cut at 5 \(\mu m\). Stains used were haematoxylin and eosin, Martius-Scarlet-Blue, Gordon and Sweet's reticulin, and a peroxidase stain (Drury and Wallington, 1967). The degree of pulmonary oedema observed on light microscopy was quantified using the following scoring system: no oedema = 0; slight oedema (broncho-vascular interstitial oedema) = 1; severe oedema (intra-alveolar flooding) = 2.

Fresh lung specimens (approximately 1.0 mm\(^3\)) for electron microscopy were fixed in glutaraldehyde solution (3% w/v) and stained using Reynold's lead citrate and uranyl acetate reagent.

**Chemical estimations**

**Haemoglobin** concentration and red cell count of whole blood were determined by Coulter counter.

**Protein** content of lung and liver homogenates was determined by the method of Lowry and colleagues (1951), using human albumin as standard.

**Lipid peroxidation** was assessed on the 5th day of exposure by measuring the formation of malondialdehyde (MDA) when lung and liver homogenates were allowed to react with thiobarbituric acid in hydrochloric acid (Buege and Aust, 1978). Results are expressed as MDA nmol/g protein.

**Reduced glutathione (GSH)** was estimated in whole blood and tissues by the method of Beutler (1975). The assay uses as chromophore 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB); this is reduced by sulphhydryl compounds to form a highly coloured yellow anion. The assay is specific for GSH in red cells as virtually all of their non-protein sulphhydryl content is present as reduced glutathione. GSH concentration was expressed as \(\mu mol/g\) haemoglobin (blood), or \(\mu mol/g\) wet tissue weight (lung and liver).

**Red cell assays**

**Hydrogen peroxide haemolysis** was measured by incubating a suspension of washed red blood cells in hydrogen peroxide (Gordon, Nitowsky and Cornblath, 1955; Nitowsky, 1956).

**Osmotic fragility** was assessed by the method of Dacie and Lewis (1970) on whole unwashed blood.

**Pentose phosphate pathway activity** was estimated using a method which measures \(^{14}\)C-carbon dioxide released when red cells metabolize \(^{14}\)C-glucose labelled in the C1 position. Both stimulated (by the addition of methylene blue) and unstimulated activities were determined. This test is specific for the pentose phosphate pathway (PPP) in the case of red blood cells because of their lack of enzymes for oxidative phosphorylation. PPP activity was expressed as the percentage of carbon-14, added as \(^{14}\)C-1-glucose, which was recovered \(^{14}\)C-carbon dioxide per 3 h per red cell (\(^{14}\)CO\(_2/3\) h/red cell).

**Enzymes**

Glucose-6-phosphate dehydrogenase (G-6-PD), 6-phosphogluconate dehydrogenase (6-PGD), NADPH-diaphorase (NADPH-D), catalase (CAT) and glutathione peroxidase (GPX) were estimated by the method of Beutler (1975). Results were expressed as iu/g haemoglobin (red cell), or iu/g protein (lung and liver).

Superoxide dismutase (SOD) activity was estimated by the method of Crapo, McCord and Fridovich (1978). Xanthine 0.5 mmol litre\(^{-1}\) and xanthine oxidase were used to generate superoxide anion at a constant rate, and ferrixytochrome c 0.1 mmol litre\(^{-1}\) was used to detect superoxide anion. With liver extracts it was found necessary to add 10 \(\mu l\) of 3-mmol litre\(^{-1}\) potassium cyanide (to inhibit interference from cytochrome oxidase) and 10 \(\mu l\) of catalase 10000 iu ml\(^{-1}\) (to inhibit interference from cytochrome c peroxidase) to the reaction mixture.

In some samples of lung and liver homogenate taken after 5 days exposure, the relative contributions of CuZn- and Mn- forms of SOD to the total SOD activity were determined. This was accomplished by performing the assay in the presence of potassium cyanide 1 mmol litre\(^{-1}\), which inhibits CuZn-SOD by about 90%. Mn-SOD was determined by subtracting the CuZn-SOD from the total SOD activity. One unit of SOD activity is that activity which results in a 50% inhibition of the rate of the control reaction. Results are expressed as u/g haemoglobin (red cell), or u/g protein (lung and liver).
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Statistical analysis

Results were compared by the Mann–Whitney U or the Kruskal–Wallis statistic for non-parametric data, or by Student’s t test, as appropriate (Sachs, 1982). Results shown are mean ± SEM; *P < 0.05, **P < 0.01 and ***P < 0.001 are significantly different from control.

RESULTS

Histology

Haematoxylin and eosin staining revealed both interstitial and intra-alveolar oedema in all oxygen exposed groups, but no significant further increase was noted with treatment for more than 24 h (table I). Peroxidase staining for lysozyme failed to detect an increase in the number of macrophages in lung sections from treated animals, and the absence of fibrin deposition was confirmed by MSB staining.

Electron microscopy of sections of lung from control and 11-day oxygen-treated animals revealed a significant increase in the ratio of type II to type I pneumocytes in the oxygen-treated rats. Furthermore, there was an increase in the number of lamellar bodies within the type II cells of treated animals (table II).

Chemical estimations

Haemoglobin concentrations were significantly decreased with oxygen exposure: control was 13.8 ± 1.4 (SD) g dl⁻¹, 3 days exposure 13.4 ± 0.9 g dl⁻¹, 5 days exposure 12.4 ± 0.5 g dl⁻¹ (P < 0.001), and at 11 days exposure 11.9 ± 0.6 g dl⁻¹ (P < 0.001).

Protein. Lung weight increased by 28% at the 3rd day of exposure to 80% oxygen and no further increase occurred with longer exposures (table III). Protein content, expressed as mg per lung wet weight, remained constant, with the exception of a significant reduction at day 5. However, when expressed as mg/g lung wet weight, there was a significant reduction with all times of exposure to hyperoxia studied.

Lipid peroxidation. The increase in products of peroxidation measured at the 5th day (fig. 1) was significant (P < 0.001) in both tissue homogenates.

<p>| Table I. Severity of pulmonary oedema produced by exposure of rats for varying periods to an 80% oxygen:20% nitrogen mixture. Pulmonary oedema was determined by light microscopy of haematoxylin and eosin-stained lung specimens. Scores: 0 = no oedema; 1 = mild oedema; 2 = severe oedema (results for rats in each group were summed and a mean determined) |
|---|---|---|</p>
<table>
<thead>
<tr>
<th>Exposure to 80% oxygen (days)</th>
<th>n</th>
<th>Oedema score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13</td>
<td>0.0</td>
</tr>
<tr>
<td>1</td>
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<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
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<td>3</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>1.6</td>
</tr>
</tbody>
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| Table II. Electron microscopic changes in alveolar type I and type II cells produced by oxygen exposure. Rats were exposed to an 80% oxygen:20% nitrogen mixture for 11 days and the electron microscopic appearance of lung specimens compared with those of air-breathing animals. Representative fields from each group were examined at the same magnification and the total number of type I and type II cells, and the number of lamellar bodies within each type II cell, determined. *P < 0.05; **P < 0.01 |
|---|---|---|---|
| Air | 80% Oxygen |
|---|---|---|
| Fields inspected | 15 | 12 |
| Type II/type I cell ratio | 0.20 ± 0.02 | 0.56 ± 0.05** |
| Lamellar bodies per type II cell | 7.80 ± 0.77 | 9.95 ± 1.02* |

<p>| Table III. Changes in lung weight and protein from rats exposed to an 80% oxygen:20% nitrogen mixture for 3, 5, and 11 days when compared with air control animals (0 days). Results are mean ± SEM. *P &lt; 0.05; **P &lt; 0.01; ***P &lt; 0.001 |
|---|---|---|</p>
<table>
<thead>
<tr>
<th>Exposure to 80% oxygen (days)</th>
<th>n</th>
<th>Lung wet weight (g)</th>
<th>Total lung protein (mg) (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>22</td>
<td>1.08 ± 0.03</td>
<td>154 ± 8</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>1.23 ± 0.15*</td>
<td>134 ± 12</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>1.25 ± 0.03**</td>
<td>123 ± 5*</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>1.34 ± 0.04***</td>
<td>142 ± 17</td>
</tr>
</tbody>
</table>
Reduced glutathione concentration. Six rats exposed to 80% oxygen in nitrogen for 5 days had concentrations of reduced glutathione in lung of $0.48 \pm 0.06 \mu mol/g$ wet weight; this was unaltered from animals breathing 21% oxygen (air controls): $0.53 \pm 0.06 \mu mol/g$ wet weight.

Persistent, and small but significant, decreases ($P < 0.05$, $P < 0.001$ and $P < 0.01$ at 3, 5 and 11 days, respectively) were noted in red cell reduced glutathione concentrations with oxygen treatment (fig. 2).

Red cell assays

Hydrogen peroxide haemolysis. There was a steady increase in hydrogen peroxide haemolysis with increasing durations of exposure to oxygen (fig. 3), indicating a progressive inability of the red cell to withstand sustained oxidative stress ($P < 0.001$).

Osmotic fragility. Red blood cells from rats exposed to 80% oxygen for 5 days were less fragile than those from control animals (fig. 3).

Pentose phosphate pathway. Stimulated and unstimulated activities of the pentose phosphate pathway were decreased following treatment with oxygen for 3 and 5 days ($P < 0.05$ and 0.01; and $P < 0.01$ and 0.01, respectively), although activity in both cases had returned virtually to normal after 11 days treatment (fig. 4). G-6-PD and NADPH-D activities were increased in the experimental animals at 5 days exposure (fig. 5).

Enzyme assays

Significant increases in the activities of SOD (3-fold), CAT (5-fold) and GPX (2-fold) in lung were noted, whilst increases in the activity of SOD and CAT only were demonstrable in liver (figs 6–8). Red cell GPX activity decreased with exposure to oxygen with no statistically significant change in either SOD or CAT activities.

All three enzymes had a higher activity in liver than in lung; the most marked difference was seen with catalase, where activity in liver was approximately 30 times that in lung, compared with three times for SOD and GPX.

There was also a wide difference in activities of GPX and CAT between the tissues studied, with lung having 1000 times more GPX activity than CAT activity, and the red cell having 100 times more CAT activity than GPX activity.

No one form of SOD was responsible for the increase in total SOD activity seen in either lung or liver, as both the CuZn and Mn forms increased by the same relative amounts (fig. 9).

DISCUSSION

Previous workers have reported changes in some or all of the antioxidative systems in a variety of
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Fig. 3. Red blood cell hydrogen peroxide haemolysis and osmotic fragility of rats breathing 80% oxygen for up to 11 days, compared with air-breathing controls (0 days). Results are expressed as percentage of the fully haemolysed sample (for H$_2$O$_2$ haemolysis) and the concentration of saline producing 50% haemolysis (mean RBC osmotic fragility).

Fig. 4. Red blood cell pentose phosphate pathway (PPP) activity of rats breathing 80% oxygen for 3, 5 and 11 days compared with air breathing controls (0 days). Both stimulated (by addition of methylene blue) and unstimulated PPP activity are shown. Results are expressed as the percentage of carbon-14 added as $^{14}$C-1-glucose which was recovered as $^{14}$CO$_2$/3 h/red cell x 10$^{-6}$.

tissues, but none has looked at them all in lung, liver and red blood cells. Moreover, none has associated changes in red blood cells with those in the lung. Such red cell biochemical markers reflecting oxidative damage within the lung may prove useful as monitors of the clinical situation.

The weight of lung varies with oxygen exposure as a result of the accumulation of oedema. An increase in lung weight of 28% was observed at 3 days, and this then remained virtually constant (table I). Crapo and Tierney (1974) observed a 45% increase in lung weight after 7 days exposure
Fig. 5. Glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and NADPH-diaphorase activities in red blood cells taken from rats breathing 80% oxygen for 5 days. For comparison, results from rats breathing air only are also shown (0 days).

Fig. 6. Superoxide dismutase activity in red blood cells and tissues taken from rats exposed to 80% oxygen for periods up to 11 days. For comparison, results from rats breathing air only are also shown (0 days).

to 85% oxygen. However, in contrast to the present study where a decrease in total lung protein of 35% was seen following 5 days exposure to oxygen, Crapo and Tierney (1974) observed an increase (68%) in lung protein after 7 days exposure. The additional 2 days may be significant as, in the present study, total lung protein had returned to control values by 11 days. These workers offered no explanation for these changes. Moreover, these workers observed no
change in total DNA content of lung after the 7-day exposure period, which suggests that there was no significant change in cell numbers.

Lung superoxide dismutase activity in the present study increased 3-fold, with similar changes in both the CuZn and Mn forms of SOD (figs 6 and 9), this was maximal at 5 days in lung and was still increased at 11 days. The response in liver occurred more slowly, with activity increasing further between days 5 and 11. Again the increase was similar in both forms of SOD. Kimball and colleagues (1976) also found similar changes in SOD activity of lung mitochondrial and cytosolic fractions in rats exposed to 90% oxygen. In another study (Crapo et al., 1980) there was a 42% increase for CuZn-SOD and 75% for
Mn-SOD activity in lung after 5 days exposure. Crapo and Tierney (1974) examined the changes in SOD activity in several tissues including lung, liver and whole blood, finding demonstrable differences between exposed and unexposed rats only in the case of lung and brain, where activity was increased, and in liver, where activity was significantly decreased. No change in red cell SOD activity was reported by these workers. In the present study an increase was noted, although this was not statistically significant. Red blood cells have no DNA or RNA and cannot synthesize protein. However, the red cell population changes and a new generation could have higher SOD activities. Another possibility would be the change in specific activity that could be produced by enzyme amplification.

The liver responds to oxidant stress in a manner similar to the lung, with SOD activity increasing by 67% and 130% after 5 and 11 days exposure to oxygen, respectively. This finding is in contrast to that of Crapo and Tierney (1974), who observed a decrease in liver SOD activity. One reason for this difference may be the units used to express enzyme activity, Crapo and Tierney (1974) giving units of activity per organ wet weight, as opposed to our units per milligram of protein. In addition, in the present study it was found necessary to use the assay modification developed by Crapo, McCord and Fridovich (1978) to overcome interference by cytochrome oxidase and cytochrome c peroxidase. There was no mention of this modification being used by Crapo and Tierney (1974).

The presence of increased concentrations of intracellular superoxide anion inevitably results in increases in peroxide concentrations. Although it is now accepted that, under normal circumstances, glutathione peroxidase is probably the main enzyme responsible for the removal of organic peroxides, catalase has an important role in the detoxification of hydrogen peroxide. Lung CAT activity was found to increase by 75% after 5 days (fig. 7). Steinberg and co-workers (1983) also showed an increase of 80% after 7 days exposure. Activity of catalase in liver increased more slowly, by 45% of control values after 11 days. The slower response of liver compared with lung was seen with both SOD and CAT. There was no significant effect of exposure to oxygen on red cell CAT activity.

Both Kimball and colleagues (1976) and Ospital, Kasumaya and Tierney (1983) found increases in lung GPX activity (approximately 120% after 7 days) with oxygen exposure. A similar change was noted earlier (at 5 days) in the present study but, unlike catalase, no change was observed in GPX activity in liver. This suggests that catalase is more important than GPX in the liver of animals exposed to oxygen, perhaps as
The tissue responses to hyperoxia are a result of the preferential accumulation of hydrogen peroxide rather than organic peroxides.

The greater activity of CAT in red cells than GPX (100 times) suggests that this cell is particularly well adapted to cope with increases in hydrogen peroxide concentration. On account of the large turnover of methaemoglobin (approximately 3% of total body haemoglobin per day) (Carrell, Winterbourne and Rachmilewitz, 1975), and the high content of iron, probably the major toxic oxygen-derived species within the red cell are the hydroxyl radical and hydrogen peroxide. This is unlike lung and liver, where GPX activity is approximately 100–1000 times that of CAT and organic peroxides would appear to be the principal toxic agents.

Red cell G-6-PD activity was increased by 45%, while 6-PGD and NADPH-D activities remained constant, after 5 days exposure to 80% oxygen (fig. 5). Kimball and colleagues (1976) and Ospital, Kasumaya and Tierney (1983) found greater increases in lung G-6-PD activity: 250% and 189%, respectively.

A 2-fold increase in products of peroxidation in lung homogenates was found in the present study after 5 days oxygen exposure (fig. 1). We are in close agreement with Freeman, Topolosky and Crapo (1982), who reported a 150% increase in TBA reactive material in lung taken from rats exposed to increased oxygen tensions. The increase (by 550%) of peroxidation products in liver lends support to the hypothesis that the liver also can be adversely affected by oxygen.

After taking into consideration the glutathione content of residual red blood cells in tissues, it was noted that reduced glutathione concentration was much lower in lung than in liver. Concentrations in lung were unaltered in rats exposed to 80% oxygen for 5 days. Kimball and colleagues (1976) showed an increase in total thiol content in lung following 5 days exposure. However, the actual concentration of reduced glutathione reported was 1.17 ± 0.18 μmol/lung—a value double that seen in the present study. This difference could be explained by red cell contamination.

The concentration of red cell reduced glutathione was decreased significantly with oxygen exposure. This is probably a result of oxidation of red cell components, with oxidized glutathione rapidly leaving the red cell by an active membrane-bound transport system. This would be expected to occur largely at the beginning of the period of exposure, as tensions around the red cell would then be greatest. Later, blood oxygen tension would decrease as a result of the defective diffusion of oxygen across the alveolar membrane as a result of the accumulation of oedema fluid. This may explain why the reduced glutathione concentration decreased significantly after 3 days and then remained constant.

Reduced glutathione (GSH) is a major low molecular weight intracellular antioxidant. It is oxidized to GSSG either directly, or indirectly, by acting as cofactor for the enzyme glutathione peroxidase. This oxidation results in an unfavorable GSH:GSSG ratio. GSH is regenerated from GSSG by glutathione reductase, and this enzyme has a requirement for NADPH which is derived largely from the metabolism of glucose-6-phosphate via the pentose phosphate pathway. At 3 and 5 days exposure, when GSH concentrations were lowest, red cell PPP activity, both stimulated and unstimulated, was significantly reduced from control values, activity returning to normal after 11 days exposure.

In view of the recorded decrease in red cell glutathione peroxidase activity, PPP activity and reduced glutathione concentration, it would appear that at 5 days, GSH is not the major defence of the red cell to oxidant stress. It is possible that some other substance, such as vitamin E, is used at this stage. Increased hydrogen peroxide haemolysis has been reported with vitamin E deficiency (Nitowsky, 1956). The observed increase in hydrogen peroxide haemolysis (fig. 2) supports this point. With minor changes in the measured red cell antioxidants, the erythrocyte membrane was unable to withstand further oxidative stress. This change was not the result of overt structural damage, as osmotic fragility was, surprisingly, decreased after 5 days exposure to oxygen. One explanation may be that peroxidation of the red cell lipid results in a stiffer membrane with altered ion and water transferring capacity (Sheridan and Block, 1985).

The occurrence of interstitial oedema in the broncho-vascular bundle was a constant finding in the lungs of oxygen-treated rats. Oedema accumulated rapidly, being found after only 24 h exposure to 80% oxygen. However, even at 11 days, no increase (from control animals) could be detected in fibrin deposition. These findings are in agreement with other reports (Kimball et al., 1976; Crapo et al., 1980; Hayatdavoudi et al.,...
1981; Johnson et al., 1981) and lead to the suggestion that the initial site of injury is the pulmonary capillary endothelium.

In electron microscopy specimens examined after 11 days exposure to oxygen, an increase in alveolar type II cells was observed (table II). Crapo and co-workers (1980) also observed this change following treatment with oxygen, suggesting this to be a repair process—both to maintain the alveolar integrity and, perhaps, to assist the biochemical response. In this respect useful information may be obtained by comparing the antioxidant enzyme responses of alveolar type I and type II cells to 80% oxygen. Type II cells are known to be biochemically active and it is possible that they have higher activities of antioxidant enzymes. Enzyme changes noted in the whole lung may thus be entirely attributable to an increase in type II cell numbers. Forman and Fisher (1981), who examined alveolar type II cells from oxygen-treated and control rats found that only CuZn-SOD was increased; all other enzymes investigated (Mn-SOD, catalase, GPX and G-6-PD) remained unaltered. As these workers expressed their results both as units/mg protein and units/mg DNA, it is possible that absolute numbers of type II cells may be an important factor. Some adaptation, however, obviously occurs within the type II cell, as an increase in the number of lamellar bodies within their cytoplasm was observed (table II). This may possibly be an attempt of the type II cell either to increase the total amount of pulmonary surfactant, or to replace that lost as a result of increased lipid peroxidation.

Our results suggest various possibilities for the prevention of oxygen-induced pulmonary damage. It would be logical to imitate the biochemical response seen in this study by the parenteral administration of SOD, with either CAT or GPX. However, these enzymes which act intracellularly have a half-life in plasma of only a few minutes (Huber and Saifer, 1977) and do not readily cross the cell membrane into the cytoplasm. Attempts to solve this problem have been made and liposome-entrapped SOD has been shown to reduce oxygen-induced damage to cultured endothelial cells (Freeman, Young and Crapo, 1983). Liposomes readily become incorporated within cell membranes, with discharge of their contents into the cytosol. This permits not only modification of the intracellular antioxidant activity, but also alteration of the cell membrane lipid composition. McDonald and colleagues (1985) suggested that conjugation with polyethylene glycol is a preferable method of delivery of antioxidant enzymes.

Treatment with antioxidants and free radical scavengers other than enzymes is another possibility. Again, delivery to the cell in sufficiently high concentrations is a potential problem and many of these compounds have their own problems of toxicity. Moreover, the use of such agents may suppress the response of endogenous antioxidants and may result in enhancement of the pathological changes.

In conclusion, a wide range of biochemical changes has been demonstrated in the lungs of rats exposed to 80% oxygen, some of which are paralleled by changes in the red cell. There is a wide interspecies variation in sensitivity to oxygen and, for obvious reasons, biochemical changes in the lungs of humans exposed to hyperoxia have not been determined. Any extrapolation of our findings in the rat to the human should, therefore, be made with caution.

REFERENCES


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