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LIPID PEROXIDATION IN BRAIN AND LUNGS FROM MICE EXPOSED TO HYPEROXIA

DANA D. JAMIESON
School of Physiology & Pharmacology, University of New South Wales, P.O. Box 1, Kensington, Sydney, Australia

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Abstract—Lipid peroxidation was measured in brains and lungs from mice which had been exposed to 100% O_2 at 445 kPa, 515 kPa and 585 kPa for 30 min. These treatments produced varying degrees of convulsive activity and negligible to moderate lung damage. Lipid peroxidation was estimated by the thiobarbituric acid method and by analysis of Schiff bases. Lipid peroxidation did not increase in either lungs or brains after hyperbaric oxygen exposure, nor was there any difference between tissues from control or hyperoxic mice in the degree of lipid peroxidation which occurred during *in vitro* incubation of homogenates at 37° in air for 150 min. Similarly, no increased lipid peroxidation was seen in lung tissue from mice exposed to normobaric 100% O_2 for either 60 or 72 hr.

In mammals the inhalation of oxygen at pressures above 2–3 atmospheres absolute (ATA) causes convulsions, and lung damage accompanied by oedema and haemorrhage. In normobaric hyperoxia only pulmonary injury is apparent. It is assumed that hyperoxic injury is caused by the metabolic production of oxygen radicals and related reactive oxygen species at an increased rate which is dependent upon the oxygen tension, and which is in excess of the capacity of the cell's natural antioxidant defence mechanisms [1–5]. Lipid peroxidation which may result from such overproduction of reactive oxygen species could be subsequently responsible for the pathological consequences of hyperoxia [6–8]. *In vitro* studies have verified that the production of superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and lipid peroxidation can be increased in homogenates, slices and subcellular organelles of several tissues including the lung, when the oxygen tension of the suspending medium is raised [9–14]. However, as pointed out in the excellent reviews by Halliwell and co-workers [15–17] the role of lipid peroxidation in the mechanism of many oxygen radical based pathologies may be overemphasized.

In addition, there are many limitations in the extrapolation of *in vitro* data to the *in vivo* situation, and thus the strongest evidence supporting the concept that lipid peroxidation is the link between excess oxygen radical production and gross pathology is circumstantial, based on manipulations which enhance or inhibit the effects of high oxygen concentrations [2]. The present experiments were undertaken in an attempt to correlate pathological changes with measurements of lipid peroxidation in brain and lung tissue from mice exposed to hyperoxia under conditions chosen to produce varying degrees of tissue damage.

MATERIALS AND METHODS

Hyperbaric oxygen treatment. Male Balb/c mice, 23–30 g were used throughout these experiments. Pressurization of mice in the hyperbaric chamber and monitoring of gases were carried out as described previously [18, 19]. Up to four mice were pressurized simultaneously and the latency before the initial and before the later sustained convulsions seen in these mice was noted, as described previously [20]. Following decompression and cervical fracture the lungs and brains of the mice were removed, weighed and analysed for thiobarbituric acid reactive substances (TBARS) and, in some experiments, for Schiff bases. Three test groups of mice were used, exposed to either 445, 515 or 585 kPa for 30 min.

Normobaric 100% O_2 *treatment.* Groups of 10 weighed mice were placed in a perspex container of 30 L capacity and maintained at >99% oxygen concentration for 62 or 70 hr. The chamber was fitted with a fan to ensure gas circulation and carbon dioxide was absorbed in sodium hydroxide solution. Gases were monitored as previously described [19]. Carbon dioxide tension did not rise above 0.3% and the chamber temperature varied between 23–28°. At the end of the hyperoxic period animals were weighed, then killed by cervical fracture and their lungs removed, weighed and analysed for TBARS and Schiff bases.

Preparation of tissues for ex vivo *analysis.* To obtain sufficient lung homogenate for duplicate samples of non-incubated material and incubated material for TBARS estimations it was necessary to pool lungs from two mice. In experiments where Schiff bases were also analysed pooled material from three to four mice was used. Thus, for all experiments reported here each measurement (for both lung and brain) consists of pooled material from two to four test or control animals. In each experiment tissues from control (air breathing) mice were processed at the same time and in exactly the same manner as the hyperoxic groups. As there was negligible variation between control groups they have been pooled together in the presentation of Results.

Lung damage in hyperoxia consists of oedema and haemorrhage and therefore the lung weight increases. This would lead to gross inaccuracy in the results if they were calculated on any index of lung weight

following hyperoxic exposures. Thus, all dilutions for lung homogenate assays have been made on a body weight basis, using the considerable data we have in the past accumulated for this strain of mice [18–20]. Lung weights were recorded as an index of hyperoxic damage [18–20]. Blood was removed by cardiac puncture and pooled blood from several mice used.

Estimation of TBARS. TBARS were estimated essentially as described by Boehme *et al.* [21], except that tissues were homogenized in 100 mM K_2HPO_4 buffer, pH 7.4. Aliquots of the homogenates were immediately precipitated and analysed (time 0 samples) while other aliquots were incubated in a shaking water bath at 37° for 150 min to test their *in vitro* oxidizing capacity.

Samples were read at 532 nM with a Hitachi Model U3210 recording spectrophotometer. For calculation of results TBARS were assumed to be malonaldehyde (MDA) and an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ used. Control and test tissues were always processed together for each experiment.

Analysis of Schiff bases. A slight modification of the methods used by Dillard and co-workers [22, 23] was used to estimate Schiff bases. Brain or lung tissues were diluted 1:5 in 100 mM K_2HPO_4 buffer, pH 7.4. Six millilitres of 2:1 spectroscopically pure carbon tetrachloride:methanol was added to 1 mL of this homogenate and the mixture rapidly vortexed for 1 min. Four millilitres of distilled water were then added and the sample revortexed for 1 min. Samples were centrifuged at approximately 3000 rpm to separate the layers, and the chloroform rich layer was removed. Methanol was then added at one-tenth the volume of the chloroform layer to remove turbidity and the samples were assayed fluorometrically using a Hitachi Model 204 spectrofluorometer.

Excitation and emission spectra of mouse brain and lung gave excitation peaks of 365 and 362 nm and emission peaks of 435 and 480 nm, respectively. These wavelengths were used for estimations of the fluorescence of brain and lung samples. Acidic quinine sulphate, 0.2 μg/mL, was used as the standard solution, and all values are relative to this solution standardized to giving a reading of 50 divisions. The standard solution showed very little (<6%) day-to-day variation over the course of these experiments. Control (air breathing) mouse tissues were always processed alongside tissues from hyperoxic mice.

Effect of blood on TBARS and Schiff base analyses. To determine the effects of blood on the biochemical determinations of TBARS and Schiff bases, homogenates of lung or brain were prepared as above and varying amounts of blood (10, 20, 50 or 100 μL for TBARS tests and 20, 50, 100 or 200 μL for fluorescence analysis) or saline were added to the homogenates. Both unincubated and 150 min incubated samples were analysed.

Considerable quantities of tissue were necessary for these *in vitro* determinations and thus tissues from several mice were pooled to make up the initial homogenates.

Chemicals. Chemicals were all analytical grade, obtained from AJAX Chemicals, NSW, Australia. Medical oxygen (Medishield, Australia) was used for all hyperoxic experiments.

Statistical analysis. Statistical analysis was performed using Student's *t*-test for unpaired data with a SPSS package.

RESULTS

Convulsive activity in hyperbaric oxygen

As previously described [20] the Balb/c mice used in these experiments show biphasic convulsive activity when exposed to hyperbaric oxygen, with the initial convulsion being indicated by a temporary loss of righting reflex which occurred after several minutes, followed by a quiet period preceding later and more sustained convulsive activity which continued intermittently until the end of the 30 min exposure period. From Table 1 it can be seen that at higher pressures the onset of convulsions, which is the first obvious sign of hyperbaric oxygen toxicity, occurred after only about 3 min at 585 kPa whereas 19 min elapsed before any convulsive activity was evident at 445 kPa. Sustained and severe convulsive activity began later at 515 kPa than at 585 kPa while only two of eight mice showed severe, sustained convulsions within 30 min at 445 kPa. Thus, it could be assumed that progressively greater amounts of brain damage were produced in the 445, 515 and 585 kPa groups.

Pulmonary damage in hyperbaric oxygen

The wet weights of lungs from control non-pressurized mice and those from animals following 30 min at 445, 515 or 585 kPa of O_2 are shown in Table 1. No weight increase occurred during hyperbaric exposure at the lowest pressure tested, while slight but significant weight gain was evident at 515 kPa and considerable oedema and haemorrhage was seen concomitant with weight increase in the 585 kPa group.

TBARS in brain and lungs of mice exposed to hyperbaric oxygen

Brain. In spite of varying amounts of convulsive activity in groups of mice exposed to hyperbaric oxygen (Table 1) there was no measurable difference in the *ex vivo* TBARS values from the control group (i.e. unincubated, time 0) (Fig. 1). The control value is from pooled data from controls for all experimental groups. Aliquots of all homogenates (from pooled tissues of two mice) were also incubated for 150 min at 37° in air to test their "oxidative potential". An approximately 20-fold rise in TBARS was detected after this time in control brain homogenates. Homogenates from hyperbaric oxygen treated mice showed almost exactly the same increase in TBARS after incubation, indicating no changes in the oxidizing potential of these samples (Fig. 2).

Lungs. Ex vivo analysis of lungs of mice exposed to hyperbaric oxygen at various pressures showed no evidence of increased lipid peroxidation (Fig. 1). Thus, TBARS values were unchanged from controls, and were the same whether no lung damage was yet evident (445 kPa group), or lung damage with oedema and haemorrhage had occurred to varying degrees (515 and 585 kPa groups) as shown in Table 1. Indeed, considering that dilutions of homogenates

Table 1. Latency to convulsions, and lung weights, after exposure to various pressures of hyperbaric oxygen for 30 min

Treatment	No. mice	Initial convulsions (min)	Sustained convulsions (min)	Wet lung weight (mg)*
Control non-pressurized	28	—	—	136 ± 2
445 kPa	8	19.1 ± 2.6	—	134 ± 2
515 kPa	10	4.9 ± 0.5	23.0 ± 1.4	170 ± 14
585 kPa	10	3.0 ± 0.5	19.1 ± 1.0	242 ± 37

All results are expressed as mean ± SE.
* Lung weights adjusted to a body weight of 25 g.

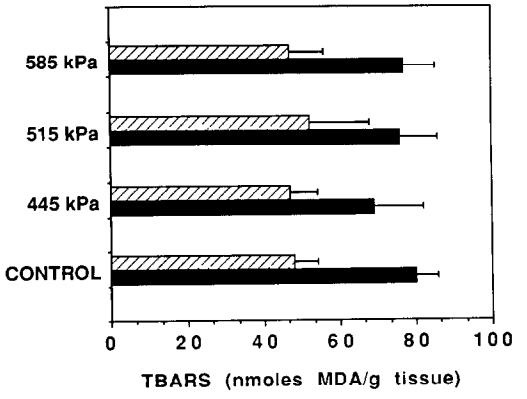

Fig. 1. Brain and lung TBARS from unincubated control, non-pressurized mice and from mice exposed to various pressures of oxygen for 30 min. This data is from the mice reported in Table 1, with pooled tissue from two mice per analysis. Standard error bars are shown, and no significant differences between TBARS from control and pressurized animals were found. Key: (■) lung, (▨) brain.

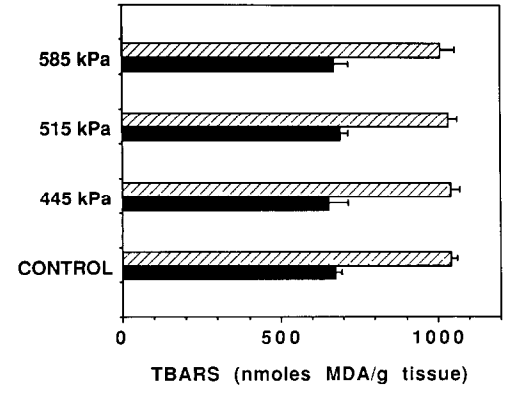

Fig. 2. TBARS in brain and lung homogenates incubated at 37° for 2.5 hr. The incubated samples were aliquots of the homogenates depicted in Fig. 1. No significant differences occurred between control and test mice. Key: (■) lung, (▨) brain.

were made on a body weight basis and different amounts of oedema and blood were present in these homogenates, the results are remarkably similar. This was also true of the lung homogenates incubated for 150 min in air at 37° (Fig. 2). As found for brain homogenates there was no difference between any group. Lungs of these mice give higher initial *ex vivo* readings of TBARS than brain tissue, and are similar to other reported values for mouse lung [24], but less lipid peroxidation occurred in the lung homogenates incubated *in vitro* than in brain homogenates, with an approximately eight-fold increase during the incubation period.

TBARS in blood of mice exposed to hyperbaric oxygen

In three experiments blood was removed by cardiac puncture from control mice and from mice exposed to 585 kPa for 30 min. Pooled blood from four mice was used for each estimation. No measurable TBARS were detected in blood of either the control group or the hyperbaric oxygen group and there was no increase in TBARS during *in vitro* incubation.

Schiff bases in brain and lungs of mice exposed to hyperbaric oxygen

In a separate series of experiments Schiff bases were measured together with TBARS in lung and brain tissue from control mice and mice exposed to hyperbaric oxygen at 515 kPa. Excitation optima were 365 and 362 nm, respectively, for brain and lung while emission spectra showed an optimum value of 435 nm for brain and 480 nm for lung tissue, values within the ranges found by Fletcher *et al.* [23].

It is obvious from Fig. 3 that there was no change in the Schiff base values in either tissue following hyperbaric oxygenation. As in the experiments reported above TBARS were again unchanged from control values (Fig. 3). Initial convulsions occurred at 4.3 ± 0.2 min and sustained convulsions at 20.9 ± 0.8 min in the 23 mice used in this series of experiments, thus the results are very similar to those reported for this pressure in Table 1. Wet lung weights for this group (176 ± 11 mg) were also similar to those reported in Table 1.

Exposure of mice to normobaric 100% O_2

Mice maintained in approximately 100% O_2 lost

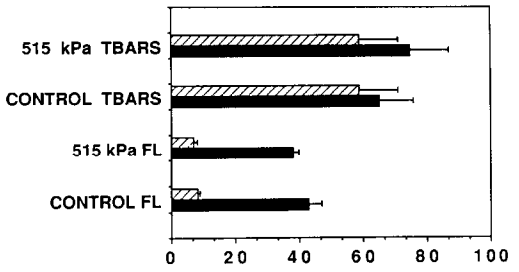

Fig. 3. In a separate experiment fluorescence of Schiff bases (FL) and unincubated TBARS from control, non-pressurized mice and mice exposed to 515 kPa oxygen for 30 min were compared. Fluorescence is expressed in arbitrary units and TBARS as nmol MDA/g wet tissue. Each result is the mean ± SE of six analyses with pooled tissue from three to four mice per analysis. No significant differences were found between control and pressurized mice. Key: (■) lung, (▨) brain.

weight while their air-breathing counterparts gained weight slightly. For estimates of lung weights for dilution purposes an average of the body weight prior to hyperoxia and at the end of the hyperoxic period was used. The body weight difference was very slight (7.5% loss) for the group of mice exposed to 100% O_2 for 62 hr. The weight loss was more severe (14%) for those mice remaining in oxygen for 70 hr, in which severe lung damage occurred (Table 2).

The lung weights of the 62 hr group were little different from their corresponding controls, but by 70 hr lungs were severely damaged in most mice and one mouse died at 69 hr and was discarded. Indeed the macroscopic appearance and weight gain of the lungs of the 70 hr group showed damage more severe than that produced by 30 min exposure to the highest pressure used in the hyperbaric experiments (cf. Table 1). However, in spite of spanning a range from virtually no damage to severe damage the TBARS values did not increase, nor was there any increase in Schiff bases (Table 2). Lungs from two to three mice were pooled for the TBARS and Schiff base data presented in Table 2. The only significant change was a slight decrease in the TBARS values in the 70 hr, severely damaged lung group due to the large amount of extra blood present in the lung as described below.

Control experiments with blood added to homogenates

It was necessary to examine the effect of adding blood to homogenates as considerable quantities of blood components enter the lungs during hyperoxic damage.

The addition of up to 50 μL blood to the 30 mg sample of tissue in each tube in these assays did not significantly alter the value of TBARS in unincubated lung or brain homogenates. Addition of 50 μL blood did cause a significant ($P < 0.05$) decrease in the incubated brain TBARS (from 1018 ± 18 to 894 ± 24 nmol MDA/g wet weight) and 100 μL blood significantly ($P < 0.05$) decreased the incubated lung values (from 589 ± 28 to 472 ± 13 nmol MDA/g wet weight).

The Schiff base analysis showed that incubation for 150 min at 37° in air caused an approximate six-fold increase in the fluorescence of brain homogenates (corresponding to the 20-fold increase in TBARS in incubated samples, cf. Figs 1 and 2). Addition of blood did not alter brain unincubated or incubated fluorescence values.

Lung tissue gave initially higher fluorescence readings, but this was not significantly increased during incubation. Added blood did not alter significantly the lung fluorescence.

DISCUSSION

For some years the unifying concept underlying pathologies attributed to oxygen radical damage has been that reactive oxygen species are formed at an excessive rate and overcome the cellular defence mechanisms. The reactive oxygen species formed during metabolism are superoxide and hydrogen peroxide, and in the presence of iron more deleterious radicals then result, such as the hydroxyl radical or the more recently implicated iron–oxygen radicals. Many investigators have supported the hypothesis that these latter highly reactive radicals then initiate lipid peroxidation, leading to cellular membrane destruction and consequent tissue damage [25–28]. Thus, lipid peroxidation has been implicated as the link between excessive production of reactive oxygen species and various pathological conditions including liver damage following carbon tetrachloride, paracetamol or halothane administration [29–32], damage induced by re-oxygenation of the hypoxic heart [27, 33], toxicity of anticancer drugs [34, 35], ageing [36], and brain and lung damage caused by hyperoxia [7, 8, 37, 38].

Table 2. Effect of breathing 100% O_2 at normobaric pressure on lung damage and indicators of lipid peroxidation

Treatment	Initial mouse wt (g)	Final mouse wt (g)	Lung wet weight (mg)	Initial TBARS	Incubated TBARS	Schiff bases
Control (air)	25.6 ± 0.5 (18)	25.9 ± 0.5 (18)	150 ± 3 (18)	86 ± 6 (7)	574 ± 13 (7)	44 ±1 (5)
100% O_2 for 62 hr	25.3 ± 0.6 (10)	23.4 ± 0.6 (10)	168 ± 5 (10)	83 ± 9 (4)	610 ± 32 (4)	42 ± 2 (2)
100% O_2 for 70 hr	27.1 ± 0.7 (8)	23.4 ± 0.6 (8)	286 ± 37 (8)	69 ± 10 (3)	482 ± 41 (3)	47 ± 1 (2)

The numbers in parentheses are the number of animals used or assays performed. Each assay represents pooled tissue obtained from two to three animals.

There is little doubt that lipid peroxidation can be deleterious to the cell, both directly and indirectly. Thus, toxic aldehydes, particularly 4-hydroxy alkenes may be formed during lipid peroxidation of membranes and subsequently affect other cellular components [33, 39, 40]. Lipid peroxidation can directly alter the biophysical characteristics of the membrane, with a decrease in fluidity [41, 42] and decrease in electrical resistance [42]. However, even when lipid peroxidation does occur the question remains as to whether lipid peroxidation is a coincidental outcome of the damage in these radical based pathologies, or is indeed the precursor to the damage [15–17].

As with other radical-based pathologies there is a great deal of circumstantial evidence in the hyperoxic field which supports the concept that oxygen radicals are formed at increased rates, which exceed the antioxidant defence mechanisms' capability to detoxify the reactive oxygen species. Support for this hypothesis is based largely on manipulations in which antioxidants or enhanced endogenous antioxidant defences have been shown to ameliorate oxygen toxicity or depletion of biological antioxidants shown to potentiate toxicity [6, 43]. However, there is considerably less evidence to prove that this overproduction of reactive oxygen species leads to lipid peroxidation which in turn induces hyperoxic pathology. Previous investigations have usually reported small, or negligible increases [37, 44–46] in lipid peroxidation in brains of animals not deficient in tocopherol, and exposed to hyperbaric oxygen. In only one case was there any positive correlation with convulsions [45]. In the present experiments there was no suggestion of increased levels of lipid peroxidation in the brain in spite of varying degrees of convulsive activity produced.

A 20-fold increase in TBARS occurred in mouse brain homogenates during incubation in air at 37° for 150 min. If antioxidant levels or other relevant parameters were altered during the hyperbaric oxygen exposure of animals then this incubated value might be expected to alter also. However, in the incubated samples the TBARS values for brain homogenates from mice exposed to hyperbaric oxygen did not differ from the homogenates from control mice. It must be pointed out that such *in vitro* incubations may have limited relevance to the *in vivo* situation where the topography of the cells and tissues are intact.

Similar negative results were obtained for lung tissue. The exposure times and pressures of oxygen were chosen to produce minimal to moderate lung damage to determine whether lipid peroxidation, if it occurred, may precede or be subsequent to permeability changes. No increases in lipid peroxidation were seen. In an earlier investigation Raskin *et al.* [47] reported decreases rather than increases in lipid peroxidation levels in incubated (60 min at 37°) homogenates from lungs of mice exposed to hyperbaric oxygen. However, in the study of Raskin *et al.* lungs were reported to be haemorrhagic, yet were diluted on the basis of the total weight. This probably seriously underestimated lipid peroxides because of dilution of tissue sample by accumulated haemorrhagic fluid, and also makes it impossible to gauge how much inaccuracy occurred since no data on the degree of lung damage was reported. Certainly the entry of oedema fluid and whole blood into the hyperoxic damaged lung presents a problem in the assay of biochemical changes in this tissue and thus in the present experiments this confounding factor has been explored to ensure, as much as is possible, that the results are free of such artefacts. In the experiments reported here all dilutions of lung tissue were made on a body weight basis using data from past experiments [18–20]. The control lung weights in the present experiments were virtually identical to these earlier studies. It is obvious that the presence of extra blood in the homogenates could alter the assayed values of lipid peroxidation products. If membranes of blood cells are themselves peroxidized by hyperbaric oxygen then higher values for lung peroxidation from haemorrhagic lungs may result. Also, it has been reported that haemoglobin can potentiate tissue damage, by increasing the level of available iron, [48] and catalysing lipid peroxidation. Conversely, it has been reported that red blood cells may protect against hyperoxic damage [49, 50] and that other blood components have antioxidant effects [21, 51–53]. Thus, it was possible that entry of blood into damaged lungs could increase or decrease the measured values for lung peroxidation.

However, it was found that hyperbaric oxygen exposure of mice did not affect the negligible amount of lipid peroxidation measured in blood, in agreement with reports that red blood cell membranes do not readily peroxidize [54, 55]. Thus, it was unlikely that an artefactual increase in lipid peroxidation would be seen in lungs due to haemorrhage.

To test further whether the presence of blood in the lung homogenates altered values for lipid peroxidation, lung homogenates were assayed with various amounts of blood added, up to amounts in excess of those found in damaged lungs in the present experiments. In the hyperbaric groups the average weight gain of the lung at the highest pressure (585 kPa) was 78%, corresponding to about 22 μL of blood added to the 30 mg tissue in each sample. This amount of added blood produced no significant difference in TBARS in unincubated lung homogenates nor in homogenates incubated for 150 min, although, as the amount of added blood was increased even further there was a progressive and significant decrease in the TBARS values in incubated samples.

As with all methods presently available for the estimation of lipid peroxidation there has been considerable criticism of the thiobarbituric acid assay [39, 56–58]. One source of criticism has been that malonaldehyde can be further metabolized *in vivo*. If indeed such further metabolism occurs it should result in an increase in Schiff bases which can be measured fluorometrically [55, 59]. Thus, in the experiments reported here Schiff bases were measured in addition to the measurement of TBARS from the lungs and brains of a further group of mice exposed to 515 kPa for 30 min. Control experiments showed that the presence of blood did not interfere significantly with the fluorometric assay. Once again, using the fluorometric assay, we found no indication

of increased lipid peroxidation in lungs or brains from hyperbaric oxygen treated mice.

While there has been almost no previous data regarding lipid peroxidation measurements from lungs of animals exposed to hyperbaric oxygen, several reports have appeared using animals exposed to hyperoxia at normal atmospheric pressure and the results have been controversial. Approximately two-fold increases in lipid peroxidation have been reported using TBARS assays in lungs from rats maintained for 5 days in 80% O_2 [60] or 7 days in 85% O_2 [11]. In both these experiments tolerance to hyperoxia was produced. A more recent study [8] also reported increases in TBARS aft · rabbits were exposed to 100% oxygen for 48 hr. By contrast, there was no increase in TBARS in lungs from newborn rabbits maintained in >90% O_2 [61] or 100% O_2 [62] unless the animals were tocopherol-deficient. Lung damage had occurred in these newborn rabbits. In agreement with these latter negative results Roberts *et al.* [63] found no rises in ethane or methane production in newborn rats exposed to 79% O_2, unless animals were fasted. In the latter case both air and hyperoxic treated animals gave similar rises above control values for this measure of lipid peroxidation yet there was greatly increased mortality in the hyperoxic group compared to the fasted air group. Yet Habib *et al.* [7] reported that only 8 hr of exposure to 100% oxygen was sufficient to significantly increase ethane production in rats, while in young adult rats exposed to 100% oxygen Török *et al.* [64] found little if any lung lipid peroxidation in contrast to the increases produced by other means such as hypoxia or ischemia.

In the experiments reported here exposure to 100% O_2 for 62 or 70 hr did not result in increased lipid peroxidation in adult mouse lungs. At 62 hr the average lung damage was very slight, with a negligible lung weight increase, while at 70 hr lung damage was severe. However, once again no increases in lipid peroxidation were recorded on the basis of both TBARS and Schiff base estimations, and during *in vitro* incubation samples from the hyperoxic groups showed no increased propensity to oxidize.

Thus, the present studies show no evidence for involvement of lipid peroxidation in the sequelae of events leading to lung or brain damage in hyperoxia. It may be argued that very small increases in peroxidation of membranes, undetectable *in vivo* or *ex vivo* by present methods, may be sufficient to precipitate severe and lethal cellular dysfunction. However, there is evidence which suggests that lipid peroxidation is not a prerequisite for cellular damage in other radical-based pathologies. Thus, dissociations of cellular damage and lipid peroxidation has now been shown for hepatotoxicity after administration of various substances including carbon tetrachloride [65] and paracetamol [66]. A similar lack of correlation was seen in hearts from pigs fed fish-oil diets, where lipid peroxidation was increased but without concomitant functional changes [67]. These recent results support suggestions [15–17] that lipid peroxidation may not be critical in oxygen radical based damage to tissue.

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