On the pulmonary toxicity of oxygen.

5. Electronic structure and the paramagnetic property of oxygen.*

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Abstract

Oxygen uptake by the pulmonary circulation is a chemical reaction. The physicochemical attributes of oxygen are critical when studying pulmonary oxygen toxicity. Extent of lung injury depends on the percentage of oxygen in an oxygen:nitrogen mix in polybaric circumstances (Shanklin, 1969). Further change in extent of lesion follows when other gases are used in the inhalant mix instead of nitrogen (Shanklin and Lester, 1972), with oxygen at 21-100% of the mix. Comparative subatmospheric oxygen levels down to 3% in hydrogen, helium, nitrogen, argon, or sulfur hexafluoride, were run with and without ventilatory distress by the Farber (1937) model, bilateral cervical vagotomy (BCV). This yielded coherent results indicating a need to consider molecular characteristics at the atomic level. Molecular mass and size, gas viscosity, and thermal conductivity yielded no obvious correlates to lung injury. Saturation of the outer electron shells of the diluents fit the empiric data, prospectively an interaction between oxygen and nitrogen from their electronegativity and closely approximate molecular mass, size, and shape. The lesion is essentially eliminated at 7% oxygen in nitrogen. At 3% oxygen, the least lesion is found with N_2, H_2, and SF_6, all gases with incomplete outer electron shells, allowing for transient, possibly polarized, covalent bonding with oxygen as the significant minority component in the mix. Argon and helium do not interfere with oxygen. With 3% oxygen in argon without BCV, the experiments ran so long (>70 hours) they were terminated once the point had been made. 3% oxygen in argon after BCV yielded a mean survival more than twice that of BCV in air, indicating a remarkable degree of nitrogen interference with oxygen in the respiratory medium of terrestrial animal life. Argon displayed other advantages for the lung compared to nitrogen. Hydrogen, nitrogen, and oxygen are diatomic molecules, a feature which does relate to the extent of lung injury, but only oxygen is paramagnetic.

Magnetic effects on lesion formation were tested: [1] with ventilatory distress induced in newborn rabbits, and [2] in young adult female white mice exposed to 100% oxygen without added mechanical distress. A noninvasive model for ventilatory distress, thoracic restraint (TR), with longer mean survivals of 40-50 hours, was employed rather than the Farber model. Parallel runs with TR, one subset receiving 100% oxygen in a plastic chamber resting on six strong ring magnets with measured fields up to +1200 gauss, the other plain 100% oxygen, were performed. Both subsets developed moderate metabolic acidosis with average weight losses circa 25%, but over different time courses, 82.89 ± 4.91 hours in magnetized oxygen, 55.4 per cent longer than the 53.34 ± 9.82 hours in plain oxygen ($\boldsymbol{p}$ <0.001). The longer survival in magnetized oxygen meant extensive lung injury (99.57 ± 0.42% pleural surface, versus 83.86 ± 14.03%), but the rate of lesion formation was 30.89 per cent faster in plain oxygen (1.5722% per hour) than in magnetized oxygen (1.2012% per hour), a difference significant at $\boldsymbol{p}$ <0.001.

The effect of oxygen without mechanical ventilatory distress was examined in female adult white mice exposed to oxygen or magnetized oxygen. Similar survivals and weight losses were achieved. The rate of lung lesion formation was different, 1.2617% per hour in plain oxygen, 46.13 per cent faster than 0.8634% per hour in magnetized oxygen. A variable magnetic field, with animals moving and breathing in chambers flooded with oxygen, has both systemic and pulmonary effects which alter the rate of lesion formation due to oxygen toxicity. Paramagnetic oxygen in a magnetic field influences the effect of oxygen toxicity on the lung but at these strengths of field it does not overcome significant mechanical disturbance.

Key words

Oxygen toxicity, lung injury, hyaline membrane disease, respiratory distress, argon, molecular attributes, electron shell saturation, paramagnetism, redefinition of hypoxia, rate of lesion formation

Introduction

The single most critical adaptation to extrauterine life of the fetus is the onset of ventilation. Until then the functions of respiration are carried out by the disposable placenta. This process entails much more than just the replacement of the interface of the villous trophoblast and the maternal intervillous space by the pulmonary airspace-capillary boundary.

Anatomic and functional maturation of the fetal lung is a long term, drawn out process. It ranges from the development of the several components of peripheral secondary respiratory lobules to the metabolic provision of those chemical substances which stabilize the newly activated air to tissue interface and the complex mix of the airspace lining fluid.

Moreover, the extrapulmonary apparatus, the numerous muscles and nerves (Campbell, et al., 1964), the form and the integrity of the rib cage and the thoracocervical girdle, and the strength, shape, and size of the laryngotracheobronchial tree have to be taken into account. The patency and size of the nasal passages also pertain.

Both clinical observation and the use of experimental models have shown the complexity of integration of the components for successful adaptation. A major disorder, clinically termed neonatal respiratory distress syndrome (RDS) (Usher, 1961), and pathologically known as hyaline membrane disease (HMD), has been the subject of descriptive reports for over a century and of experimental work since shortly before World War II. It is especially common in prematurely born infants, in males, and in premature infants delivered by abdominal section. This report concerns some late 20th century experiments and some performed in early 2012.

The simple fact that oxygen uptake by the pulmonary circulation is a chemical reaction implies necessary consideration of the physicochemical attributes of both oxygen and hemoglobin, the primary oxygen carrier to the organs and tissues. The latter comes to the lung in volume by dramatic changes in the intrathoracic circulation at birth (Patten, 1960), flooding the pulmonary capillary bed which was previously largely unfilled and collapsed. This vascular subcompartment is partially sequestered from the active fetal circulation. There is one critical developmental requirement for it, namely, to have fashioned the capacity to receive and contain the requisite blood volume in transition after just a few breaths of air.

Paradoxically, the physicochemical properties of oxygen crucial to respiratory competence are the basis for toxic injury to the lung. Reported here are two attributes which appear to control the intrapulmonary dynamics of the toxic oxygen effect, one of which seems to have systemic effects as well.

Atomic oxygen has an incomplete outer electron shell, six electrons where eight can be accommodated (the L shell; neon is the atomic form of a completely filled L shell). This is the source of its combinatorial prowess. The effect of this comparative state has been explored by experiments on newborn rabbits at oxygen percentages below that of air, with and without ventilatory distress induced by bilateral cervical vagotomy (BCV). The results of these experiments were coherent and prompted the study of a second attribute of oxygen, its highly particular paramagnetic property.

BCV results in fairly short mean and median survival times. This reduces the sensitivity of the test model by which to measure the effect of other factors. A slower but effective model was developed from an overall consideration of the larger ventilatory apparatus. The method chosen by which to examine the effect of magnetic fields on oxygen toxicity was that of thoracic restraint, with mean and median survival times four and more times those for BCV. Studies with and without the magnetic field effect were conducted in 100% oxygen by which to guarantee severe lung injury.

As a further examination of the paramagnetic effect but without mechanically induced ventilatory distress, young adult female white mice were treated with 100% oxygen in a similar way, with and without a magnetic field.

Materials and methods

There are several important objectives to the design of a model for human disease. Since the ill patient is intact throughout pathogenesis and in restitution and repair, models in the whole animal offer the advantage of bringing to bear on the matter those other aspects of systemic and wider regional response to the agents of disease and the processes by which the lesions and dysfunctions of the particular disorder evolve. An ideal model allows for the assessment of other factors which are capable of modulating or accelerating the principal vectors of the lesions or the state of malfunction which occurs. The results reported here come from [1] use of what is now properly called the classical model, bilateral cervical vagotomy (Farber, 1937; Shanklin and Berman, 1964), and [2] a derived model which, as noted above, has longer mean and median survivals during which the influence of a magnetic field effect was measured. In all cases, pregnant rabbit does or young adult female white mice were obtained by contract from local dealers in accordance with the rules and regulations of the applicable institutional animal care committees.

The classical model:

The first detailed use of bilateral cervical vagtomy to induce pulmonary edema in adult rabbits was by Farber (1937). When the technique of Farber was replicated in newborn rabbits the complete profile of clinical distress and the gross appearance and the histopathology of HMD was produced on a varied scale of extent which was dependent in part on time of survival, sex of the animal, and birth weight (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967). This model served to elucidate two major attributes of oxygen as the initiating etiological agent of HMD. The first was that the interaction between oxygen and nitrogen was an effect of their relative percentage in the inhaled gas mixture and not their partial pressure (Shanklin, 1969). The apparent effect of partial pressure was determined to be an attribute of sea level physiology. This was established by differential percentage compositions at 0.2, 0.6, 1.0, and 3.0 atmospheres absolute (ATA). Data of this sort demonstrates that BCV is a suitable model system by which to study those attributes of oxygen which pertain to and dominate the development of pulmonary toxicity.

In contrast to the human disorder, the classical Farber model in the newborn rabbit for hyaline membrane disease, BCV, often has only a partial lesion, sometimes only unilateral, often delineated along lobar and lobular boundaries (Figure 1). This attribute allows for quantitative assessment by intermediate findings (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967; Shanklin, 1969). At higher oxygen percentages most lesions cover all of the lung (100%). This model was used for the study of subatmospheric oxygen levels.

Subatmospheric oxygen tension:

The classical model also demonstrated the effect of other gases substituted for nitrogen in the environment following BCV. In brief, argon, helium, and sulfur hexafluoride have very different effects compared to nitrogen on oxygen at 21-100% (Shanklin and Lester, 1972).

There is a generally accepted belief that subatmospheric oxygen tensions (<21%) are hypoxic although this is usually referenced against the nitrogen:oxygen binary. Also, usually, this detail is left out of the equation (Shanklin, 1970, 1978)..This is further established by studies with and without BCV at oxygen percentages of 3 and 7 per cent. The 3-7% range covers the limit of hypoxia tolerated by newborns of various animal species described by Gordon Avery in 1963. Avery concluded that *hypoxia* was not a cause of hyaline membrane disease. This work confirms Avery's interpretation. As indicated, the oxygen percentage was determined by an in line Beckman D-2 oximeter (Shanklin, 1969; Shanklin and Lester, 1972). A total of 765 newborn rabbits were utilized in the subatmospheric experiments in this category, including 171 controls in air after BCV. Full exposition of statistical significance is provided for the runs at 3% oxygen in tabular form and as illustrations. All subsets have a positive time vector and negative values are not possible for gross lung change. Accordingly, the t test assessments of weight loss and lung injury are given as one-tailed and the birth weight t values are given as two-tailed population estimates. All gases used in this experiment were obtained through Air Products Industries, Allentown, Pennsylvania.

Hydrogen was not used at 7% oxygen as the general advisory from the U.S. Bureau of Mines at the time stated this ratio would not always quench propagation of a spark or flame, a consideration perhaps overly attended to with abundant air moving through the hood (MSDS #1009, 1994)! Premixed 3% oxygen and 97% hydrogen was used to eliminate any possible hazard from mixing these gases on site.

Derivative slow models for pathogenesis:

The use of neurectomies to disrupt the ventilatory function is not limited to bilateral vagotomy. The recurrent laryngeal nerves (BRLN) are retrograde branches of the vagus and independent study has shown the earlier part of the survival curve following BCV is due to the laryngeal effect of interruption of both recurrent laryngeal nerves (Shanklin and Sotelo-Avila,, 1967). The phrenic nerves and the external respiratory nerves of Bell also play important roles in ventilation. These methods, which will not be detailed here, result in a slower onset of respiratory distress but both require invasive surgery to achieve the effect. Both methods have a considerable effect on the lower thorax at the end of the rib cage and a non-invasive method was designed to influence ventilation from this site, that of *thoracic restraint* (TR).

Briefly, the thoracic circumference was measured by wrapping a string around it at the level of the xiphoid process and this distance was measured on soft half inch cloth adhesive tape. A transverse line was then marked at 90% of circumference and the tape was trimmed to twice this length. Just prior to placing the subject in the assigned chamber, the chest was wrapped snugly with the lower edge of the tape at the xiphoid and pulled so that a double layer was created compressing that zone of the thorax to 90% of original circumference. This did not overtly impede movement in the chambers and no immediate reaction was observed. The end result over time was similar to that following external respiratory neurectomy which has its physiologic effect through destabilization of the thorax against the force of downward excursion of the diaphragm.

A total of 74 newborn rabbits, 60 New Zealand white and 14 Florida White, were used in the thoracic restraint experiments. Experiments with New Zealand White rabbits were used to establish the parameters of the model (60 animals) as direct comparison to numerous other experiments which over time had involved several thousand newborn rabbits of the New Zealand White strain. The Florida White rabbit is an offshoot of the New Zealand strain, developed in Florida in the late 1970s. Mature does are 10-15 per cent smaller and the number of pups in each litter varies but tends to be slightly fewer. The difference in the birth weights of these related strains is shown in Table 1. Based on the observed distinctions between the two strains, the results for TR in 100% plain (not magnetized) oxygen were not commingled.

The newborn rabbits were weighed on arrival in the laboratory from the animal care room and were marked on the head and back by indelible ink for color coding. After isolation from the doe for a stabilizing period of about one hour, they were reweighed and the thoracic restraint tape was put on as noted above and the animals placed in a clear plastic chamber with a capacity of 2532 ml which contained a high wall water dish for humidity control and a humidity sensor to match the conditions of earlier experiments on newborn rabbits. One chamber rested on an array of six ring magnets held tightly by their magnetic force, arranged in a 2 x 3 configuration. In one row they were placed with the negative pole of #1 and #3 up and the positive pole of #2 up; the second row was reversed. Oxygen was fed through a hole above the animals connected to the flow meter at 2.0 liters/minute which came into the chamber over the water dish; the pressure of flow was released at the far end by a series of four 0.25 inch holes. The other chamber was the same except for the magnets, and both were on a constant heat warming table by which to maintain chamber temperature at 34-36 C, relative humidity circa 50%. When an animal died, it was promptly removed and after the lid was replaced the chamber was flushed at a flow of 5.0 L/min for two minutes.

Young adult female white mice:

The mice were color coded on the head and back by indelible ink markers and weighed upon arrival in the laboratory, and immediately assigned to clear plastic chambers with a tight lid for oxygen

flow. The chambers had a 480 ml capacity, enough for up to four animals. Female mice were employed to eliminate any possible effect due to sex of animal; in general, in the newborn rabbit experiments over time, males developed more extensive lesions. A total of 38 mice were used, 18 in plain 100% oxygen and 20 in a chamber on top of four 3 inch doughnut ring magnets which were positioned tightly together in a quadrilateral array by their own magnetic force, #1 and #3 with the negative pole up, and #2 and #4 with the positive pole up (Figure 2). Multiple measurements were by a hand held magnetometer probe. Nine measurements were made as follows: at each end, both corners and in the middle, and across the central axis of the chamber, at both edges and over the center point. The recorded maximum was +1200 gauss. The chambers were held down tightly against the magnets by 2-inch strapping tape across the lid and onto the table surface. One hole had been drilled in one end to receive the oxygen tube and the opposite end had four to five 0.3 cm holes for release of the pressure of the flow of oxygen at 1.0-1.5 liters/minute. When an animal died, it was promptly removed and after the lid was replaced the chamber was flushed at a flow of 5.0 L/min for two minutes. Medical grade oxygen for the newborn rabbit TR series and the mice was obtained from Praxair Distribution Southeast, LLC, Gainesville, Florida.

Second series, 7% oxygen in nitrogen:

The subatmospheric experiments described above did not regularly incorporate weighing the lung at autopsy. The striking lack of gross lung pathology at 7% oxygen in nitrogen prompted further study of this gas mixture on weight loss as a measure of metabolic effect and the relative mass of the lung. Thus, an additional full series of newborn rabbits were tested in 7% oxygen in nitrogen, 58 in all, 28 after BCV and 30 without vagotomy. This series was subjected to the usual observations plus lung weight at autopsy and weight loss over the time of each survival. The run without vagotomy was typical of many others without BCV, prolonged survival well beyond the final animal death in the post-BCV subset, leading to sacrifice of those remaining at some point in time, often about twice the time interval of the final demise in the post-BCV subset. Thirteen of the non-BCV died prior to the sacrifice point, yielding a final set of 41 for analysis of weight loss and lung weight. The others were autopsied in the usual way but are not part of the statistical analysis.

Statistical treatments of the data:

Spreadsheet development, principal descriptive statistical treatment, and the graphic plots were performed using ProStat 4.51 and 5.50 for Windows (Poly Software International, P.O. Box 80, Pearl River, NY 10965). Means are reported with standard errors of the mean (S.E.M.). Calculation of regression equations and determination of values of ***p*** by the T-test were performed using Abstat 5.0 for DOS (Anderson-Bell Corporation, P.O. Box 745160, Arvada, CO 80006).

Results

An overall total of 875 experimental animals was examined in this series of studies, 837 newborn rabbits and 38 young adult female white mice. The Farber (1937) model for the induction of neuropathic pulmonary edema, adapted to newborn rabbits (Shanklin and Berman, 1964, Shanklin and Sotelo-Avila, 1967), was employed in 531 newborn rabbits. This model has demonstrated versatility in the assay of various modulating factors, including hypobaric, normobaric, and hyperbaric oxygen environments (Shanklin, 1969) and the role of gases substituted for nitrogen at 21-100% oxygen (Shanklin and Lester, 1972). The gases employed in the latter were helium, neon, argon, and sulfur hexafluoride. The survival curves which result from these applications of BCV, when plotted in linear fashion, survival time on the ordinate and percentage remaining on the abscissa, have exponential characteristics (Shanklin, 2010). However, when plotted in semi-log format, with $\log_{10}$ per cent surviving on the abscissa, a general triphasic format emerges (Shanklin and Lester, 1972; Shanklin, 2010; Shanklin, 2012), the initial sharp decline of which (Phase A) is explained by severance of the recurrent laryngeal nerves, a component of the cervical vagus which can be isolated independently in the rabbit (Shanklin and Sotelo-Avila, 1967). Typical mean survivals range from 6-8 hours with medians approximating half of those values. The general supplemental time frame air control post-BCV in this report, with N = 126, had a mean survival

of 6.87 ± 1.14 hours and a median of exactly 3.0 hours. The internal concurrent air controls (N = 45) had a mean of 7.03 ± 1.80 hours with a median of 3.2 hours.

This phase A of the overall pattern of survival disappears with the use of non-vagotomy induction of ventilatory distress. The principal alternative models studied in newborn rabbits were bilateral phrenicotomy to denervate the diaphragm, interruption of both external respiratory nerves of Bell (EN), to denervate the *serratus anterior*, and thoracic restraint (TR) as described above, which has a result very similar to bilateral EN. This muscle serves to counter the downward sweep of the diaphragm by contraction from its scapular attachment, a small modulation of respiratory effort. The defining work here on thoracic restraint, with a similar pathological outcome, confirms the role of this muscle during ventilation. In the absence of measurements of the dynamics of tidal air flow, it may be that this aspect of thoracic function becomes evident only in situations of ventilatory distress.

The use of various gases as diluent with oxygen at 3-21%, with and without BCV, resulted in a remarkable pattern of lung injury, a total absence at 7% oxygen in nitrogen post-BCV (Figure 3). Birth weight means and medians were in the range of 38.0-51.0 grams for all 21 subsets (Table 2). The survival patterns and extent of lung injury are shown in Table 3.

Birth weights

Since the birth weights in the subatmospheric study, with the greatest differences in outcome for survival and lung injury in this study, fall into a fairly broad range (14.0 - 79.7 grams) with means over a more narrow scale (38.36 - 50.26), a two-tailed t test of the extreme means might delineate the entire varied distribution. These extremes are 3% oxygen in nitrogen without BCV, 50.26 ± 1.84 grams (N = 30), and 7% oxygen in sulfur hexafluoride, also without BCV, 38.36 ± 2.22 (N = 32). The t test yields $t = 4.1276$, $\boldsymbol{p} = 0.0001$, which would be significant except they are not in fully comparable subsets (different oxygen levels) although both are non-BCV. By contrast the five mean birth weights in the 3% oxygen post BCV group are 48.96 ± 1.51 (nitrogen), 45.83 ± 2.03 (hydrogen), 43.01 ± 1.91 (helium), 45.35 ± 1.40 (argon), and 39.73 ± 1.53 (sulfur hexafluoride).

Here the difference between the extremes is 9.23 grams, with $t = 4.2938$, $p < 0.0001$, whereas the difference between nitrogen and argon is only 3.13 grams, with $t = 1.2372$ and $\boldsymbol{p} = 0.2208$. The end result of lung injury, however, is in the opposite direction, in that larger animals live longer and tend to have more lung injury (Shanklin and Berman, 1964). Attention should be paid, nevertheless, to the distribution of animals by birth weight in these two subsets. The nitrogen subset has a kurtosis of +0.2668, sulfur hexafluoride, -0.2037, indicating opposing shifts in the distribution, with a coefficient of skewness for sulfur hexafluoride of -0.03718 against one of +0.05932 for nitrogen subset. These indices indicate the difference in means, plus the contradiction of lung injury, and their position in different subsets, is an artefact without significance, the $\boldsymbol{p}$ value notwithstanding.

Oxygen at 3% after BCV

All five diluents were in this subset, hydrogen, helium, nitrogen, argon, and sulfur hexafluoride. Both of the diatomic gases, hydrogen and nitrogen, yielded extremely short median and mean survivals. The median for hydrogen was 2.10 hours, for nitrogen, 1.5 hours. The respective means were 2.72 ± 0.45 hours and 2.36 ± 0.51. However, as Table 4 illustrates, there is a difference in the molecular diameter between these diluents, hydrogen at 2.34 A and nitrogen at 3.15, the latter 7% larger than oxygen at 2.92 A (Weast, 1968; Lide, 1997). Hydrogen is less of a block of oxygen on the basis of size and the resultant lung lesion is very much worse than that of nitrogen, 12.06 ± 3.42 per cent of pleural surface versus 2.71 ± 0.92 per cent for nitrogen ($t = 4.4575$, $\boldsymbol{p} < 0.0001$). The other mean survivals also had high degrees of significance, helium ($\boldsymbol{p} < 0.0001$), argon ($\boldsymbol{p} < 0.0001$), and sulfur hexafluoride ($\boldsymbol{p} = 0.0005$).

The pattern of gross lung injury was distinct since 3% oxygen in sulfur hexafluoride had the widest range of lung injury, a spread of 90.33 per cent, despite which the raw difference between nitrogen and SF_6 was significant at $t = 1.751$, $\boldsymbol{p} = 0.0425$. The raw results for both helium and argon were distinct and significant (helium, $t = 4.146$, $\boldsymbol{p} = 0.0001$; argon, $t = 2.8022$, $\boldsymbol{p} = 0.0034$).

The 3% oxygen series was the only opportunity to examine the possible role of the shape of diatomic molecules. As noted in Table 4, nitrogen and oxygen are very nearly the same size and nitrogen has an 7% greater "diameter," possibly indicative of a receptor or other lung tissue surface factor capable of holding either as the basis for the stochastic competition between them (Shanklin, 1969). The notably smaller hydrogen molecule does have an effect at this level of oxygen percentage (Figure 4). Their survival curves after vagotomy are essentially identical but very different survivals are obtained without vagotomy. The respective areas under their curves are 55.5 quantitative exposure units [QEU (Shanklin, 2010)] (nitrogen) and 176.5 QEU (hydrogen), a 3-fold difference not explained by the other molecular attributes noted in Table 4.

Oxygen at 3% without BCV

The survivals for hydrogen, helium, argon, and sulfur hexafluoride were substantially longer than for nitrogen, with a range of 19.97 (hydrogen) to 61.84 (argon) hours. The least difference, that of hydrogen, nevertheless was highly significant with t= 4.4187, $\boldsymbol{p} < 0.0001$. The other survivals were all at a greater degree of significance (argon, t = 14.6132, $\boldsymbol{p} << 0.0001$; helium, t = 12.9975, $\boldsymbol{p} << 0.0001$; sulfur hexafluoride, t = 10.89, $\boldsymbol{p} << 0.0001$).

Lung injury from 3% oxygen in nitrogen was the same whether vagotomy was performed or not (t = 0.2246, $\boldsymbol{p} = 0.4119$) but there was a decrease with all of the other diluents. The *difference between* BCV and no-BCV, for argon, was minimal and not statistically significant, -1.81% (t = 0.3338, $\boldsymbol{p} = 0.3698$) but the change in the others clustered around $\boldsymbol{p}$ values of 0.05: hydrogen, 0.0472; helium, 0.0573; and sulfur hexafluoride, 0.0348.

The difference in lung injury in 3% oxygen without vagotomy. against nitrogen, has a different profile. As is shown in Figure 4, hydrogen, nitrogen, and sulfur hexafluoride have means (± S.E.M.) which are on the 3% limit line, an approximate equality confirmed by three 2-way t tests with $\boldsymbol{p}$ values from 0.1071 to 0.3304. Both noble gases yielded very different lung injury compared to nitrogen: helium, t =2.31299, $\boldsymbol{p} = 0.0121$ and argon, t = 2.93739, $\boldsymbol{p} = 0.0023$.

Thus, when the 3% oxygen data in Table 3 are taken together statistically and graphically, a definitive pattern is seen to emerge when the extent of lung injury was correlated with the electron density of the outer shells of the molecules involved (Table 5; Figures 4 through 7). Figure 4 places the findings against the level of outer electron shell saturation. Figure 5 shows this in a different format, with emphasis on the distinction due to the effect of vagotomy. Figure 6 has direct comparisons for survival in nitrogen and hydrogen with and without vagotomy. There is no difference in survival after BCV between them but hydrogen clearly interferes far less with the survival potential of 3% oxygen than nitrogen when there was no vagotomy. Figure 7 is a direct comparison between hydrogen and helium. The helium effect is more pronounced without vagotomy.

Oxygen at 7% after BCV

Survival was less varied than in the 3% series with the maximal difference, between nitrogen and helium, not significant with t = 1.3225, $\boldsymbol{p} = 0.0956$. This indicates the remaining two mean survivals fall short of statistical validity. However, the difference in extent of lung injury, when contrasted to none in all 25 newborn rabbits in 7% oxygen in nitrogen following BCV, against a helium lesion close to that in 3% oxygen, 26.79 ± 5.41 per cent, yielding a t value of 7.9578, $\boldsymbol{p} < 0.0001$, is a remarkable observation. Even the much smaller difference between nitrogen and sulfur hexafluoride, 3.14 per cent, is significant, with t = 2.1958 and $\boldsymbol{p} = 0.0161$. The small difference between argon and sulfur hexafluoride, both of which are significantly different from nitrogen (data not shown), 4.86 per cent, is significant to a similar degree, t = 2.1877, $\boldsymbol{p} = 0.0162$. Such clear distinctions are additional evidence of the stochastic competition between oxygen and nitrogen.

Oxygen at 7% without BCV

The original subatmospheric or low level experiments did not include a series of 7% oxygen in nitrogen without vagotomy This was remedied by a subsequent second series in part to obtain

weight loss data and the rate of lesion formation in 7% oxygen in nitrogen without BCV (Table 6). Survival after BCV was identical up to 5 hours with the original series but with an earlier conversion to phase B and a shorter overall survival range (data not shown). However, the rate of lesion formation without BCV fitted well into the overall scheme shown in Figures 8a- 8d. These comparisons well illustrate the special advantages of argon-oxygen mixtures. When plotted on an arbitrary scale of progressive increase in oxygen percentage on the abscissa the rates of lesion formation are lower with nitrogen at 7% but higher at 60% oxygen (Figure 8a). When the nitrogen effect is compared between post-BCV and no-BCV subsets, the rate values of the latter fit well on the curve of the former (Figure 8b). When the post-BCV effect of argon is compared to the no-BCV subset, the latter is substantially lower than the general curve of the former (Figure 8c). Finally, when the rates of lesion formation for all four instances of no-BCV, the data much favor the argon effect (Figure 8d). Table 3 also conveys an argon effect. The mean gross lung change across the 3 and 7 per cent spectrum is a steady decline, from a mean of 14.25% (3% oxygen, post-BCV) down to 2.90% (7% oxygen, no BCV). The differences between these means, when plotted on linear coordinates, has a logistic profile, perhaps indicating a recruitment of a defense factor in the process (data not shown).

Similar general considerations were examined in the sulfur hexafluoride series (data not shown). The 3% and 7% four part subset results in closely approximate rates of lesion formation, all under 1.0% per hour. As was previously shown (Shanklin and Lester, 1972), sulfur hexafluoride and oxygen in mixes from 21% to 100% oxygen have a more complex relationship in which the air equivalent (20% oxygen) appears as an anomaly in the extent of lung injury. Calculations of the rate of lesion formation confirm this and when that data point is removed the remainder have a near linear minimally (and negatively) hyperbolic function with the nadir at 7% oxygen (data not shown). This anomaly has not been further explored so far.

There is an additional aspect, the proportion of each subset wherein many animals had no overt gross lung lesions at all. This will be described under the discussion as an interpretative finding.

The progression of the nitrogen-oxygen series

Again, in combination with several data points from earlier papers (Shanklin, 1969; Shanklin and Lester, 1972), a pattern of survival and mean lung injury appears when the oxygen-nitrogen mixtures are considered (Table 6). The only substantial survival in any oxygen-nitrogen mixture was in the no BCV 7% subset, 30.65 ± 2.99 hours, although mean survival in both the 10% and 15% subsets fell in the range of 10-15 hours, and three different air controls were all close to 7 hours. Very interestingly, the rates of lesion formation from 30% to 20/21% oxygen all fell in a range from zero ro 1.6667% per hour, a very narrow range for the spread of oxygen percentage. Thence, with higher oxygen levels, 30 to 100 per cent, both the mean injury and the rate of lesion formation, in accordance with prior observations on excess oxygen, rose progressively to 12.2344% per hour (100% oxygen), but not linearly. A plateau was in the middle range, 30-60% oxygen, with lesion formation rates between 3.8 and 4.2 per cent per hour. There is an internal very complex relationship in this middle range which, with the anomaly in the oxygen-sulfur hexafluoride studies, is outside of this presentation and will be examined in a future communication. The narrow range of fairly short mean survivals, however, is further evidence for a competitive relationship between oxygen and nitrogen.

The time dependency of weight loss

The second series of 7% oxygen in nitrogen afforded another comparison. The weight loss means at 7% are shown in Table 7. Although the percentage weight loss for the no-BCV subset is almost twice that of the post-BCV series, the rates of weight loss are reversed. There is a marked difference in the mean survivals of these two groups which accounts for the data. When the raw data is plotted over their appropriate time frames (Figure 9) what appears is a more fundamental aspect: loss of body mass is directly time dependent. One implication of this observation is when two groups appropriate for comparison have closely similar survival patterns, a difference in loss of body mass can be attributed to another factor, not to elapsed time.

Thoracic restraint as pathogenetic model

The defining series involved 60 New Zealand White newborn rabbits, thirty each in air and 100% oxygen. The overall ranges of birth weight, survival, weight loss, and rate of weight loss per hour were closely matched in both subsets, as shown in Table 8. The principal distinction in outcome was an expected increase in the extent of lung injury with the addition of 100% oxygen to the TR model, slightly more than a doubling. The mean gross lung change in air was 16.95 ± 4.19 per cent of pleural surface against 38.97 ± 6.496 per cent in 100% oxygen. The difference is significant, with t = 2.982, p = 0.0021, despite the wide variation indicated by both S.E.M. Thus, the usefulness of this slow motion model can be said to be established. With median and mean survivals in the range of 60 hours, ample time became available during which the influence of other factors could be studied.

Thoracic restraint and 100% oxygen with and without a magnetic field

As indicated above, important differences between New Zealand White and Florida White newborn rabbits required independent analysis of the results of TR in the latter. Despite the smaller sample further distinctions between those in magnetized 100% oxygen and plain 100% oxygen emerged (Table 9). Although the range, median, and mean birth weights differ in that the animals in plain oxygen occupy a lower segment of the general scale (Table 2), they are within the overall values found generally in the larger general experience reported here. The mean birth weights are not significantly different (t = 1.506, $\boldsymbol{p} > 0.1$). By contrast, survival is markedly different. Mean survival in plain 100% oxygen was 53.34 ± 9.82 hours, in magnetized 100% oxygen, 82.89 ± 4.91. The difference, 29.55 hours, is statistically very significant with t = 2.6912, $\boldsymbol{p}<0.02$, despite the wide variance indicated by the respective S.E.M.(Figure 10). Statistically, the extent of gross lung injury seems to be different *ab initio*, a numerical value of 15.71 per cent, but both subsets have medians of 100% and the difference is largely due to one animal in plain 100% oxygen with zero gross lung change; t = 1.1193 with $\boldsymbol{p} > 0.2$.

However, the rate of formation of the pulmonary lesion is very different. That of animals in plain 100% oxygen is 1.5722% per hour versus 1.2012% per hour for magnetized 100% oxygen, a ratio of 1.3089 or 30.89 per cent faster.

The difference in survival affects weight loss in a similar fashion (plain 100% oxygen: 26.27 ± 2.98 per cent; magnetized 100% oxygen: 25.17 ± 3.15, essentially the same) to very different rates per hour. For plain 100% oxygen, this was 0.4925% per hour; and for magnetized 100% oxygen, 0.3036% per hour, a ratio of 1.6222, a 24% higher rate than found for lung injury, likely some aspect of metabolism in that the progressive starvation state yields severe metabolic acidosis. This difference is indicative of systemic effects from the magnetic field in addition to those in the lung, which is not large enough an organ to drive overall metabolic homeostasis.

Young adult female white mice and a magnetic field

Chamber observations were made at frequent intervals throughout all experimental runs. There was a tendency for moisture to accumulate on the under surface of the lid, especially in the subset of plain oxygen. While this provided for chamber humidity it was wiped off when excessive and before the fur of the mice became damp. Animal weights were very similar between the two groups and the small difference in means was not significant, t = 1.2606, $\boldsymbol{p} = 0.1078$ (Table 10).

The mean weight loss for the plain oxygen subset was 28.05 ± 2.57 per cent and 31.55 ± 1.49 per cent for magnetized oxygen. This difference is not significant, t = 1.1918, $\boldsymbol{p} = 0.1206$. Likewise, the mean gross lung change was not significantly different, that for plain oxygen was 61.67 ± 10.91 per cent and for magnetized oxygen, 55.75 ± 10.45, t = 0.3917, $\boldsymbol{p} = 0.3488$. This is due to the wider variability than that seen in newborn rabbits.

Magnetized oxygen resulted in longer survival and less lung injury in the mice (Table 10). Mice in magnetized oxygen lived a mean 15.691 hours longer than plain oxygen, a difference which is statistically significant with t = 2.5547, $\boldsymbol{p} = 0.0075$.

When the result is considered on the basis of rate of lung injury per hour, magnetized oxygen is much slower due to the longer mean survival. The comparative rates of lesion formation were: plain oxygen, 1.2616% per hour, and magnetized oxygen, 0.8634% per hour, the former a 46% faster effect. These effects and values compare reasonably with those for newborn rabbits. They are in the same direction and as ratios (magnetized oxygen to plain oxygen) they bracket the value of 0.7. A lesser formation of lung injury in magnetized adult female mice, as well as in newborn rabbits, is indicative of systemic effects of the magnetic field as well as some localized protection for the lung. The overall difference in survival is shown in Figure 11. This approach meant the onset of respiratory distress was induced in part by the oxygen effects *per se.* There is evidence the intrathoracic rise in tissue oxygen tension itself interferes with ventilatory neuroregulation since nerves are known to be susceptible to high oxygen levels, with a reduction in functional capacity (Shanklin, et al., 1972).

Discussion

This is a preliminary report from studies of developmental biophysics in which fundamental atomic attributes are strongly correlated with the result of induction of ventilatory distress leading to gross pathological lesions in the lungs of newborn rabbits and young adult female white mice. The experimental work reported here is complex and detailed, requiring numerous instances of statistical treatment of the data in gross, referent the many subsets under specific consideration. The animal database is large, 875 in total. This is the largest collection when compared to the previous major reports from this laboratory. The databases in papers from this program, from1964 to the present, have utilized a total of 3085 newborn rabbits in many capacities and with numerous experimental subsets.

Of this total, 837 were newborn rabbits and 38 were white mice. After adjustment for an air-BCV control group in common with the report on thyroid factors, 126 animals, the working total of newborn rabbits in this report is similar to that in as the 1972 report on the effects of the second gas, 722 animals (Shanklin and Lester, 1972).

This is especially appropriate because this report is the logical sequel to it.

Although complex and detailed, the fundamental result is rather straightforward. This is the first report demonstrating the relationship between certain molecular attributes of oxygen itself to the formation of pulmonary lesions. Especially important are the saturation of the outer electron shell of oxygen and its derivative feature, its paramagnetic property. Molecular interaction can be determined from biological experiments (Shanklin, 2011). And, atomic attributes can be shown to be determinative in whole animal experiments under the circumstances reported here in greater detail (Shanklin, 2012b).

Neonatal hyaline membrane disease (HMD) and adult respiratory distress syndrome (ARDS) are the prototypes of pulmonary oxygen toxicity. They encompass the dilemma of the physiologic necessity for lung uptake of oxygen for systemic metabolism and energy production and the pulmonary toxicity of oxygen *per se.* Hyaline membrane disease (HMD) is a common and sometimes lethal disorder, especially in premature newborns (Shanklin, 1965; Shanklin, 1971). A characteristic x-ray pattern is produced (Wolfson, et al., 1969).

Previous work in the 20th century slowly moved away from the early misconception that the so-called hyaline membranes were the result of aspiration of amniotic fluid contents (Blystad, et al., 1951; Cameron and De, 1949; Dick and Pund, 1949; Farber, 1937; Hadders and Dirken, 1955; Johnson, 1923; Johnson and Meyer, 1925; Latham, et al., 1955; Potter, 1950; Potter, 1952; Potter, 1961; Potter and Craig, 1975; Shanklin, 1959; Shanklin, 1976). Firstly, they are mostly protein but do not actually fulfill the definition of hyalin such as that seen in amyloid degeneration (Taylor, 1988) and the term *hyaline membrane disease* is a simplification of an earlier designation as *hyaline-like* membranes (Latham, et al., 1955). Secondly, their form in the lung is highly variable and the earliest lesions, especially in very immature infants and in males, is necrosis of the terminal bronchiolar

epithelium (Shanklin, 1971). This aspect will be explored more completely in a subsequent communication. Johnson in 1923 called specific attention to their resemblance to the pulmonary lesion of viral influenza, no doubt stimulated by the then recent global pandemic which had high mortality and distinctive features, viz, hyaline-like membranes! The historic and horrendous Cocoanut Grove fire in Boston in November 1942, which resulted in 492 deaths, was the cause of especially serious lung injuries from inhalation of hot air, many of which resembled so-called hyaline membrane disease (Moritz, et al., 1945). The direct inhalation of hot gas is a form of energy transfer and the pulmonary changes (Wilhelm and Mason, 1960) differ from the secondary lung lesions seen in persons dying from extensive dermal burns (Sochor and Mallory, 1963). Somewhat similar are the lung lesions from inhalation of phosgene partially recreated experimentally by the installation of dilute hydrochloric acid (HCl) (Winternitz, et al., 1920). Since HCl is a monoprotic acid, oxidation *per se* is not part of its effect on the lung. The coagulation changes the integrity of the air-lung interface. This commonality of result, since oxygen is required to support ordinary combustion, another form of energy transfer, is a reminder that the protein exudation which occurs in cutaneous burns does so because of changes in vascular permeability (Wilhelm and Mason, 1960).

There is significant evidence the lesions in hyaline membrane disease can be induced by oxygen enrichment (Shanklin and Wolfson, 1967; Shanklin, 1969). The question then becomes whether the 10-fold rise in intrapulmonary oxygen tension which follows immediately upon initial lung expansion (Shanklin, 2010) is a chemical burn *per se* for peripheral lung tissue, augmented by interference with the intrathoracic neural regulatory system also immersed in this new ventilatory milieu (Shanklin, et al., 1972). If so, then the next question to be answered is at what level the neuropathic effect is accomplished.

Although this could be attained by high oxygen tension in the vagus trunks, left and right, from the transthoracic permeation of oxygen, a more direct and variable effect would likely follow from disseminated involvement of dozens to hundreds of the peripheral afferent fibers which progressively merge to form the vagus trunks. This is a candidate recruitment prospect for the varied extent of lesion seen in both the bilateral cervical vagotomy model and in the thoracic restraint and other slow motion models when animals are exposed to higher percentages of oxygen. It could also explain the occasional clinical experience of delayed onset of respiratory distress in the premature newborn. The combination of short survival spans and varied extent of lesions in the animal model, in this possible view of pathogenesis, would argue for a range of susceptibility in the peripheral secondary pulmonary lobules, perhaps similar to that shown in Figure 1.

The findings of comparative study at subatmospheric oxygen levels down to 3%, using hydrogen, helium, nitrogen, argon, and sulfur hexafluoride, with and without BCV, gave rise to the need to consider fundamental molecular characteristics at the atomic and/or quantum level, as explanation for the results. Examination of molecular mass, molecular size, thermal conductivity, and viscosity yielded no correlates to lung injury. The common diatomic format of hydrogen, nitrogen, and oxygen seems to have some effect, especially when overt ventilatory distress is absent (when vagotomy is not performed), but this factor does not explain the congruence between the findings and outer electron shell saturation. When this is examined for each of the diluents the saturation was found to fit the empiric data. This indicates a prospect for interaction between oxygen and nitrogen from their difference in electronegativity over their closely approximate molecular mass and size, enhanced by their shape. At 3% oxygen, the least lesion is found with nitrogen, hydrogen, and sulfur hexafluoride, all gases with incomplete outer electron shells, allowing for at least transient, possibly polarized, covalent bonding with oxygen as the significant minority component in the mix.

Argon and helium do not interfere with oxygen in this manner. When 3% oxygen in argon is used without BCV, the experiments ran so long (70+ hours) they were terminated once the point had been made. 3% oxygen in argon after BCV yielded a mean survival twice that of BCV and air, indicating a remarkable degree of nitrogen interference with oxygen in the respiratory medium of all animal life. And, since of the two, oxygen and nitrogen, only oxygen is paramagnetic, a test of the behavior of dioxygen in a varying magnetic field was necessary and appropriate.

The other aspect for consideration, as briefly mentioned before, is the frequency of animals with no overt gross lung lesion (Table 11). By simple (not weighted) averages, nitrogen has the most zero lesions, 68.09%, which is not particularly surprising since it is the most effective antidote for lesions formation overall (Table 3) as well as the most efficient interference with survival (Table 3) of all of the diluents. The second best proportion of zero lesions is from sulfur hexafluoride, a simple average of 56.34%. This comports with the generally low mean lesion formation from this diluent, which is roughly similar to that from argon overall (Table 3). The possibility that sulfur hexafluoride might be used in appropriate clinical conditions, however, is tempered by the absolute requirement the source has to be 100% free of disulfur decafluoride, S_2F_{10}, which has significant biotoxic properties.

It should be noted that many of the subsets, especially those with low oxygen percentage (3% and 7%), have the S.E.M. derived statistically, because gross lung lesions tend to develop late in the course of the experiments at final survival times considerably longer than the mean. The reverse of the data in Table 11, of course, is the number of positives.

A fundamental question answered in the affirmative by these studies was whether magnetic fields have physicochemical effects on oxygen in tissue in a fashion similar to what has been shown for open flames.

Studies have considered the effects of magnetic fields on flame combustion which is a chemical reaction involving oxygen (Ueno, et al., 1985; Ueno and Haruda, 1987; Wakayama, 1991; Wakayama, 1993); oxygenation in capillaries (Bali and Awasthi, 2010); and also on organic photochemical reactions (Turro and Kraeutler, 1980), which, taken together, indicate magnetic influences on the flow and orientation of oxygen as gas and in solution.

A variable magnetic field added to whole animal models of pulmonary oxygen toxicity changes the outcome in two overt ways: [1] survival is enhanced, but despite this, [2] the rate of formation of lung injury is reduced by 24% in newborn rabbits and by 31% in young adult female white mice. Thus, the toxic effect of oxygen is reduced systemically and in the lung by low strength magnetic field effects on inhaled paramagnetic 100% oxygen.

Unsaturated hemoglobin, abundant in the pulmonary arterial flow, is also paramagnetic (Pauling and Coryell, 1936), due to the oxidative state of unbound atoms of iron. This fact alone argues that the interaction between oxygen content of the terminal air space and the influx of unsaturated venous return has to be part of the action, thus also part of the problem. Indeed, the profound congestion or hyperemia which is the dominant aspect of the histological lesion of hyaline membrane disease might be considered a defensive action on the part of the organism, maximizing the amount of unsaturated hemoglobin by which to neutralize the adverse activity of oxygen which follows from its paramagnetic molecular state. The first volumetric surge of blood into the lung after birth is from reverse flow of low oxygen tension blood through the ductus arteriosus during the change from fetal vascular pO_2 values (Shanklin, 2010) to neonatal levels following effective onset of air breathing. The diversion of right ventricular outflow into the lungs, added to the reverse ductus flow, has an erectile effect (Jaykka, 1958), yielding some rigidity to pulmonary tissue. This factor is more significant in the premature infant because there is a progressive rise in pulmonary circulation towards term (Patten, 1960; Shanklin, 1959), thus less of a change after birth at or around a gestational age of 40 weeks.

Summary

The experimental study of pulmonary oxygen toxicity has been both driven and informed by clinical scenarios which often reflect the paradox of the necessity of oxygen for aerobic metabolism combined with adverse effects on the lung and other tissues. The pathophysiology of oxygen toxicity is complex. Studies in whole animal models make use of the adaptations they undertake during the oxygen challenge and other forms of induced distress. The long term use of a basic tool, the bilateral cervical vagotomy model of Farber, adapted to newborn rabbits, has been productive

on several levels. The device of neural interruption in rabbits, as a model for humans, is assisted by the fact the respiratory innervation of both species is identical as to the location and the branching of nerves as well as their neuromuscular and reflex functions. Of the extrinsic physical attributes of gases substituted for nitrogen, neither molecular weight, size, nor gas viscosity explain the behavior of oxygen beyond the percentage applied. One aspect of these molecules, the monoatomic/diatomic configuration, does have some effect at the level of contact surface interaction. A more fundamental aspect, the degree of saturation of the outermost electron shell, well matches the difference in extent of lung injury at subatmospheric oxygen tensions, invoking the paramagnetic quality of dioxygen. Direct testing of this concept in a varied magnetic field up to 1200 gauss demonstrates clear effects on the toxicity of oxygen to the lung and to the whole animal, modulated by the magnetic field. Magnetized oxygen injures the lung but at a measurably slower rate, strongly indicative of the interplay of the paramagnetic property of oxygen through transient covalent bonding with the other gases with covalent properties. This includes hydrogen, nitrogen, and sulfur hexafluoride from the panel of five tested in the experiments described in this preliminary report.

The noble gases, helium and argon, do not interfere with the adverse effects of oxygen on the lung and in the whole animal on its adaptive metabolism. The paramagnetic work described here is not a seemingly likely basis for clinical adaptation due to uncertainties of the power of the magnetic fields required to favorably influence a premature infant with a birth weight anywhere from 500 to 2500 grams, plus the probable interference of the apparatus with currently conventional clinical monitoring devices and systems. The especially favorable outcome for lung injury when argon replaces nitrogen in cases of experimental ventilatory distress is by itself a clear suggestion for possible adaptation to clinical scenarios of a comparable type.

Conflict of interest statement

The author declares that there are no conflicts of interest.

References

Air Products Industries, 1994. Material safety data sheet #1009 - hydrogen. Revision 4. Allentown, Pennsylvania

Avery, G., 1963. The effect of hypoxia on newborn animals with reference to hyaline membrane disease. Pediatrics 32, 801-807.

Bali, R., Awasthi, U., 2010. Study of an oxygenation process in capillary in the presence of magnetic field. Int.J.Math.Anal. 4, 1697-1706.

Blystad, W., Landing, B.H., Smith, C.A., 1951. Pulmonary hyaline membranes in newborn infants. Statistical, morphologic and experimental study of their nature, occurrence and significance. Pediatrics 8, 5-21.

Cameron, G.R., De, S.N., 1949. Experimental pulmonary œdema of nervous origin. J.Pathol.Bact. 61, 375-387.

Campbell, E.J.M., Dickinson, C.J., Howell, J.B.L., 1964. The immediate effects of added loads on the inspiratory musculature of the rabbit. J.Physiol. 172, 321-331.

Dick, F, Jr., Pund, E.R., 1949. Asphyxia neonatorum and the vernix membrane. Arch. Pathol. 47, 307-316.

Farber, S., 1937. Studies on pulmonary edema. I. The consequences of bilateral cervical vagotomy in the rabbit. J.Exp.Med. 66, 397-404.

Hadders, H.N., Dirken, M.N.J., 1955. On the origin of the pulmonary hyaline membranes. J.Pathol.Bact. 70, 419-425.

Jäykkä, S., 1958. Capillary erection and the structural appearance of fetal and neonatal lungs. Acta Pediatrica 47, 484-500.

Johnson, W.C., 1923. Pneumonia in newborn infants with lesions resembling influenza. Proc.N.Y.Path.Soc. 23, 138.

Johnson, W.C., Meyer, J.R., 1925. A study of pneumonia in the stillborn and newborn. Am. J.Obstet.Gynecol. 9, 151-167.

Latham, E.F., Nesbitt, R.E., Jr., Anderson, G.W., 1955. A clinical pathological study of newborn lung with hyaline-like membranes. Bull.Johns Hopkins Hosp. 96, 173-198.

Lide, D.R., ed., 1997. CRC Handbook of chemistry and physics, 78th ed., 1997-98, CRC Press, Boca Raton, Florida, p. 6-201, ISBN 0-8493-0478-4.

Moritz, A.R., Henriques, R.C., McLean, R., 1945. The effects of inhaled heat on the air passages and lungs. Am.J.Pathol. 21, 311-332.

Patten, B.M., 1960. The changes in circulation following birth. Am.Heart J. 6, 192-205.

Pauling, L., Coryell, C.D., 1936. The magnetic properties and structure of hemoglobin, oxyhemoglobin and carbonmonoxyhemoglobin. Proc.Nat.Acad.Sci.USA 22, 210-216.

Potter, E.L., 1950. Pathology of prematurity. J.Am.Med.Women's Assoc. 5, 391-396. .

Potter, E.L., 1952. Pathology of the fetus and newborn, Year Book Publishers, Chicago, pp. 249-252.

Potter, E.L., 1961. Pathology of the fetus and infant, 2nd ed., Year Book Publishers, Chicago, pp. 288-292, LCCCN 61-10996.

Potter, E.L., and Craig, J.M., 1975. Pathology of the fetus and infant, 3rd ed., Year Book Publishers, Chicago, pp. 288-293, LCCCN 75-16021, ISBN 0-8151-6760-1.

Shanklin, D.R., 1959. Cardiovascular factors in development of pulmonary hyaline membrane. A.M.A Arch.Pathol. 68, 49-57.

Shanklin. D.R., 1965. Hyaline membrane disease. Clin.Med. 72, 477-490.

Shanklin, D.R., 1969. On the pulmonary toxicity of oxygen. I. The relationship of oxygen content to the effect of oxygen on the lung. Lab.Invest. 21, 439-448.

Shanklin, D.R., 1970. The special relation between oxygen and nitrogen in pulmonary injury. Pediat.Res. 4, 469.

Shanklin, D.R.,1971. Oxygen and the lungs of newborn infants. Int.J.Clin.Pharmacol. 5, 20-25.

Shanklin, D.R., 1976. Criteria for diagnosis of hyaline membrane disease. Arch.Pathol. 100, 345-346.

Shanklin, D.R., 1978. Redefinition of "hypoxia" and the pulmonary oxygen-nitrogen receptor. Fed.Proc. 37, 867.

Shanklin, D.R., 2010. On the pulmonary toxicity of oxygen. III. The induction of oxygen dependency by oxygen use. Exp.Mol.Pathol. 89, 36-45.

Shanklin, D.R., 2011. Argon and the pathophysiology of pulmonary oxygen toxicity. 42nd Middle Atlantic Regional Meeting, American Chemical Society, College Park, Maryland, May 21-23, 2011, #415, p. 262.

Shanklin, D.R., 2012a. On the pulmonary toxicity of oxygen. 4. The thyroid arena. Exp.Mol.Pathol. 92, 140-154.

Shanklin, D. R., 2012b. Pulmonary oxygen toxicity is modulated by its paramagnetic property. 43rd Middle Atlantic Regional Meeting, American Chemical Society, University of Maryland Baltimore County, May 31-June 2, 2012, #225, p. 169.

Shanklin, D.R., Berman, P.A., 1964. An experimental model for hyaline membrane disease. Biol.Neonat. 6, 340-360.

Shanklin, D.R., Lester. E.P., 1972. On the pulmonary toxicity of oxygen. II. The effect of the second gas. Biol.Neonat. 20, 140-158.

Shanklin, D.R., Sotelo-Avila, C., 1967. The effects of components of vagotomy on the lung and the effects of anesthesia on vagotomy induced lung change. Biol.Neonat. 11, 61-86.

Shanklin, D.R., Stein, S.A., Thompson, D., Banks, J., 1972. Effects of varied oxygen tension on properties of excised squid giant axon. Biol.Bull. 143, 477-478.

Shanklin, D.R., Wolfson, S.L., 1967. Therapeutic oxygen as a possible cause of pulmonary hemorrhage in premature newborns. New Eng.J.Med. 277:833-837.

Sochor, F.M., Mallory, G.K., 1963. Lung lesions in patients dying of burns. Arch.Pathol. 75, 303-308.

Taylor, E.J., ed., 1988. Dorland's Illustrated Medical Dictionary, 27th ed., W.B. Saunders Co., Philadelphia, p. 780, col. 1, ISBN 0-7216-3154-1.

Turro, N.J., Kraeutler, B., 1980. Magnetic field and magnetic isotope effects in organic photochemical reactions. A novel probe of reaction mechanisms and a method for enrichment of magnetic isotopes. Acc.Chem.Res. 13, 369-377.

Ueno, S., Esaki, H., Haruda, K., 1985. Combustion processes in strong DC magnetic fields. IEEE Trans.Mag. 21, 2077-2079.

Ueno, S., Haruda, K., 1987. Effects of magnetic fields on flames and gas flow. IEEE Trans.Mag. 23, 2752-2754.

Usher, R.H., 1961. The respiratory distress syndrome of prematurity. Clinical and therapeutic aspects. Pediat.Clin.North Amer. 8, 525-538.

Wakayama, N.I., 1991. Behavior of gas flow under gradient magnetic fields. J.Appl.Physics 69, 2734-2736.

Wakayama, N.I., 1993. Magnetic promotion of combustion in diffusion flames. Combus.Flame 93, 207-214.

Weast, R.C., ed., 1968. CRC Handbook of chemistry and physics, 49th ed., 1968-69, The Chemical Rubber Co., Cleveland, Ohio, p. F-151, LCCN 13-11056.

Wilhelm, D.L. Mason, B., 1960. Vascular permeability changes in inflammation: the role of endogenous permeability factors in mild thermal injury. Brit.J.Exp.Pathol. 41, 487-605.

Winternitz, M.C., Smith, G.H., McNamara, F.P., 1920. Effect of intrabronchial insufflation of acid. J.Exp.Med. 32, 199-204.

Wolfson, S.L., Frech, R., Hewitt, C., Shanklin, D.R., 1969. Radiographic diagnosis of hyaline membrane disease. Radiology 93, 339-343.