

On the pulmonary toxicity of oxygen:

III. The induction of oxygen dependency by oxygen use*

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Abstract

Oxygen is central to the development of neonatal lung injury. The increase in oxygen exposure of the neonatal lung during the onset of extrauterine air breathing is an order of magnitude, from a range of 10-12 to 110-120 Torr. The contributions of oxygen and the volume and pressure relationships of ventilatory support to lung injury are not easily distinguished in the clinical setting. Sequential changes in inspired air or 100% oxygen were studied in 536 newborn rabbits without ventilatory support. Bilateral cervical vagotomies (BCV) were performed at 24 hours post natal to induce ventilatory distress which eventuates in hyaline membrane disease. The sequences applied yielded evidence for an induced state of oxygen dependency from oxygen use which was reflected in differences in survival and the extent of pulmonary injury. The median survival for animals kept in air throughout was 3 hours. Oxygen before vagotomy or during the first 3 hours afterwards extended the survival significantly but produced more extensive, more severe, and more rapid lung lesions. Returning animals to air after prior oxygen exposure reduced the number of survivors past 10 hours and shortened the maximum survival in those groups. These features indicate the development of a dependency of the defense mechanisms on the availability of oxygen at the higher level for metabolic and possibly other aspects of the pulmonary and systemic response to injury, beyond the usual physiological need. Subset analysis revealed additive and latent effects of oxygen and demonstrated a remarkable rapidity in onset of severe lesions under some circumstances, illustrating the toxicity of oxygen *per se*.

Introduction

Significant difficulties in the management of respiratory distress syndromes, especially in the premature newborn, are not new (Egan, 1977a; Shanklin, 1971). The problems in treatment revolve around a common if not completely understood linkage between ventilatory support and supplemental oxygenation (Nash et al, 1967; Northway and Rosan, 1969; Northway et al., 1967). The principal initial concerns over ventilatory therapy were: (a) the mechanical aspects and risks at certain pressures or cycles, including prolonged use (Egan, 1977b; Taylor, 1978), and (b) the more complex problems of weaning (Gregory, 1971; Downs, et al., 1973; Cogswell, 1975; Sahn, 1976; Egan, 1977c).

These problems occur against a background of dynamic changes in the metabolism and oxygen consumption of newborns, whether mature, premature, or dysmature (Jonxis et al., 1968; Moss, 1965; Orzalesi, 1967; Schwartz, 1968; Scopes and Ahmed, 1966). Indeed, metabolic and other problems often arise during ventilatory support (Egan, 1977d), after weaning (Downs, et al., 1973), or during periods of reduced supplemental oxygenation, which, both from chemical index criteria and clinical judgment, often lead to changes in or reinstatement of ventilatory support and enhancement of the inhaled oxygen content (Northway et al., 1967; Cogswell et al., 1975). Moreover, there is evidence of systemic and pulmonary depletion of anti-oxidant ascorbic acid during oxygen challenge (Shanklin and O'Dell, 1966; Shanklin, et al., 1967). Consideration of these issues within the scope of clinical efforts to support ventilation date from about 1948 (Barach, 1948).

The concurrent concerns are essentially the same (Chess et al., 2006), with emphasis on such techniques as elective high frequency oscillatory ventilation (Cools et al., 2009), early nasal continuous positive airway pressure (te Pas et al., 2007; Morley and Davis, 2008), extracorporeal membrane oxygenation [ECMO] (Petrou and Edwards, 2004), and the addition of small amounts of nitric oxide [NO] to the inhalant gas (Da-Silva and Dellinger, 2004). Indeed, the overall problem remains as great as or even more prevalent than in decades past with renewed emphasis on the public health aspects, seeking the prevention of premature birth as the best prophylaxis for neonatal respiratory distress (Morken, 2010). Persistent high frequencies of prematurity (Blumenthal and Lyell, 2009; Martin et al., 2009) and recent increased rates of twinning in the United States (Hardin et al., 2009; Sunderam et al., 2009); both of these factors add measurably to the importance of the problem.

Similarly, the generally considered contribution of inadequate pulmonary surfactant to either the susceptibility to or as a pathogenetic factor in hyaline membrane disease, is undergoing reassessment through the now better understood genetic complexity of the component proteins (Lyra and Diniz, 2007). Efforts to find better ways to administer surfactant as a preventative, such as intra-amniotic injection, are inconclusive *per se* (Abdel-Latif, et al., 2010). Moreover, the recent studies have not fully dealt with earlier work demonstrating the passage or collapse of the layer into the terminal airspaces by the very first phase of post injury edema (Bolande and Klaus, 1964) or the presence of abundant lamellar bodies in type II pneumocytes despite well formed lesions of hyaline membrane disease (deMello, et al., 1987). DeMello, et al. (1987), found an absence of intraluminal tubular myelin rather than a loss of lamellar bodies in type II pneumocytes in these circumstances.

More recent and concurrent concerns over oxygen toxicity incorporate these fundamental physiological aspects in the context of detailed knowledge of the mechanisms of cell injury, including cytokines and related substances, and a better understanding of how oxygen is managed in lung tissue.

Distinctions between oxygen factors (Shanklin and Wolfson, 1967) and mechanical factors in weaning or in long-term use of ventilators are not easily made in the clinical setting (Egan, 1977e). The evidence of their connection with the then described lesion complex of bronchopulmonary dysplasia is fairly extensive and convincing (Northway and Rosan, 1969; Northway, et al., 1967; Balentine, 1982). Nevertheless, various reports are not in agreement on the relative significance or the separability of these factors (Balentine, 1982; Northway and Rosan, 1969; Northway, et al., 1967; Robertson, et al., 1964). This is of particular interest in light of the increasing importance of genetic mutations, more especially in the healing phases (Shanklin, et al., 2008).

The present experiments were designed to examine the oxygen factor independent of methods of ventilatory support. Bilateral cervical vagotomy produces a ventilatory distress syndrome in newborn rabbits similar in overt clinical behavior to the seemingly spontaneous human disease (Shanklin and Berman, 1964). The lesion complex contains all the elements of the human disorder known as hyaline membrane disease (Shanklin and Berman, 1964), making the model a good comparative test system for oxygen factors in small infants. An earlier report contained data to

suggest that the median survival of newborn rabbits following vagotomy was improved by an increase in oxygen content beyond air at one atmosphere absolute (Shanklin, 1969). This change was not linear or simple. Although the median was higher when 100% oxygen was used, the maximum observed enhancement was at 60% oxygen (Shanklin, 1969). The system and results previously described in detail (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967; Shanklin, 1969; Shanklin and Wolfson, 1970) all used newborn rabbits kept in air between birth and vagotomy, with experimental changes of environment instituted upon completion of vagotomy. Additionally, animals maintained in air continuously have a median postvagotomy survival of almost exactly three hours (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967; Shanklin, 1969), a convenient interval for shifting the sequence of gas mixtures used. The median is used in preference to the mean because of the hyperbolic character of the survival curves when plotted rectilinearly (Shanklin and Berman, 1964). A decreasing triphasic linear plot results when the semilogarithmic technique is used with survival on the logarithmic ordinate, starting with 100% survival at time zero.

This study concerns changes in the proportion of groups surviving vagotomy by three hours or more, the consequences of returning to air *after* oxygen use, and the extent of lung lesions produced by the various combinations (Shanklin, 1972). Ventilatory support was not used in any of the experimental work reported here.

Materials and methods

Pregnant New Zealand and Dutch rabbits at term were induced by oxytocin (Shanklin, 1966). Immediately following delivery the newborns were allocated randomly to incubator boxes with either air or 100% oxygen, separate from the doe. This period, to 24 hours of age, is termed the *primary* treatment. The incubator system was maintained at 34°C and a relative humidity of 50-60% as previously described (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967; Shanklin, 1969; Shanklin and Lester, 1972). The experimental protocols were approved by the relevant animal research committees in each of the institutions housing the facilities as recipients of the grants-in-aid as noted.

At 24 hours all animals were subjected to bilateral cervical vagotomy as previously described (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967). For animals kept in oxygen this meant brief air exposure. Almost all vagotomies took less than one minute to complete, including positioning and restraining the subject for the procedure. A few drops of dilute local anesthetic were used in all cases (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967).

Secondary random reassignment following vagotomy was established. Thus, animals in oxygen before vagotomy were either kept in oxygen or put in air; animals in air before vagotomy were reassigned to either air or oxygen. As mentioned, previous work had established that three hours was the median survival in air following vagotomy (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967; Shanklin, 1969). This time point was used for a final *tertiary* allocation between oxygen and air. All survivors at three hours postvagotomy were reassigned to either air or 100% oxygen. The sequences generated by this plan are shown in Table 1. Confirmation of 100% oxygen before and after opening the subchambers for reassignment or removal of dead animals was

by Beckman D-2 oximeter.

Close observation of all animals was maintained. Animals were removed after death and were autopsied according to the methods and procedures previously reported (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967; Shanklin, 1969). In brief, the extent of lesion was determined by a grid approximation formula on the pleural surface of the lungs, with each of the four principal lobes considered separately. These values were combined by a weighted formula. The result is a percentage of total pleural surface. The determination was performed under a dissecting microscope at 15-30X to verify airlessness and fluid collection of each lobular zone. Lungs were fixed in Bouin's fluid and the lesions were then confirmed microscopically.

A total of 536 newborn rabbits was used. In contrast to prior experiments (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967; Shanklin, 1969), experimental groups were expanded so that 30 or more animals would survive *beyond three hours* postvagotomy, not just postvagotomy alone.

Assignment of animals from given litters to a group was as before, with special attention to large litters and by weight (Shanklin, 1969). The sex ratio of individual groups was left to random sort by the minimum test group size as noted above (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967; Shanklin, 1969) inasmuch as the sex of the animal is readily determined only at autopsy. In addition, although other contemporaneous experiments involved the two major comparative groups, AAA and AOO, there were no representatives of those litters reported here in the other six groups and, although those results were similar and supportive, they are not part of the core data of this report, lacking the proper internal controls.

Analysis by litter, weight, and sex showed no inherent bias to the distribution, with random, nonsystematic placement between comparable, adjacent, and sequential groups. In the text to follow, the hyphen in the notations (e.g., AA-, OO-, et al.) indicates the comparison does not concern the tertiary phase.

The following time intervals postvagotomy are used in the text: (a) *early*, 0-3 hours; (b) *intermediate*, 3-10 hours; and (c) *late*, 10+ hours. This means, in effect, there is no exact coordination between the timing or duration of phases A, B, or C determined by graphic plots, and the exposure sequences. The overall pattern and consistency of the plots is indicative of the fundamental response(s) of the animals to the scheduled gas exposures. Graphic plots were undertaken with survival percentages on the ordinate (y-axis) and elapsed time on the abscissa (x-axis). The slopes of phases A, B, and C were calculated from the log scale conversions. Where useful, χ^2 analysis and Student's *t test* has been applied for small sample comparisons. Quantitative assessment of the joint effects of time and oxygen levels have been estimated by application of the graphic analysis method *area under the curve* (AUC), not heretofore applied to lesion formation, but fully appropriate in this context for the examination of semilog plots. The analytic nature of the data, expressed in proportion and time of survival, make the reference term for AUC in this context, *percent survival-hours*, both cumbersome and potentially confusing. Accordingly, the term *quantitative exposure units* (QEU) will be substituted.

Global analysis by statistical means (\pm s.e.m) has little meaning in light of the differences in the slopes and intercepts of the three phases and was not used. Rather, the comparisons are by survival against time and by extent of pulmonary lesion for each time frame. Time sequence analysis will use both the 3 and 10 hour postvagotomy intervals (Shanklin and Berman, 1964; Shanklin and Lester, 1972) and the relationships developed from the above mentioned semilogarithmic plots for animals with only air (Figure 1) and with only oxygen (Figure 2) during the primary prevagotomy period.

Formal analysis and graphs were done with the aid of ProStat 4.51 for Windows (Poly Software International, Inc., P.O. Box 80, Pearl River, NY 10965); preliminary graphs and the slopes of regression equations were calculated using Abstat 5.0 for DOS and 1.96 for Windows (Anderson-Bell Corporation, P.O. Box 745160, Arvada, CO 80006); χ^2 calculations were cross verified at: www2.lv.psu.edu/jxm57/irp/chisquar.html.

Six pregnant New Zealand does were used in a satellite project. A small abdominal window over one horn of the uterus was opened and secured along the margins under I.V. pentobarbital and the uterine horn moved into the opening and lightly sutured to the abdominal wall for stability. After orientation of a fetus was determined by palpation, a smaller window, about 3 x 3 cm across, was developed by close dissection and the right side of the fetal thorax was carefully secured by two sutures, one in the subclavicular zone and the other at the diaphragm. The field was then flooded by nitrogen from a standard storage tank and a Clark type microelectrode* was pushed into the underlying right lung and the intrapulmonary pO₂ measured by the method described by Crompton, et al (1965) and Kwan, et al (1972). The litters from these does were not used in any of the experiments described in this report.

Results

Eight different sequences were possible using the format described and all were used. There are four each following prevagotomy air or oxygen. These are delineated in Table 1. The results are presented from two perspectives, (a) the effect of changing the gas environment on survival, and (b) the effect of this on the extent of lung injury.

Comparative survival following changes in the gas environment:

The eight gas sequences resulted in differences in survival (Table 2, Figures 1 and 2). Inspection of Table 2 immediately suggests three key comparisons. These are: [1] AAA and AAO, [2] AOA and AOO, and [3] OOA and OOO. Furthermore, there is one other connection in the data between the AOA/AOO and the OOA/OOO pairings. Comparison by difference in percentage are given as the proportion of change beyond the lower datum by the higher figures found by experiment.

* Product no longer available; likely closest items: www.lazarlab.com or www.instechlabs.com

A-A-A versus A-A-O:

The prevagotomy (primary) and the first three hours postvagotomy (secondary) environments are the same, *air* followed by *air*. Their survival experience at the end of these is closely similar, 48.8% and 50.8% respectively. Changing the gas to oxygen after 3 hours showed a trend to prolongation of life beyond 10 hours (AAO) from 17.7% to 23.3%, a 31.7% increase but hardly conclusive by itself.

A-O-A versus A-O-O:

Here the primary period was in *air* and the secondary period in *oxygen*. Again, survival beyond 3 hours was closely similar, 59.6% and 61.0% respectively, an improvement in survival to this point of 21.1% compared to the AAA/AAO pairing, attributable to oxygen for the first three hours after vagotomy.

The comparison in the intermediate period for possible oxygen dependency is AOA versus AOO. The series AOA has a much shorter mean for the interval 3-10 hours, 3.9 ± 0.3 hours against 5.4 ± 0.2 hours. Nevertheless, because these are comparatively large groups, the difference in survival is significant, with $t = 4.167$, d.f. = 85, and $p < 0.0001$.

Further improvement in survival after 10 hours post vagotomy occurred in the 21 animals *put back into air* for the remainder of their course, 32.2% against 20.4% for those in AOO. This latter figure is halfway between the 10+ hour survivals for AAA and AAO, and statistically indistinguishable from them (data not shown). The nearly a third survival beyond 10 hours for the AOA cohort is likely due to limits in programming of the metabolic and reactive states of the experimental challenge, and the 59.8% improvement is the second largest in Table 2.

The late survival comparison shows late foreshortening of survival by the oxygen. The 10+ hour interval, AOA has a mean survival of 36.8 ± 5.0 hours versus 25.9 ± 3.8 hours for AOO. The difference of 10.9 hours between small groups is of borderline significance, $t = 1.737$, d.f. = 25, and $p = 0.1$.

O-O-A and O-O-O:

Oxygen for 24 hours prior to and for 3 hours after vagotomy had a consistent positive effect on survival, 85.7% and 83.3% respectively. Their average, 84.5%, is substantially above that of either of the two cohorts just described, a 68.9% rise. This is the group or class cohort experience which looks different when the inner profiles of phases A, B, and C are examined (Figures 1 and 2), in the context of the data presented in Table 3.

Only one of the four pairings showed a disparity for the 3 hour point, OAA and OAO, at 35.5% and 25.6% respectively. Interestingly, their overt graphical behavior beyond 3 hours post vagotomy is almost identical but when this is reassessed by internal time range data, major differences are found (Table 4). The χ^2 value from the referent 2x2 table = 28.937; at one degree of freedom, $p < 0.000001$, very highly significant. This seems conclusive enough: continuous oxygen prior to vagotomy and

for three hours thereafter has a strong survival effect on newborn rabbits with severe ventilatory distress. Not entirely by contrast, however, failure to continue oxygen treatment by returning 30 newborn rabbits to *air*, had a devastating effect. Only four of the 30 survived past 10 hours in *air* (13.3%) while nine kept in oxygen did so (30.0%) This difference is also clearly shown in Figure 2. The two-fold difference in mean survival after 10 hours can be interpreted in two ways: [1] the final oxygen exposure after three hours for OAO was a factor in sustaining phase B despite the earlier onset, subordinating the three hours of exposure to air only as a minor factor, or [2] the relative lack of oxygen in OAA after three hours in the secondary exposure promoted a collapse of the metabolic and reactive forces which otherwise would have sustained the animals beyond 10 hours after vagotomy. In fact, as will be demonstrated below, due to their different slopes, the aggregate exposure time in phase C (area under the curve [AUC] - Table 5) is not as strikingly different.

Other comparisons:

Secondary oxygen effects in isolation are indicated by the comparison of survival after 3 hours post vagotomy between AO-, AA-, OO- and AO-. Again, prevagotomy oxygen has a strong positive effect on survival. Positive secondary effects occur between AA- and AO- and between OA- and OO-. Negative secondary effects occur when OA- is compared to OO- from the reverse perspective. All four pairings in Table 6 are significant.

When AA- is compared to AO-, pure oxygen for 3 hours immediately after vagotomy reduces mortality in that interval from 50.5% (94/186) to 39.4% (74/188). Although a small difference numerically, it is significant, $\chi^2 = 4.7$ at 1 degree of freedom, $p = 0.04$.

By contrast, in the series OOO we observe that 30 out of 36 went past 3 hours, and in the series OOA, 30/35. When combined, the proportion is 60/71 or 84.5%. Thus, first order time analysis shows significant life prolongation postvagotomy by the combination of primary and secondary oxygen exposure. The direct statistical comparison in a 2 x 2 table results in $\chi^2 = 26.05$, $p << 0.0001$. The observed median survival in the series OOO was 6.43 hours, more than double that for AAA.

Figure 2 also shows clearly that after a total experimental exposure to oxygen (OOO), phase C is one of rapid collapse, a point reinforced in Table 3 by a log slope of -0.190, more than twice any other entry.

Intercept points:

The values of P_1 and P_2 are of interest but not readily compared because of further changes in environment after three hours. What is most striking is the comparison of P_1 and P_2 between AAA and AOO, i.e., oxygen continuously after vagotomy. The values for P_1 are 10% survival, 9 hours and 10% survival, 11 hours respectively. This supports the conclusion of moderate enhancement of survival for postvagotomy oxygen. The values for P_2 are 2.1% survival, 67 hours and 1.3% survival, 43 hours respectively, indicating that severe lesions ultimately reduce *late* survival.

Analysis of P_1 and P_2 for OAA versus OAO demonstrates further distinctions. Here the return to

oxygen after 3 hours in air brings on phase B much sooner, at 7 hours with 30% surviving. The slope of phase B for both was -0.018, closer to that of AAA (-0.012) and OOO (-0.011) than the other cohorts. Point P_2 occurs much sooner, at 57 hours and 6.2% survival, than for either pure sequence. The comparison to series OAA is especially instructive. P_1 for OAA is 13 hours, 11.5% surviving, and P_2 is an early 42 hours, 3.5% surviving. Accordingly, remaining in air after prevagotomy oxygen leads to very poor maximal survival times. This supports the conclusion of a dependency induced by oxygen during the first 24 hours after birth.

Additive effects:

The possibility of additive effects of pre- and post-vagotomy oxygen are shown first by the pairing of OO- and AO-. Mortality for the first three hours after vagotomy in the former group is reduced further from 74/188 (39.4%) to 11/71 (15.5%). This is highly significant, $\chi^2 = 13.27$, d.f. = 1, $p << 0.001$. There is clearly an additive effect for the first three hours after vagotomy over this 27 hour period for the first three hours after vagotomy by the use of 100% oxygen.

This is offset by the comparison of OA- and OO-, a test of lingering effect of prevagotomy oxygen. Reversing the emphasis shows that oxygen in the secondary period, 0-3 hours after vagotomy, does add somewhat to the survival potential but it has lesser effects early and late.

Comparative environmental exposure and lung effect from changes in the gas environment:

The operational concept of the *area under the curve* has found considerable value in therapeutics, by which the effective availability of drugs or other substances can be compared (Chang and Wong, 1998; Le Floch, et al., 1990). Here it is applied to the phases of the survival curves drawn from the origin, the intercept points between phases A and B and phases B and C, and the termination (Figure 3).

The contrast between the total air exposure (AAA) and oxygen only after vagotomy (AOO) is dramatic as they have almost identical areas under the experience curve by group. The benefit over time of prevagotomy oxygen is also clearly shown between AOO and OOO and not necessarily by added time of exposure. The protective effect of secondary air in OAO reduced the mean lesion but extended total exposure time beyond OOO by 36%. The reversal pair cohorts, AAO and OOA, ended with the same area under the curve and the same mean animal lung lesion, despite a large difference in the duration of phase C, 5.0 and 32.0 hours respectively. This seems counterintuitive with the most severe lesions usually appearing during phase C (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967), but is best understood as a marker for complexity of the reactions in tissues in response to changing gas environments. Another group with a similar mean lung lesion per animal, AOA, had the lowest exposure experience by area under the curve (452.95 QEU) in the entire system.

Comparative lung injury from changes in gas breathed:Extent of early lesions:

The mean time frame extent of lung injury is shown in Table 7 with the mean survival in hours for each of the three time segments. Exposure to air at one atmosphere absolute resulted in a fairly low order of pulmonary lesion averaging about 8% of total pleural surface. The extent of change is smaller in the first three hours ($3.8 \pm 1.0\%$) after vagotomy, and rises measurably after 10 hours ($44.8 \pm 7.7\%$). Complete or 100% pulmonary lesions were never seen in previous total air experiments (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967; Shanklin, 1969; Shanklin and Lester, 1972), and none occurred in this study. The highest value achieved was 80% during phase C in the tertiary time frame. By contrast, pure oxygen at one atmosphere absolute after vagotomy rather consistently results in near total or total lesions, especially after a few hours (Shanklin, 1969). In this set of experiments the mean lung injury in the first three hours was $68.50 \pm 17.33\%$ in the OOO series and $96.20 \pm 3.80\%$ in the OOA cohort (OO-). These observations are highlighted by the observation that seven out of the first 20 animals to succumb after vagotomy from the OOO series had complete or 100% lesions, all of them in the first 1.12 hours after surgery. The average extent of the lesion in these 20 cases was 64.8%.

Larger series for comparisons of the first three hours after vagotomy come from the combinations of AAA and AAO for air and OOO and OOA for oxygen. Direct comparison of this series for AAA shows no difference in severity of lesion between 0-3 hours ($3.8 \pm 1.0\%$) and 3-10 hours ($5.5 \pm 1.8\%$), $t = 0.82$, d.f. = 92, $p \gg 0.1$. Late survivors show an average increase from 4-5% lesions to 45% lesions, at an average survival time of 44 hours. By contrast, substantial pulmonary injury occurred in the OOO group before 3 hours ($68.5 \pm 17.3\%$), with a modest further change for the 3-10 hour sample ($82.3 \pm 6.5\%$). Overall survival is much enhanced and the proportion surviving beyond 10 hours is significant, $\chi^2 = 12.338$, $p < 0.001$. However, the actual mean survival for the post-10 hour group was 32.5 ± 8.84 hours for OOO against 43.9 ± 5.5 hours for AAA. The extent of lung injury did not rise over this further passage of time. The average lesion after continuous oxygen is about 80%. Greatly enhanced lesions thus were present early and maintained throughout. The foreshortening of late survival may be due to severity of the pulmonary lesions but oxygen related metabolic phenomena cannot be ruled out.

The severity of pulmonary injury reflected in the first three hours after vagotomy is low in most instances when air, the prevagotomy gas, is followed by air for three hours. The 0-3 hour lesion following air only (AA-) is $3.8 \pm 1.0\%$ of the lung with a mean survival (for animals dying in the interval) of 1.94 ± 0.08 hours. The 0-3 hour lesion following oxygen before vagotomy and air afterward (OA-) is higher, averaging $11.4 \pm 1.9\%$ of the lung at a mean survival of 2.31 ± 0.21 hours. The difference in lesion formation is statistically significant, $t = 3.54$, d.f. = 74, $p = 0.001$. The difference in survival is not significant, making the comparison of the extent of injury all the more important.

This consideration leads to an important preliminary conclusion: oxygen for 24 hours prior to ventilatory distress induced by vagotomy enhances very early lung

Extent of intermediate lesions:

The intermediate lesions (3-10 hours) between AAA and AAO are also much more severe, indicating the impact of oxygen in the earlier part of the tertiary period of ventilatory distress. As noted, the mean survivals for this period are essentially the same, 5.2, 5.1 hours, making the contrast in lesions even more dramatic. The average extent for AAO is 68.0 ± 7.3 per cent; that for AAA is 5.5 ± 1.8 per cent. This difference is highly significant with $t = 8.315$, d.f. = 72, and $p \lll 0.001$. This degree of change is rather reminiscent of the quick effect seen in cohorts OOO and OOA.

Extent of late lesions:

The intermediate lesions (3-10 hours) are less consistent for oxygen, with two of the four having intermediate results (OAO, 44.0 ± 7.6 per cent; AAO, 68.0 ± 7.3 per cent). In all four instances, however, when air is the terminal gas exposure, the intermediate lesions are less than the late effects. As for oxygen, however, two show less change between intermediate and late effects. The series OOA shows 33.0 ± 6.5 per cent intermediate and 46.5 ± 9.9 late lung injury, which is not significant, $t = 1.142$, d.f. = 28, $p \gg 0.1$. Series AOA shows 30.0 ± 6.5 per cent intermediate injury versus 43.3 ± 10.27 per cent late lesion, unequal results but again, not significant, $t = 1.098$, d.f. = 29, $p \gg 0.1$. The trends could support a real difference with larger samples of each. However, the data from these specific runs points to the importance of oxygen exposure immediately after the onset of ventilatory distress.

Similar long term latent effects are seen when OAA is compared to OAO. The intermediate lesions, 3-10 hours, are: 9.25 ± 2.81 per cent for OAA and 44.0 ± 7.6 per cent for OAO. The late lesions are 31.2 ± 7.14 and 90.3 ± 3.9 per cent respectively. The progression for OAA is significant, $t = 2.86$, d.f. <29, and $p = 0.01$. The change for OAO occurs at a very different position on the injury scale and is very significant, $t = 5.837$, d.f. = 30, $p \lll 0.001$. Of especial interest are the observations: (a) that the sequence OAO has the lowest extent of lesion during the intermediate interval 3-10 hours of the four groups which eventually produce severe lesions beyond 10 hours, and (b) the mean late lesion is by far the worst result of the same group of four. Thus the lung change in this run is the most severe and the most dramatic. Latent oxygen effects are important and the three hour break appears to be insufficient to repair prior damage or to prevent progression of the lesion to a greater extent.

A dramatic shift is seen in these results when AOO is compared to AOA. The return to air after 3 hours much ameliorates the extent of lesion. The intermediate lesions are: AOO, 83.8 ± 3.0 per cent; AOA, 30.0 ± 6.5 per cent. This difference of 53.0 per cent has $t = 7.377$, d.f. = 85, $p \lll 0.001$. The late lesions are still very different. Those of AOO retain the high severity of 85.9 ± 6.0 per cent. Those of AOA approximate the range of all other animals in air as the final gas exposure, 43.3 ± 10.2 per cent. This comparison has $t = 3.601$, d.f. = 25, $p < 0.001$, also significant despite the smaller sample. These observations confirm the additive or cumulative effects of oxygen during the period of ventilatory distress. Moreover, they emphasize that returning to air has to last longer than three hours or, perhaps, the timing with respect to progression of the lesion has to be different for best effect.

Late oxygen only (AAO) has significant effects on lung injury, eventuating in severe late lesions (10+ hours) with an extent comparable to the most severe seen in any of these experiments, 78.6 ± 8.9 per cent of lung surface at a mean survival of 29.3 ± 6.7 hours. Even the much longer survival of AAA (43.9 ± 5.5 hours) does not produce an equally severe lesion (44.8 ± 7.7 per cent), a difference which is significant, $t = 2,878$, d.f. = 18, $p \approx 0.01$ (one-tailed test).

Finally, the comparison of OOO versus OOA confirms this trend. The intermediate lesions for the interval 3-10 hours are: OOO, 82.3 ± 6.52 per cent and OOA, 33.0 ± 6.0 per cent. The late lesions for 10+ hours are 75.2 ± 6.97 and 46.5 ± 9.9 per cent respectively. The difference for OOO, 7.1 per cent, is not significant, $t = 0.745$, d.f. = 28, $p < 0.1$. The difference for OOA is larger at a lower position on the scale but is similarly not significant, $t = 1.167$, d.f. = 28, $p > 0.1$.

Working backward, the terminal gas exposure bears a consistent relationship to the magnitude of late (10+ hours) lesions. In pure oxygen 3 hours or more after vagotomy the resulting injury clusters around 80% of lung surface with many 100% lesions. In air 3 hours or more after vagotomy the resulting injury clusters around 40% of lung surface, with somewhat greater variability which relates to earlier events. Thus the late oxygen effect on pre-existing ventilatory distress is especially injurious to the lung.

The oxygen content of fetal lung:

The six measurements were almost identical: 10, 11, 11, 12, 12, 12 Torr. The sample does not lend itself to statistical analysis (arithmetic mean = 11.33 Torr) but served as a bench mark for interpretation.

The effect of birth weight:

There was considerable variation in birth weight which could serve as a marker for nutritional and physiological reserves against the oxygen effects. Table 8 provides outline birth weight information for cohorts AAA and AOO from the extended database.

An XY plot of survival against birth weight for cohort AAA (not shown) has the expected dense cluster in the first three hours and a wide scatter otherwise; the regression slope was +0.237206. A similar plot of gross lung lesion against birth weight yielded a regression slope of +0.269536 when all animals were included and +0.288796 when those with zero lesions were excluded. The same treatment of the AOO cohort (not shown) yielded regression slopes of +0.0764254 for survival, +0.0815197 for gross lung lesion, and -0.0758406 for gross lung change with zero lesions (5) excluded. The main conclusion here is the considerable effect oxygen after vagotomy has on the system, diminishing the modest influence of birth weight on the outcome. The negative slope for those animals under AOO showing overt pathological changes is of interest. When animals with only 60-100% lesions are considered, a group of 137, the regression slope for lung lesion formation was -0.0954235, a small accentuation of the trend.

Finally, similar plots for OOO, continuous oxygen (data not shown), add to the conundrum. The regression slope for survival against birth weight was +0.395412, the highest determined. This also

enhances the role of oxygen in furthering survival at the cost of disease progression. By contrast, the regression slope for lung lesions by birth weight in OOO was a strong -0.774967 . It is beyond the matrix of data from this study to fully interpret this particular finding but one suggestion is, under the duress of continuous oxygen, greater size does facilitate the defensive resources against oxygen toxicity.

Discussion

No prior study of the effect of pre-vagotomy oxygen has been undertaken. A brief summary of the results of these experiments would be: continuous oxygen before and after vagotomy both prolongs life and enhances the lung lesion at certain times and circumstances. At others, survival is reduced and the very last animals to succumb do not always show the most severe lesions. This, at an experimental level, is the dilemma of the neonatal therapist. Although these experiments were not designed to adduce evidence for the *particularities* of the tissue process of oxygen effects, there were findings relevant to an understanding of oxygen in the pulmonary environment.

The early onset of severe lung injury in the OOO series is further evidence for the dissociation between mortality and disease in the vagotomy model (Shanklin, 1969). In the light of previous evidence for significantly worse lesions after 10 hours in pure air experiments (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967), observations confirmed here, the change in the severity of lesions early in the pure oxygen runs points to the inherent toxicity of oxygen toward the lung (Shanklin, 1969; Shanklin and Lester, 1972). The large numbers of late survivors with severe lesions is taken to mean that oxygen enrichment drives the organism metabolically, sufficient to maintain life despite severe respiratory distress (Allen and Rasmussen, 1971), at least for certain intervals of time.

Three hours was chosen as a critical departure point because it was the median survival for animals after vagotomy continuously in air. This was confirmed from a number of other studies. When the AAA cohort was expanded later to 200, the result was 98 surviving past 3.0 hours (49.0%). The series AAO also fulfills the conditions of air up through 3 hours post-vagotomy, 30 surviving out of 59, or 50.9%. If these are combined for statistical purposes, the result is 128/259, or 49.5%. The consistency validates the assignment and the experimental template (Table 1).

The exact pathophysiological meaning of the dramatic change in slope of phase B of the mortality curve (Figures 1 and 2) is unclear, but evidence of equally profound differences when estrogen treated males and females are considered separately (Shanklin and Wolfson, 1970), supports the hypothesis of a marshaling of various remnant defensive resources in that portion of each experimental group.

As Figure 1 suggests, the most singular aspect of this comparison is that phase B has essentially the same slope in both groups, AAA and AAO. This parallelity is seen in several other comparisons noted below. Without indicating just what is occurring, the pattern is suggestive of a specific and likely important adaptation.

There are many reports on a variety of subjects which bear on the findings and interpretations from

these experiments. Arguably the most singular is that of Urschitz et al (2004). Concerned with the variations in manual therapeutic oxygen delivery to premature infants, a factor generally considered to be the cause of retinopathy of prematurity (also known as retrolental fibroplasia), they have devised an algorithm and software to automate the oxygen content inspired by infants or delivered by ventilation devices. The control is feedback from pulse oximetry which they recommend setting at 87-96% SpO₂.

This concern is an important one but they make no mention of possible effects on the lung of providing more oxygen in later stages of respiratory distress than many lungs can tolerate. Moreover, this broader issue is not new. The connection between retrolental fibroplasia and hyaline membrane disease was first made over 50 years ago (Ingalls, 1954). The concept applied by Ingalls was this: “*On biologic principle alone one doubts whether an agent with such a severe morbidity has no mortality* (p. 1020).”

The role of oxygen in retinopathy of prematurity is well known (Urschitz et al 2004). Less appreciated is the role of oxygen as an etiologic agent in hyaline membrane disease (Shanklin and Wolfson, 1967). The primary toxicity of oxygen toward the neonatal lung has been emphasized from several clinical and research perspectives. Claireaux (1975) summarized much of the earlier work. Other pertinent reports are those of Ambrus, et al (1968), Northway et al (1969), Frank et al (1977), Frank et al (1978), Mortola (1983), Fisher et al (1991), Langley and Kelly (1994), Kelly and Lubec (1995), Phillips et al (1995), Han, et al (1996), Schock et al (2001), Collard et al (2004), and Higgins et al (2007). One paper stands out from the earlier work on these issues by the authors’ clear conclusion that the oxygen was more of a problem than the ventilatory measures used (Nash et al, 1967).

From one perspective, it seems, given that oxygen is a vital substance for living organisms, and especially for humans, the use of descriptive language other than *etiology*, *cause*, or *causative agent*, for oxygen, has the tendency to keep the issues slightly out of focus. The term oxygen toxicity is in common use (Turrens et al, 1984; Nishiki et al, 1976) but so also are alternative phrases such as *hyperoxic injury* (Kelly and Lubec, 1995), *oxygen-induced lung injury* (Ambrus et al, 1968; Phillips et al, 1995), *oxidative damage* (Collard et al, 2004), and *oxidative stress* (Schock et al, 2001), inter alia. The important paper by Claireaux (1975) used the neutral phrase, the *effect of oxygen* on the lung, in the context of what he termed the exudative phase of oxygen toxicity.

Among the consistent general findings at autopsy in cases of human hyaline membrane disease prior to the period of increasing intensive care (Shanklin, 1971) was the profound vascular congestion, with occasional focal hemorrhage as a counterpoint (Shanklin and Wolfson, 1967). The same is true in this neonatal animal model of hyaline membrane disease. As was pointed out by Fisher et al (1991) and others: “*The normal lung has a very large blood flow in relation to its tissue mass, far in excess of that required for its metabolic needs* [p. 676].”

Whether this is to be termed *congestion* or *hyperemia* depends on one’s point of view. Pathologists often see such vascular findings as congestion whereas the dynamics of organ blood flow are perhaps better considered as hyperemia. The seminal paper of Rudolph and Heymann (1967) on the fetal circulation stated the fetal lung receives 3.9% of the cardiac output at a flow rate of ≤ 0.5

ml/g/min. There is certainly a mixture in pulmonary arterial flow from vena caval return and reflux from the ductus arteriosus bearing the placental return shunted through the foramen ovale, in fetal life. As such, the oxygen tension of the umbilical arterial flow may be expected to be higher than in the lung. This has been established by measurement in utero as 15 Torr (McGrath et al, 1986). Direct measurement of the pO₂ of fetal lung tissue at 10-12 Torr is consistent with these considerations. The neonatal lung has proportionately less air dead space than the adult or older child and the expanded terminal airspaces will reflect values in the range of 100-120 Torr, an order of magnitude change after birth.

Postnatally, the rise in peripheral arterial resistance prompts considerable retrograde flow through the ductus arteriosus, a factor which aids in lung expansion through an erectile function. Not often noted, however, is this flooding of the lung by blood also brings to it those defensive factors for which the blood is the principal repository. The role of intense infusion of blood from the general circulation in disease is well illustrated by the classical term for early lobar pneumonia: *red hepatization*. Indeed, gross photographs of neonatal human lung from the preintensive care era, and of newborn rabbit and guinea pig lungs from experimental models, are the image of lungs looking like nearby liver. The association of this process with the blood borne antioxidant enzyme catalase has been known for over a century (Winternitz and Meloy, 1908).

Much more recently there has been much interest in the composition of the epithelial lining fluid (ELF) of the lung, that part of the structure which is in direct contact with the inhalant mix, above and beyond the physiology and pathophysiology of surfactants (Collard et al, 2004; Cross et al, 1994; Nishiki et al, 1976; Schock et al, 2001). Catalase is one ingredient of epithelial lining fluid but as the probable total volume of this thin layer in the adult lung is less than 10 ml spread across a surface area in excess of 880,000 cm² (Cross et al, 1994), and appears in the ELF only late in development (Kaarteenaho-Wiik and Kinnula, 2004), this is not much of a defense for a premature newborn experiencing a sudden order of magnitude increase in pulmonary oxygen content, from 10-12 up to 110-120 Torr. There are no reported measurements of the volume of the ELF in newborns, mature or premature, but from the topography and comparative size of the lungs referent the adult, a volume in excess of 1 ml would be remarkable.

Much of the injury to the lung from oxygen appears to be through lipid peroxidation (Collard et al, 2004; Niishiki et al, 1976; Turrens et al, 1984). Malondialdehyde has been cited as a specific marker for lipid peroxidation, rising from a control level of $0.345 \pm 0.09 \mu\text{M}$ to a mean of $1.3 \pm 0.31 \mu\text{M}$ and a peak of $2.5 \mu\text{M}$ on the fifth day (Collard et al, 2004). A comparison study on the release of hydrogen peroxide (H₂O₂), a principal product of activated oxygen species, against lipid peroxidation under hyperbaric oxygenation, concluded the rate of lipid peroxidation was the primary response, rather than the rate of formation of H₂O₂ (Niishiki et al, 1976).

The significance of these investigations was extended by the report of Turrens, et al(1984). Catalase and superoxide dismutase were encapsulated in artificial liposomes injected into rats exposed to 100% oxygen. When injected prior to oxygen challenge this treatment increased the time to the midpoint of the survival curve [LT50] by 62.5% and the maximum survival time was doubled. The lungs became heavier but pleural effusion was greatly diminished. Their results suggested that superoxide dismutase (SOD) and catalase worked in unison, shunting off superoxide anion and

H₂O₂ respectively. The increase in fluid was not further noted, i.e., the relative amounts of blood or edema. There are no studies of time compartment changes in the peroxidation of pulmonary lipids but Turrens, et al (1984) provided some evidence on the rapidity and duration of effects of additive liposomes. The half life of directly injected catalase and SOD differed, at 23 and 6 minutes respectively; when encapsulated in liposomes, the half lives became 2.4 (catalase) and 4.0 hours (SOD). Although not plotted in a semilog fashion the survival curve for the joint liposome treatment was suggestive of a triphasic response. As postulated by Winternitz and Meloy in 1908, the direct correlation between the tissue engorgement by erythrocytes and the level of the antioxidant enzyme catalase is a signal statement on the role of the latter toward the cause or causes of the former.

Further correlation here to antioxidant factors now known to be active in pulmonary oxygen toxicity is unwarranted since these experiments were not revealing thereon. These points have been made: (1) the lung is injured by excess oxygen, very possibly by the dramatic rise attendant on simply breathing air upon birth, (2) the injury and survival potential arising from it can be modified by changes in the oxygen percentage of the inhaled gas and by various sequences between air and pure oxygen during exposure, and (3) there is a continuing effect for survival and enhancement of the lung lesion. Continuous prevagotomy oxygen does influence the immediate postvagotomy oxygen effect. Survival beyond the 3 hour point is enhanced whereas, for those fewer animals dying during that brief period, the lung lesion is also increased. This neatly summarizes the paradox of the oxygen effect: enhancement of average survival by the shifting of many deaths to the post-3 hour group, with a worsening of the injury and under some circumstances, a foreshortened maximum survival. How the latter is derived from the former bears further study.

Summary

Newborn rabbits with ventilatory distress produced by bilateral cervical vagotomy at age 24 hours, without ventilatory support, had variable results which indicate several effects of oxygen on the system. Three principal effects were observed: [a] changes in survival, [b] changes in the extent of the lung injury produced, and [c] a state of oxygen dependency beyond the usual physiological need, after use of oxygen itself. The clinical dilemma of intermixture of therapeutic oxygen and ventilatory support, by whatever means, is thus clarified in the sense that oxygen has to be considered a major factor in how the lung responds to the extrauterine environment, that is, the problems are not exclusively those from the mechanics and modalities of physical resuscitation and ventilatory support. The actions of oxygen under the condition of absence of ventilatory support are complex. This is not unreasonable given the numerous ways in which oxygen participates in tissue metabolism and cell maintenance and in light of the difficulties in studying how the lung actually transports oxygen into the neonatal body.

Oxygen dependency may be due to (1) alterations in pulmonary and systemic intermediary metabolism, including aspects of the cytochrome chain, (2) disturbance or depletion of the pulmonary antioxidant reserve, (3) local effects of structural change which reduce the efficiency of oxygen transfer, or (4) some combination of these, or other factors not currently under active consideration.

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Table 1: Experimental template for sequence shifting of gas exposures.

Birth to 24 hours after birth	Vagotomy to 3 hours post vagotomy	Continuously from 3 hours post vagotomy	Sequence code designations
Air	Air	Air	A-A-A
Air	Air	Oxygen	A-A-O
Air	Oxygen	Air	A-O-A
Air	Oxygen	Oxygen	A-O-O
Oxygen	Air	Air	O-A-A
Oxygen	Air	Oxygen	O-A-O
Oxygen	Oxygen	Air	O-O-A
Oxygen	Oxygen	Oxygen	O-O-O

Table 2: Survival following different sequences of exposure to air or oxygen.

Experimental groups (Primary)			Post vagotomy (Secondary)		Post 3 hours postvagotomy (Tertiary)			
Code	Sample	Gas	Gas	Died 0-3 hr	Gas	Survived 3+ hr	Died* 3-10 hr	Died* 10+ hr
A-A-A	127	Air	Air	65 (51.2%)	Air	62 (48.8%)	51 (82.3%)	11 (17.7%)
A-A-O	59	Air	Air	29 (49.2%)	Oxygen	30 (50.8%)	23 (76.7%)	9 (23.3%)
A-O-A	52	Air	Oxygen	21 (40.4%)	Air	31 (59.6%)	21 (67.8%)	10 (32.2%)
A-O-O	136	Air	Oxygen	53 (39.0%)	Oxygen	83 (61.0%)	66 (79.6%)	17 (20.4%)
O-A-A	48	Oxygen	Air	17 (35.5%)	Air	31 (64.5%)	20 (64.6%)	11 (35.4%)
O-A-O	43	Oxygen	Air	11 (25.6%)	Oxygen	32 (74.4%)	21 (65.7%)	11 (34.3%)
O-O-A	35	Oxygen	Oxygen	5 (14.3%)	Air	30 (85.7%)	26 (86.7%)	4 (13.3%)
O-O-O	36	Oxygen	Oxygen	6 (16.7%)	Oxygen	30 (83.3%)	21 (70.0%)	9 (30.0%)

* Died during the interval 3-10 hours and 10+ hours post vagotomy are percentages of those surviving beyond 3 hours post vagotomy.

Table 3. Intercept points, duration of phase, and slopes of triphasic (semilog) survival plots.

Experimental groups	Intercept points (Per cent at hour)			Slope by phase (log scale)		
	P ₁	P ₂	End	A	B	C
A-A-A	10.0/9.0	2.1/67	0.0/72.5	-0.111	-0.012	-0.059
<i>Duration (hr)</i>	9.0	58.0	5.5			
A-A-O	6.0/15.0	2.7/46.0	0.0/51.0	-0.081	-0.011	-0.086
<i>Duration (hr)</i>	15.0	31.0	5.0			
A-O-A	16.5/7.2	10.0/35.0	0.0/66.0	-0.109	-0.008	-0.007
<i>Duration (hr)</i>	7.2	27.8	31.0			
A-O-O	10.0/11.0	1.3/43.0	0.0/59.8	-0.091	-0.028	-0.007
<i>Duration (hr)</i>	11.0	32.0	16.8			
O-A-A	11.5/13.0	3.5/42.0	0.0/49.5	-0.072	-0.018	-0.073
<i>Duration (hr)</i>	13.0	29.0	7.5			
O-A-O	30.0/7.0	6.2/57.0	0.0/77.5	-0.075	-0.018	-0.073
<i>Duration (hr)</i>	7.0	50.0	20.5			
O-O-A	15.0/7.0	2.8/28.0	0.0/60.0	-0.118	-0.035	-0.014
<i>Duration (hr)</i>	7.0	21.0	32.0			
O-O-O	12.5/13.0	2.4/77.0	0.0/79.0	-0.069	-0.011	-0.190
<i>Duration (hr)</i>	13.0	64.0	2.0			

Table 4: Internal time range survivals post vagotomy, O-A-A and O-A-O cohorts.

Experimental group	Mean survival 3-10 hr	Mean survival 10+ hr
O-A-A	5.16 ± 0.40 hr	23.62 ± 4.53 hr
O-A-O	5.14 ± 0.36 hr	47.00 ± 7.42 hr

Survival as mean ± s.e.m.

Table 5. Mean gross lung change per animal as per cent pleural surface lesion and total area under the curve of experimental exposure as per cent survival-hours. The s.e.m. of the gross lung change are omitted as the table and Figure 3 are intended to show their relative position and not significance of lung change by group.

Experimental group	Total area under the curve	Mean gross lung change per animal
A-A-A	639.77	8.034
A-A-O	762.90	41.831
A-O-A	452.95	42.815
A-O-O	645.12	79.861
O-A-A	717.50	14.208
O-A-O	1128.55	36.300
O-O-A	767.90	43.571
O-O-O	830.40	78.225

Table 6: Short term secondary survival effects up to 3 hours post vagotomy.

Experimental group	Survival beyond 3 hours post vagotomy (%)	Paired experimental groups	Comparative survival (χ^2 ,p)
AA-	92/186 (49.46%)	AA- and AO-	4.723, p<0.05
AO-	114/188 (60.64%)	AO- and OO-	13.336, p<0.001
OA-	63/91 (69.23%)	OA- and OO-	5.105, p<0.05
OO-	60/71 (84.51%)	AA- and OA-	9.524, p<0.01

Table 7. Mean lung injury by primary, secondary, and tertiary time frames as percentage of lung lesion with the mean group survivals in hours (*italicized*).

Experimental group	Lung injury 0-3 hr (%)	Lung injury 3-10 hr (%)	Lung injury 10+ hr (%)
AAA <i>Mean survivals</i>	3.8 ± 1.0 <i>1.94 ± 0.08</i>	5.50 ± 1.8 <i>5.10 ± 0.23</i>	44.8 ± 7.7 <i>43.90 ± 5.50</i>
AAO <i>Mean survivals</i>	12.2 ± 3.5 <i>1.92 ± 0.15</i>	68.0 ± 7.3 <i>5.20 ± 0.30</i>	78.6 ± 8.9 <i>29.30 ± 6.70</i>
AOA <i>Mean survivals</i>	55.4 ± 8.4 <i>2.10 ± 0.19</i>	30.0 ± 6.5 <i>3.90 ± 0.30</i>	43.3 ± 10.2 <i>36.80 ± 5.40</i>
AOO <i>Mean survivals</i>	67.3 ± 4.9 <i>1.69 ± 0.10</i>	83.8 ± 3.0 <i>5.44 ± 0.21</i>	85.9 ± 6.0 <i>25.9 ± 3.80</i>
OAA <i>Mean survivals</i>	9.1 ± 2.1 <i>1.91 ± 0.25</i>	9.3 ± 2.8 <i>5.15 ± 0.66</i>	31.2 ± 7.1 <i>23.63 ± 4.32</i>
OAO <i>Mean survivals</i>	11.4 ± 1.9 <i>2.31 ± 0.21</i>	44.0 ± 7.6 <i>5.14 ± 0.40</i>	90.3 ± 3.9 <i>38.40 ± 7.00</i>
OOA <i>Mean survivals</i>	96.2 ± 3.8 <i>2.38 ± 0.24</i>	33.0 ± 6.0 <i>4.85 ± 0.29</i>	46.5 ± 9.9 <i>29.60 ± 10.60</i>
OOO <i>Mean survivals</i>	68.5 ± 17.3 <i>2.00 ± 0.34</i>	82.3 ± 6.5 <i>6.08 ± 0.96</i>	75.2 ± 7.0 <i>32.55 ± 8.24</i>

Lung injury values have been rounded to one decimal place.

Table 8. Birth weight ranges and means from the expanded study.

Experimental group	Birth weight range	Mean birth weight (± s.e.m.)
AAA (N = 200)	18.8 - 68.3 grams	42.54 ± 0.94 grams
AOO (N = 195)	19.6 - 67.7 grams	43.95 ± 0.69 grams

Legend: Figure 1:

Survival profiles for animals in air for 24 hours prior to vagotomy. All four groups closely track down to the first intercept point between phase A and phase B. The shortest is group A-O-A ($\blacktriangle-\blacktriangle$) in which phase A includes all three hours in secondary exposure to oxygen and then 4.5 hours after returning to air. The net effect of this not only shortens phase B compared to the others but this then results in the longest phase C of all four primary air only cohorts. The starting point for phase C of the A-O-A cohort is with three to four times the proportion of survivors at this time. AOO ($\triangle-\triangle$) displays a different triphasic profile which lacks the sharp decline into the third and final phase. A-A-A as ($\square-\square$) and AAO as ($\bullet-\bullet$).

Legend: Figure 2:

Survival profiles for animals in oxygen for 24 hours prior to vagotomy. The four groups track down to the first intercept point but not as precisely as for the air prevagotomy set. Interestingly, the two that track the closest and which reach the intercept point at an identical time, are OAA ($\bullet-\bullet$) and OOO ($\square-\square$). Group OAO ($\triangle-\triangle$) follows them precisely for roughly half the time but then diverges sharply into phase B. The final group, OOA ($\blacktriangle-\blacktriangle$), displays a different triphasic profile which lacks the sharp decline into the third and final phase. The similarity between this and AOO in Figure 1 is of interest respecting long term effects on the total body economy and survival in this model of neonatal ventilatory distress.

Legend: Figure 3:

Bar graph showing relative position of each group by total area under the curve (*quantitative exposure units*) and comparative gross lung change per animal as per cent of pleural surface lesion. The complexity of the reactions which produce the lesion is illustrated by the three middle range degree of lung injury (40-45%) in the AOA, AAO, and OOA cohorts, with two very close values for QEU (AAO and OOA) and the third at circa 60% of their QEU (AOA).





