THE EFFECTS OF VARIOUS OXYGEN CONCENTRATIONS IN THE ANAESTHETIC VEHICLE ON INDUCTION TIMES AND SURVIVAL TIMES WITH HALOTHANE ANAESTHESIA

BY

R. E. RAWSTRON AND C. J. EVANS

SUMMARY

Studies were made to test the effects in mice exposed to halothane of various percentages of oxygen in the anaesthetic vehicle on induction and survival times. Over all, increasing oxygen concentrations in the vehicle were associated with longer induction times with 4 per cent and 10 per cent halothane and with longer survival times with 10 per cent halothane. It is not possible from the results to define accurately the respective roles which haemoglobin, oxygen saturation, and the carriage of oxygen in solution in the plasma, play in prolonging induction and survival times.

Previous work showed that using oxygen instead of air as the vehicle with 10 per cent halothane increased the induction times and survival times of mice, or their tolerance to halothane (Rawstron 1968, 1969). Other work, designed to test the effects of oxygen on the survival of dogs shocked by exsanguination and anaesthetized with ether or halothane (Freeman, 1962) showed that survival time was increased significantly but that 30 per cent oxygen was as efficient as 100 per cent.

Three studies are presented in this paper. Two of these tested the effects of various levels of oxygen between 21 and 100 per cent in the vehicle with 10 per cent halothane on the induction times and total anaesthetic times until death occurred (survival times) of female albino mice. The third study, also on female albino mice, tested the effects on induction times with 4 per cent halothane when the oxygen level in the vehicle ranged from 10 to 100 per cent.

MATERIALS AND METHODS

Mice.

All the mice used in these studies were albinos of partially inbred stock (Glaxo Laboratories, Palmerston North) approximately 9–10 months old and each having had four litters.

In study 1, the mice were unselected before testing. In study 2, the mice were weighed initially and mice between 17 g and 40 g were used. Random samples were boxed in groups of five for final testing. In study 3, the mice after matching for weight and haemoglobin concentration into nine groups, were boxed and numbered.

Haemoglobin estimations.

A haemoglobin estimation was made on each mouse, using the method previously described (Rawstron, 1968). In study 1, this estimation was made on blood obtained from the heart at immediate postmortem examination. In studies 2 and 3, tail blood was used.

Oxygen mixtures.

The oxygen mixtures were supplied by NZ Industrial Gases Ltd. Standard air and 100 per cent oxygen gases were used. The non-standard gases were mixtures of nitrogen and oxygen. During use the gas cylinders were stored at room temperatures. Analysis for actual oxygen percentages were made in study 3. These were 10% = 10.1%, 15% = 14.5%, air = 21%, 35% = 34.3%, 50% = 49.4% and 100% = 99.5%. As similar analysis had not been made in the first two studies, the first figure quoted for each gas will be used in the text.

Anaesthetic methods.

The equipment used was the same as that used in previous studies except for a different Fluotec...
Mark 2 MJ vaporizer (Rawstron, 1968). The mice were anaesthetized singly in a 550 ml jar using 10 per cent or 4 per cent halothane with a 2 l./min flow of the vehicle, by placing the mouse in the jar, setting the vaporizer dial to 10 per cent or 4 per cent and turning on the oxygen mixture. Following each test, the jar was filled with water to displace the anaesthetic mixture, emptied and dried. The halothane in the vaporizer was emptied and replaced with fresh halothane at room temperature after every 10 administrations in study 1, after every 5 administrations in study 2, and every 9 administrations in study 3.

In each study a Latin square of the oxygen concentrations was prepared for selecting the sequence of oxygen concentrations for each day. In study 3, each mouse was anaesthetized once weekly for 6 weeks, using a different concentration at each testing.

All determinations of induction and survival times were made by one author (R.E.R.).

**Determination of induction time.**

Induction time was considered to be complete when there was loss of righting reflex, head-drop and cessation of limb movements.

**Determination of death.**

Death was considered to have occurred when all respiratory movements ceased. This usually occurred after a period of respiratory arrest, changing to gasping breathing of decreasing amplitude, and followed by bladder evacuation.

Data of the number of mice, treatments, mean haemoglobin concentrations and mean body-weights of studies 1 and 2 are shown in table I. Similar data for study 3 are shown in table IV.

**Study 1 (November 1965).**

In the analysis of induction times one animal was omitted as it was found to be pregnant. Two mice were omitted from the analysis of survival times owing to abnormally prolonged survival (Dixon, 1953).

An analysis of variance was made. The mean induction and survival times with their standard errors from this analysis are shown in table II.

This analysis showed that with 10 per cent halothane in oxygen there was a significant increase in induction time ($P = 0.05$) and survival time ($P = 0.01$) when the oxygen content in the vehicle was increased from 21 to 40 per cent. Comparisons of times between 21 per cent oxygen and oxygen concentrations above 40 per cent showed a significant increase in induction time ($P = 0.05$) but a non-significant effect with survival. Comparisons of induction times and survival times with 40 per cent oxygen and with 60, 80 and 100 per cent oxygen were non-significant.

Tests for haemoglobin as a co-variant were made. While these tests did alter the mean induction and survival times a little, the differences in the adjusted means were non-significant.

<table>
<thead>
<tr>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers</td>
<td>Oxygen (%)</td>
</tr>
<tr>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>11</td>
<td>60</td>
</tr>
<tr>
<td>12</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>
TABLE II

The numbers of mice with their mean induction and survival times and standard errors from the analysis of variance at each oxygen concentration.

<table>
<thead>
<tr>
<th>Study 1; halothane 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers of mice</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>10</td>
</tr>
</tbody>
</table>

The coefficients of variation (per mouse) for induction time was 9.9 per cent and survival time was 25.6 per cent.

Study 2 (March 28 to April 15, 1969).

An analysis of variance was made on the results in this study. No animals were omitted from this analysis. Some variations in survival times were due to two complete tests done on March 31 (40 mice) which gave much longer survival times at all concentrations. None of these mice were eliminated from the analysis of data. The mean induction and survival times with their standard errors are shown in table III and figure 1.

This analysis showed that there was a significant increase in induction time with 10 per cent halothane in oxygen (P = 0.05) and survival time (P = 0.05) when the oxygen content in the vehicle was increased from 21 to 35 per cent. Comparison of times between 21 per cent oxygen concentration and above 35 per cent showed a significant increase in induction time (P = 0.01) and survival time (P = 0.05). Comparisons of induction times and survival times between 35 per cent oxygen and 50 and 100 per cent oxygen were non-significant.

TABLE III

The numbers of mice with their mean induction and survival times and standard errors from the analysis of variance at each oxygen concentration.

<table>
<thead>
<tr>
<th>Study 2; halothane 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers of mice</td>
</tr>
<tr>
<td>58</td>
</tr>
<tr>
<td>58</td>
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<td>58</td>
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<td>58</td>
</tr>
</tbody>
</table>

The coefficients of variation (per mouse) for induction time was 15.03 per cent and for survival time 22.24 per cent.

Study 3 (October 1969).

In this study induction time with 4 per cent halothane for each mouse was tested with each oxygen concentration. Each mouse was tested once weekly to allow elimination of possible metabolic products from the inhaled halothane (Van Dyke, Chenoweth and Van Poznak, 1964).

Data for the experiments, together with the induction times and standard deviations are shown in table IV.

On theoretical grounds it was considered that there would be a curvilinear regression with the maximum increase in induction time being between 10 and 35 per cent and a corresponding smaller increase between 35 and 100 per cent oxygen. This follows the pattern of the oxygen dissociation curve for blood. The precise nature of the curve was not determined but a test for linearity of regression gave a test quotient of 17.6 which, by exceeding the significant limit F. d.f. 4,261, indicated its non-linear nature. It was possible to fit two significant linear regressions to
EFFECTS OF VARIOUS OXYGEN CONCENTRATIONS

TABLE IV
Data of mice exposed to 4 per cent halothane together with the mean induction times and standard deviations at each oxygen concentration.

<table>
<thead>
<tr>
<th>Study 3</th>
<th>Numbers</th>
<th>Oxygen (%)</th>
<th>Haemoglobin and range (g/100 ml)</th>
<th>Weight and range (g)</th>
<th>Induction time (sec; mean and SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>10</td>
<td>13.4 9.2-15.3</td>
<td>33.8 29-40</td>
<td>24.5 3.8</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>15</td>
<td>13.4 9.2-15.3</td>
<td>33.8 29-40</td>
<td>27.6 3.6</td>
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</tr>
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<td></td>
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<td>45</td>
<td>50</td>
<td>13.4 9.2-15.3</td>
<td>33.8 29-40</td>
<td>33.0 4.2</td>
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<td>33.8 29-40</td>
<td>34.6 4.3</td>
<td></td>
</tr>
</tbody>
</table>
the saturation of the haemoglobin rises from 97 to 100 per cent (19.5 ml oxygen per 100 ml of blood to 20.1 ml) and the oxygen in solution in the plasma increases from 0.30 ml/100 ml of blood to 1.92 ml (Comroe et al., 1962).

In human anaesthesia when respiration is spontaneous, it has been shown that to ensure an arterial oxygen tension of 100 mm Hg, and so ensure normal haemoglobin saturation, inspired oxygen concentrations of 30–35 per cent are needed (Nunn, 1964, 1966). Studies on dogs shocked by exsanguination (Freeman, 1962) have shown that using 30 per cent oxygen in place of 21 per cent in the vehicle with ether or halothane anaesthesia significantly increased survival rates in these dogs but that these survival rates were not further improved by using 100 per cent oxygen. If the findings in humans during anaesthesia are applicable to the experiments on exsanguinated dogs it seems possible that the benefit derived by using higher oxygen concentrations in the vehicle is due to achievement of normal saturation of the haemoglobin in these dogs.

**Induction time.**

Study 3 will be considered first as it gives a more complete picture of events due to the greater range of oxygen concentrations used in the vehicle with 4 per cent halothane. The results showed a positive correlation between induction times and oxygen concentrations with a steady increase between 10 and 21 per cent and a similar but smaller rate between 21 and 100 per cent oxygen. While 4 per cent halothane will lower the oxygen content in the anaesthetic mixture this gives only a small reduction, from 21 to 20.2 per cent.

If it is assumed that near-normal arterial oxygen saturation occurs during induction with 21 per cent oxygen with 4 per cent halothane, then the increases in induction times associated with increases in the oxygen concentrations from 10 to 21 per cent will be due to increase in the haemoglobin saturation to near normal while the increases in induction times with increasing oxygen concentrations from 21 to 100 per cent will be due predominantly to increases in oxygen carried in solution in the plasma. It would appear, therefore, that in this study both components responsible for oxygen saturation of the blood (haemoglobin and oxygen in solution) may significantly affect induction.

It is doubtful if studies 1 and 2 fully support this contention as it is possible that when using air with 10 per cent halothane some oxygen unsaturation of the blood could have occurred. First, 10 per cent halothane will reduce the oxygen content with air from 21 to 19 per cent. Second, as 10 per cent halothane causes more respiratory irritation than 4 per cent halothane, possible respiratory interference may have resulted in some blood oxygen unsaturation. Induction times (tables II and III) show a marked rise when using concentration of oxygen from 21 to 35/40 per cent, and a small overall rise with concentration of 35/40 to 100 per cent. If oxygen unsaturation of the blood did occur during induction with 10 per cent halothane in air, then part or all of the rise in induction time when using 35/40 per cent oxygen instead of 21 per cent oxygen will be due to saturation of the haemoglobin to normal levels, because increases in oxygen content in solution in the plasma above 0.3 ml/100 ml blood will have begun at some concentration higher than 21 per cent oxygen.

As individual comparisons of induction times associated with oxygen concentrations between 35/40 per cent and above were non-significant, this may mean that with 10 per cent halothane (studies 1 and 2) increases in oxygen in solution in the plasma lead to non-significant differences in induction time.

**Survival time.**

As previous studies showed that 6 per cent halothane administered over 30 minutes and 4 per cent halothane administered over 120 minutes did not kill all test mice, it was decided for practical reasons to continue with 10 per cent halothane as the test concentration in these studies on survival times (Rawstron, 1968, 1969). These studies had shown that when using 100 per cent oxygen instead of air with various halothane concentrations there was an increased tolerance of the mice to halothane.

The results of the present studies on survival times show a similar pattern to those on induction times (correlation P = 0.01), there being a marked rise in survival time from when the oxygen concentration was raised from 21 to 35/40 per cent,
and a small overall rise when it was raised from 35/40 to 100 per cent. It is very likely that haemoglobin unsaturation occurred with the use of 10 per cent halothane in air because of the decrease of oxygen concentration to approximately 19 per cent in the mixture and possibly also for reasons mentioned previously (Nunn, 1964, 1966).

The marked increase in survival times when oxygen concentrations of 35/40 per cent were used in the vehicle instead of air could be due to the improvement in the degree of saturation of haemoglobin to near normal levels. Increases in the content of oxygen in solution in the plasma above 0.3 ml oxygen per 100 ml blood will have begun at some oxygen concentration higher than 21 per cent and possibly closer to the 35/40 per cent level. If haemoglobin saturation almost reached normal levels when the mice were exposed to oxygen concentrations approximating 35/40 per cent with 10 per cent halothane, then it seems likely that increases of the content of oxygen in solution in the plasma had little effect in increasing survival times, because analysis showed that the survival times obtained with using oxygen concentrations above 35/40 per cent were not significantly altered when compared with survival times obtained with oxygen levels of 35/40 per cent in the vehicle.

Finally, it would appear that during exposure to 4 per cent halothane, the significant increases in induction time that occurred when the oxygen content of the vehicle was increased from 10 to 100 per cent were due to increased haemoglobin oxygen saturation and to the increase in oxygen in solution in the plasma. It is considered that some haemoglobin unsaturation would be expected when 10 per cent halothane was used in air, so that the significant increases which occurred in induction times and survival times with increase in the oxygen concentrations in the vehicle were probably due to improvements in the degree of haemoglobin saturation to near normal levels, and that further increases in blood oxygen saturation due mainly to increases of oxygen in solution in the plasma played only a small and possibly non-significant part.

It would be necessary to make estimations of arterial oxygen levels with various oxygen concentrations in the vehicle in order to determine the point of normal haemoglobin oxygen saturation, and so to define the relative roles of haemoglobin oxygen saturation and plasma oxygen content in prolonging induction and survival times. This relationship may also vary with varying concentrations of halothane as suggested by the results of the studies of induction times.

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The late A. C. Glenday, M.A., B.Sc., Mrs. W. Singer, B.Sc., and K. G. Couchman, F.I.M.L.T., were responsible for the statistical work.

REFERENCES


Pp. 358; indexed. Price 78s. (£3.90).

Anaesthetists have always been interested in the design of equipment. Indeed up to some thirty years ago the high road to immortality was to design a piece of apparatus which would pass into general use. As monitoring techniques have become important and the possibility of designing new apparatus of permanent usefulness less, the interest of anaesthetists has turned from mechanical contrivances to electronic apparatus and particularly that for monitoring. In this field, however, few have the basic knowledge necessary for the design of new apparatus and for many the "black box" must remain for ever something magical whose activity they must take on trust. This is not, however, a satisfactory situation and anything which gives some indication of how the "black box" works is welcome.

Among those who have contributed profoundly to our knowledge of electronics and physics in relation to anaesthesia, Dr D. W. Hill of the Research Department of Anaesthesia in the Royal College of Surgeons must be numbered. It is therefore most appropriate that he should have written a book on this subject.

As the title implies, the volume deals with all aspects of the application of electronics to the practice of anaesthesia, surgery and, for that matter, to medicine too, for anyone interested in electrocardiography will profit greatly from reading what Dr Hill has to say on recording electrodes and their effects on the form of the electrocardiograph. The chapter on recording systems for physiological signals is a "must" for anyone contemplating the purchase of a recording device. The same applies to the chapters on pressure transducers and those on measurement of blood flow and of cardiac output, for those with an interest in this field. And all anaesthetists will want to study on pp. 79 et seq. a clear diagram and explanation of the von Recklinghausen oscillotonometer. The section on measurement of gas flow, volume and respiratory rate will be likewise most helpful to those who seek to do research in this superficially straightforward but in fact rather difficult field. The same applies to the remaining chapters of the book which deal with electronic methods of measuring other physiological parameters. The chapter on gas and vapour analysis should be carefully read by everyone who seeks to present himself for the Primary FFARCS examination. Such candidates should also seek to master the principles governing electrode systems for measuring pH, Pco, and Po in blood, though in this section there is perhaps rather more detailed information than the average anaesthetist need possess even at this stage of his career. The chapter on pacemakers and bladder and anal stimulators, like that on defibrillators, will likewise commend itself to a wide section of the medical profession in many disciplines. The problems of diathermy and electrical safety in the operating room are clearly discussed, as are those which underlies the design of oximeters, densitometers and colorimeters. The section on measurement of body temperature will also be of interest to anaesthetists, though again they may not wish to absorb the detail of the electronics fully set out here. The final chapter on radioactive isotopes provides a useful general introduction to this field for those who wish to assess their possibilities in research work.

There is no doubt that this volume will become a standard work of reference on the shelves of the library of every department of anaesthesia. Everyone who seeks to teach clinical measurement must have a copy. The actual student studying for his Primary FFARCS will need to be selective in his reading and will probably fare better if his tutor predigests the material.

The book is copiously illustrated. The extensive reference lists at the end of each chapter add greatly to its value. The index is comprehensive. In short, this book is a most valuable and much needed addition to the literature of our specialty.

A. R. Hunter