EFFECTS OF HIGHER OXIDES OF NITROGEN ON THE ANAESTHETIZED DOG

BY


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SUMMARY

The physiological derangements during and following the administration of higher oxides of nitrogen have been studied in dogs anaesthetized with pentobarbitone. The dogs were exposed to concentrations of nitric oxide or nitrogen dioxide between 0.1 and 2.0 per cent over periods between 5 and 136 minutes. Despite the inhalation of 98 per cent oxygen, death was always associated with a critical reduction in arterial oxygen content. This was caused by one or more of the following three factors: methaemoglobinaemia, low arterial $P_{O_2}$, and acidaemia which caused a shift of the oxyhaemoglobin dissociation curve. The reduction of arterial $P_{O_2}$ was caused by an outpouring of fluid into the alveoli.

There is a wealth of information on the effects of prolonged inhalation of higher oxides of nitrogen in concentrations of less than 0.01 per cent, which occur as an industrial hazard (von Oettingen, 1941; Patty, 1962; Becklake et al., 1957; Gray, 1959; Stokinger, 1965). However, few of these studies are relevant to a clinical situation in which considerably higher concentrations may be inhaled for short periods from a nitrous oxide cylinder contaminated with higher oxides of nitrogen. This latter clinical picture is described by Clutton-Brock (1967) and in the historical records reviewed by Smith (1967).

One of the most striking features observed during the inhalation of contaminated nitrous oxide during anaesthesia has been the rapid development of cyanosis, which was not relieved by the inhalation of 100 per cent oxygen. There is little doubt that this was due to the formation of methaemoglobin and this was, in fact, demonstrated in the case reported by Clutton-Brock (1967), and was described in animals by Haldane (1926). It may be calculated that conversion of sufficient haemoglobin to produce visible cyanosis requires the retention of at least 200 ml of the higher oxides of nitrogen. Since cyanosis has been reported after only 5 minutes of anaesthesia, when the patient would have been unlikely to have breathed more than 25 litres of nitrous oxide, it follows that the contamination must have been of the order of 1 per cent by volume or higher.

Retrospective analysis of a cylinder can never indicate the actual concentration of nitric oxide inhaled by a patient because of the marked fractionation of nitric oxide and nitrous oxide (Austin, 1967) which causes the percentage of nitric oxide in the effluent gas to fall rapidly as the cylinder is discharged.

Published articles give an unconvincing account of the difference in toxicity between nitric oxide and nitrogen dioxide (Pflesser, 1941). It seemed to us that this was to be expected in the presence of oxygen, since some nitric oxide is converted to nitrogen dioxide, and nitric oxide is formed when nitrogen dioxide reacts with water.

In the light of these uncertainties, we have undertaken a study of the toxic effects of higher oxides of nitrogen in the anaesthetized dog. After the survival of one dog which breathed 0.1 per cent nitrogen dioxide in oxygen for 2 hours 16 minutes, we used concentrations within the range...
0.5–2 per cent. Both nitric oxide and nitrogen dioxide were employed although we were unable to detect any appreciable difference in the toxicity of the two gases.

A detailed study of the haematological changes is reported elsewhere in this number (Toothill, 1967), and Shiel (1967) has contributed an account of the morbid anatomical changes. Relevant features of the chemistry of the higher oxides of nitrogen have been reviewed by Austin (1967), and the influence of the anaesthetic gas circuit on the inspired gas mixture has been discussed by Kain (1967).

Throughout this paper the term “nitrogen dioxide” is used to mean an equilibrium mixture of nitrogen dioxide (NO$_2$) and its dimer dinitrogen tetroxide (N$_2$O$_4$).

**METHODS**

**Subjects.**

A *non-survival series* consisted of eight anaesthetized dogs which received various concentrations of nitric oxide or nitrogen dioxide according to the schedule shown in table I. Three of these dogs breathed spontaneously while the others were ventilated artificially. Those which survived the exposure were sacrificed before recovering from anaesthesia.

A *survival series* consisted of four anaesthetized mongrel dogs which received 0.5 per cent nitrogen dioxide for periods ranging from 5 to 45 minutes, all breathing spontaneously. Following exposure, the three surviving dogs were allowed to recover from anaesthesia. By the time they had regained consciousness they showed no obvious signs of distress and were eventually sacrificed.

**Anaesthesia and gas circuitry.**

In each case anaesthesia was induced with intravenous pentobarbitone (20–40 mg/kg). An endotracheal tube was passed and the cuff inflated to give an airtight fit with the trachea. No inhalation anaesthetic was given except for dog 5 in which 2 per cent nitric oxide was administered in a carrier gas mixture of 70 per cent nitrous oxide in oxygen (artificial ventilation). The gas circuit was of the non-rebreathing type with expired air vented to the exterior through a 5-metre length of wide-bore p.v.c. tubing. During spontaneous ventilation, gas was drawn from a polyethylene reservoir bag through a Hook & Tucker unidirectional valve. The transparent reservoir bag was made in three layers to prevent leaks. During artificial ventilation, a Beaver ventilator was used to drive a bag-in-bottle system, which drew gas from the polyethylene reservoir bag and passed it to the dog by means of an inflation valve.

**Preparation of the inspired gas mixture.**

Nitric oxide was obtained from a cylinder of the pure gas supplied by Air Products Ltd. It was metered through a calibrated flowmeter and allowed to mix with oxygen supplied at 3–6 l./min. The gas then passed to the polyethylene reservoir bag and thence to the dog. The transit

<table>
<thead>
<tr>
<th>Table I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Summary of exposures and results.</strong></td>
</tr>
<tr>
<td>Exposure</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Non-survival series</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Survival series</td>
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<td></td>
</tr>
</tbody>
</table>
time through the apparatus between the point of mixture of nitric oxide and oxygen and the lungs was of the order of 3.5 seconds. Considerable oxidation to nitrogen dioxide occurred as indicated by the contents of the reservoir bag which were visibly brown when more than 1 per cent nitric oxide was administered.

Nitrogen dioxide was obtained from a cylinder of the liquid supplied by Cambrian Chemicals Ltd. Three methods of dispensing were used. In the first run the liquid was injected into a carrier gas stream by means of an automatic syringe. This was inconvenient and in later experiments gaseous nitrogen dioxide was delivered through a calibrated flowmeter while the laboratory was maintained at a temperature above the boiling point of liquid NO$_2$ (21°C). Finally, in the survival series, we injected the calculated quantity of liquid nitrogen dioxide into a measured volume of oxygen in a polyvinyl chloride Douglas bag. Liquid nitrogen dioxide was obtained from the cylinder by connection of a Mantoux syringe to the neck of the cylinder, inverting the cylinder, opening the valve and aspirating the liquid directly into the syringe. None of the manoeuvres involving liquid nitrogen dioxide was easy and an added complication was the corrosive action of this gas on nylon and most metals. Polyethylene and polyvinyl chloride did not appear to be attacked but nitrogen dioxide was clearly soluble in polyvinyl chloride.

Safety precautions.

Participants in this study were exposed to frequent contact with higher oxides of nitrogen and therefore an attempt was made to limit the concentrations which they inhaled. Care was taken to ensure that all gas circuits were airtight and a Siebe-Gorman protective breathing apparatus was kept available during the administration of higher oxides. The set was in fact required on one occasion when a gas line became accidentally disconnected and a quantity of nitric oxide escaped into the room.

Measurements.

Catheters were placed into the femoral artery and either the pulmonary artery or right ventricle and pressures were continuously recorded. Recordings were also made of oesophageal and airway pressures, and of the electrocardiogram, using a Devices transducer/multichannel recording system.

Blood samples were taken at intervals for measurements of arterial oxygen tension, oxygen content, haematocrit, haemoglobin, methaemoglobin concentration, pH, carbon dioxide tension, standard bicarbonate and base excess.

Oxygen tension was measured polarographically using an oxygen sensitive electrode, oxygen content was measured polarographically (Linden, Ledsome and Norman, 1965). Haemoglobin was measured spectrophotometrically using the cyanmethaemoglobin method and methaemoglobin was measured spectrophotometrically using the method described in this symposium (Toothill, 1967). pH, Pa$_{CO_2}$, standard bicarbonate and base excess were measured using the interpolation technique on micro-Astrup apparatus.

In one experiment, compliance and airway

<table>
<thead>
<tr>
<th>Dog 1.—0.1 per cent NO$_2$ in O$_2$ (spontaneous respiration, haemoglobin 12 g per cent).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duration of exposure</strong></td>
</tr>
<tr>
<td>(min)</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>(breathing 100% oxygen)</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>35</td>
</tr>
<tr>
<td>76</td>
</tr>
<tr>
<td>136 (stop NO$_2$)</td>
</tr>
<tr>
<td>64 min after end of exposure</td>
</tr>
</tbody>
</table>

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**TABLE II**
results were measured using the timed constant
gas flow ("pufflation") technique (Don and

RESULTS

Mortality.

A concentration of 0.1 per cent NO₂ (dog 1)
did not cause death after 136 minutes of exposure,
during which the dog remained in surprisingly
good condition without serious changes in arterial
PO₂ or appreciable methaemoglobin formation.
However, the Pco₂ rose progressively from 34.0
to 79.5 mm Hg, and this rise, together with the
concomitant "metabolic" acidosis, resulted in a
substantial lowering of blood pH to 7.08 (table II).
Dog 1 was sacrificed 64 minutes after the end of
the exposure in good general condition.

A concentration of 0.5 per cent NO or NO₂
(dogs 6, 8, 9, 10, 11, 12) produced a rapid fall in
arterial PO₂, a rise in methaemoglobin concentra-
tion and a rise in arterial Pco₂, in spite of artificial
ventilation which had been sufficient to cause
hypocapnia during the control period (table III).
Although no dog died during the administration
of 0.5 per cent nitric oxide or nitrogen dioxide,
dogs in which the administration was continued
for more than 24 minutes died with overt
pulmonary "oedema" at intervals, after exposure,
varying from 7 to 120 minutes (table I). Exposures
of 15 and 22 minutes resulted in post-anaesthetic
respiratory distress which gave rise to anxiety for
about 2 hours but which resolved without therapy.
The dogs then appeared healthy in spite of the
appearance of some froth about the mouth,
indicative of pulmonary "oedema". Apparently
normal recovery from anaesthesia followed an
exposure lasting 5 minutes.

A concentration of 2.0 per cent nitric oxide or
nitrogen dioxide resulted in death during
exposures ranging from 15 to 50 minutes (table I).
In one case (dog 4) death was apparently hastened
by the use of the "pufflation" technique for the
measurement of pulmonary compliance and air-
way resistance.

The series was too small for the application of
statistical criteria to differentiate between the
mortality during artificial as opposed to sponta-
naneous respiration, and of nitric oxide in contrast
to nitrogen dioxide. However, the use of artificial

<table>
<thead>
<tr>
<th>Duration of exposure (min)</th>
<th>Methaemoglobin (percentage of total Hb)</th>
<th>Calc. oxygen capacity (vols. %)</th>
<th>pH</th>
<th>Pco₂ (mm Hg)</th>
<th>Base excess (mm.equiv/l.)</th>
<th>Po₂ (mm Hg)</th>
<th>Derived So₂ (%)</th>
<th>Calc. Co₂ (ml/100 ml)</th>
<th>Measured Co₂ (ml/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>16.7</td>
<td>7.53</td>
<td>18</td>
<td>-3.8</td>
<td>632</td>
<td>100</td>
<td>18.6</td>
<td>18.9</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>14.4</td>
<td>7.34</td>
<td>39</td>
<td>-4.4</td>
<td>75</td>
<td>94</td>
<td>13.8</td>
<td>13.6</td>
</tr>
<tr>
<td>17</td>
<td>14</td>
<td>14.4</td>
<td>7.27</td>
<td>45</td>
<td>-7.1</td>
<td>38</td>
<td>64</td>
<td>9.4</td>
<td>6.7</td>
</tr>
<tr>
<td>22</td>
<td>17</td>
<td>13.9</td>
<td>7.11</td>
<td>61</td>
<td>-13.4</td>
<td>31</td>
<td>42</td>
<td>5.9</td>
<td>3.2</td>
</tr>
<tr>
<td>25</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>27</td>
<td>—</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>32</td>
<td>3</td>
<td>16.2</td>
<td>7.00</td>
<td>57</td>
<td>-19.4</td>
<td>28</td>
<td>30</td>
<td>5.0</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Table IV

<table>
<thead>
<tr>
<th>Dog</th>
<th>Contaminant gas</th>
<th>Concentration (%)</th>
<th>Type of ventilation</th>
<th>Duration of exposure before initial observation of cyanosis of the tongue (min)</th>
<th>Po₂ (mm Hg)</th>
<th>Methaemoglobin (percentage of total Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NO₂</td>
<td>0.1</td>
<td>Spont.</td>
<td>136</td>
<td>309</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>NO</td>
<td>2.0</td>
<td>Spont.</td>
<td>8</td>
<td>421</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>NO</td>
<td>2.0</td>
<td>Spont.</td>
<td>5</td>
<td>99</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>NO</td>
<td>2.0</td>
<td>Artif.</td>
<td>3</td>
<td>181</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>NO</td>
<td>2.0</td>
<td>Artif.</td>
<td>6</td>
<td>153</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>NO</td>
<td>0.5</td>
<td>Artif.</td>
<td>7</td>
<td>75</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>NO</td>
<td>2.0</td>
<td>Artif.</td>
<td>2</td>
<td>93</td>
<td>24</td>
</tr>
</tbody>
</table>
ventilation was, in general, associated with shorter survival times.

**Formation of methaemoglobin.**
Cyanosis of the tongue was noted as early as 2 minutes after inhalation of 2 per cent nitrogen dioxide (table IV). In each case cyanosis was caused by formation of methaemoglobin rather than desaturation.

Methaemoglobin was formed roughly in proportion to the concentration of higher oxides of nitrogen and duration of exposure (fig. 1). Other factors being the same, methaemoglobin was more rapidly formed in dogs ventilated artificially. 100 per cent conversion was attained in one dog (No. 2) immediately prior to death. Reconversion to haemoglobin was demonstrated after administration of methylene blue (1 mg/kg) (fig. 2). However, many dogs died with levels of methaemoglobin which could not have of themselves contributed to mortality (5 per cent in dogs 3, 5 and 11; 3 per cent in dogs 6 and 8).

**Arterial Po**
Severe reduction of arterial Po, occurred in all dogs inhaling 0.5 per cent or more of either higher oxide. Levels as low as 18.5 mm Hg were attained during the inhalation of 98 per cent oxygen and at the prevailing pH (range 6.8–7.2) this would result in saturations of 10–20 per cent. Two examples are shown in figure 3.
TABLE V
Blood chemistry at the time of death.

<table>
<thead>
<tr>
<th>Dog</th>
<th>Methaemoglobin (percentage of total Hb)</th>
<th>Oxygen capacity (ml/100 ml)</th>
<th>Pco₂ (mm Hg)</th>
<th>pH</th>
<th>Base excess (m.equiv/l.)</th>
<th>Po₂ (mm Hg)</th>
<th>Calc. So₂ of active Hb (%)</th>
<th>Measured oxygen content (ml/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>100</td>
<td>“0”</td>
<td>&gt;200</td>
<td>6.81</td>
<td>-9.9</td>
<td>178</td>
<td>“97”</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>5*</td>
<td>19</td>
<td>&gt;200</td>
<td>6.69</td>
<td>-9.2</td>
<td>60</td>
<td>53</td>
<td>5.5</td>
</tr>
<tr>
<td>4</td>
<td>5*</td>
<td>14</td>
<td>47</td>
<td>7.24</td>
<td>-8.5</td>
<td>19</td>
<td>22</td>
<td>4.1</td>
</tr>
<tr>
<td>5</td>
<td>5*</td>
<td>33†</td>
<td>45</td>
<td>7.18</td>
<td>-13.1</td>
<td>20</td>
<td>20</td>
<td>5.3</td>
</tr>
<tr>
<td>6</td>
<td>3*</td>
<td>16</td>
<td>57</td>
<td>7.00</td>
<td>-19.4</td>
<td>28</td>
<td>32</td>
<td>2.9</td>
</tr>
<tr>
<td>7</td>
<td>78</td>
<td>7</td>
<td>91</td>
<td>7.03</td>
<td>-14.6</td>
<td>46</td>
<td>61</td>
<td>4.6</td>
</tr>
</tbody>
</table>

* Treated with methylene blue.
† Large terminal rise in haemoglobin possibly associated with splenic contraction.

Arterial oxygen content.
At low Po₂, the arterial oxygen content approximates to the product of oxygen capacity and haemoglobin saturation. The former was in this study primarily an inverse function of methaemoglobin formation, while the latter was primarily a function of Po₂ and pH. Measured values of oxygen content accorded well with the levels of active haemoglobin, Po₂, and pH.

Arterial oxygen content was critically reduced in all samples which were drawn within a few minutes of death (table V). Such samples were obtained in dogs 2 to 7 inclusive and ranged from 2.9 to 5.5 ml/100 ml.

In dogs 2 and 7 the prime defect was methaemoglobinaemia. In dog 3, the most serious defect was the exceptionally low pH which would cause gross shift of the dissociation curve. Dog 4 had a combined defect of considerable methaemoglobin and very low arterial Po₂. In dogs 5 and 6 the low arterial oxygen content was mainly due to the combination of low arterial Po₂ and pH.

A rise of total haemoglobin concentration (mean 3 g/100 ml) occurred during exposure.

Pco₂ and acid-base changes.
Respiratory acidosis occurred in all dogs breathing spontaneously. In dog 1 (breathing 0.1 per cent nitrogen dioxide) the rate of rise was 0.3 mm Hg/min. Dogs 2 and 3 (breathing 2.0 per cent nitric oxide) showed a rise of 3 mm Hg/min (fig. 4). Artificial ventilation was by no means successful in preventing the rise of Pco₂ in all cases. Thus in dog 7 (breathing 2.0 per cent nitrogen dioxide) the rise was 5 mm Hg/min. Pco₂ tended to continue rising after exposure was terminated. Table V records the terminal arterial Pco₂ in those dogs from which a sample was obtained.

“Metabolic” acidosis seems a singularly inappropriate term for the base deficit which occurred in all dogs (fig. 5). Terminal values
ranged from 8.5 to 19.4 m.equiv/l. (table V). In
dog 8, lactic acid increased by only 0.8 m.equiv/l.
compared with a total base deficit change of 5.4
m.equiv/l. during the same period. Base deficit
continued to fall following termination of
exposure.

\[ \text{pH} \]
changes were due to the combination of
respiratory and "metabolic" acidosis. Very low
values were obtained, particularly in the two dogs
breathing 2.0 per cent nitric oxide spontaneously
in whom the \( \text{Pco}_2 \) exceeded 200 mm Hg (table V).
Nevertheless in dogs 5 and 6, although the rise
of \( \text{Pco}_2 \) was stemmed by the use of artificial
ventilation, gross acidaemia resulted from a pre-
dominantly "metabolic" acidosis.

### Table VI

<p>| Changes in airway resistance and lung compliance during administration of 2 per cent nitric oxide (dog 4). |
|-------------------------------------------------|-------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Time</th>
<th>Airway resistance (cm H(_2)O/l./sec)</th>
<th>Lung compliance (ml/cm H(_2)O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control A</td>
<td>2.0</td>
<td>55.5</td>
</tr>
<tr>
<td>Control B</td>
<td>2.0</td>
<td>45.8</td>
</tr>
<tr>
<td>6 min</td>
<td>8.0</td>
<td>33.5</td>
</tr>
<tr>
<td>14 min</td>
<td>16.0</td>
<td>27.6</td>
</tr>
</tbody>
</table>

Respiratory mechanics.

Measurements of compliance and airway resis-
tance were performed in dog 4, using the timed
constant-flow method, and the results are shown
in table VI. The tracings obtained before and
after administration of 2 per cent nitric oxide
are shown in figure 6. Lung compliance was
halved, and airway resistance increased to eight
times the control value. Similar changes may be
presumed to have occurred in those dogs (fig. 7)
which demonstrated increased airway pressures
without increase in intrathoracic pressures while
ventilated with constant volume.

In the dogs which were allowed to breathe
spontaneously, respiration became shallow and
gasping after a very short exposure to either agent,
resulting in respiratory failure, as manifest in
rising values of \( \text{Pco}_2 \). In the dogs which were
artificially ventilated, gasping attempts at sponta-
neous ventilation often took place within
minutes of the beginning of administration of the
gases.

Pulmonary oedema.

The inhalation of higher oxides of nitrogen
produced pulmonary oedema in all the dogs of
both survival and non-survival series. The fluid
filling the alveoli was only revealed by histology
in dogs 1 and 9 but, in the remaining dogs, there
was macroscopic fluid in the tracheobronchial tree
at postmortem (Shiel, 1967) and sometimes on
removal of the endotracheal tube. At postmortem
of dog 7, there was a massive outpouring of about
200 ml of greenish-brown fluid from the lungs.
This was found to have a pH of about 5.0. Dog
11 also had a massive quantity of fluid and its
pH was 6.7 at the time of death, 2 hours after
exposure. Surface tension was not measured but
bubbles showed a stability comparable to that of
fluid expressed from a normal lung biopsy.

Cardiovascular effects.

Attempts at measurement of cardiac output
were fruitless due to spectral overlap between
methaemoglobin and indocyanine green, the dye
normally used in this laboratory.

Systemic arterial pressure fell dramatically
within 3 minutes of exposure in all cases except
for dog 1. The maximum fall was from 174/102
to 68/52 mm Hg within 2 minutes, a reduction
of mean arterial pressure from 138 to 60 mm Hg
(fig. 7).

Partial but transient recovery of arterial
pressure occurred in two dogs during sustained
exposure, otherwise a progressive decline con-
tinued throughout the period of observation.
Agonal elevations of arterial blood pressure were
not seen.

Pulmonary arterial pressure changes were
variable but the tendency was towards a progres-
sive but gradual fall.

Heart rate showed marked reductions asso-
ciated with the decreased arterial pressure,
although in some dogs there was a transient
increase in heart rate immediately prior to the
terminal fall in blood pressure.

Electrocardiograms showed no changes until
the agonal period, except in dog 2 which
developed transient multifocal ventricular extras-
ystoles, at a time when the arterial \( \text{Pco}_2 \) was in
excess of 150 mm Hg. The common finding
during the terminal period was a sudden transi-
Recordings of electrocardiogram, pulmonary arterial pressure, systemic arterial pressure, airway pressure, and intrathoracic pressure, taken during "pufflation". The first record was obtained before, and the second 20 minutes after commencing the administration of 2 per cent nitric oxide. Note. The amplification of the airway pressure recording has been halved on the righthand trace. Time (sec) indicated below arterial pressure trace. Data from dog 4.

FIG. 6

Recordings of electrocardiogram, right ventricular pressure, systemic arterial pressure, airway pressure, and intrathoracic pressure, showing commencement of exposure to 2 per cent nitrogen dioxide (artificial ventilation). Duration (min) of exposure indicated below arterial pressure trace. Data from dog 7.
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Figure 8

Outline of mechanisms by which higher oxides of nitrogen reduce the arterial oxygen content (and possibly perfusion) to produce hypoxia.

The cause of death.

Since each dog at the time of death (table V), had a reduction of arterial oxygen content to a level which was not compatible with life, we conclude that, under the circumstances of our study, death was caused by arterial hypoxaemia. However, the mechanism of the hypoxaemia varied between one dog and another. Three mechanisms may be distinguished (fig. 8).

Methaemoglobinemia caused a reduction in blood oxygen capacity which was of itself incompatible with life in certain dogs (e.g., dog 2). However, reconversion to haemoglobin with methylene blue did not necessarily ensure a return to a safe level of arterial oxygen content when PaO2 and pH were low.

Low arterial PaO2 was sufficient to cause a serious and probably lethal reduction of arterial oxygen content in some dogs even had the pH been normal and methaemoglobinemia absent (e.g., dog 5). Low values of arterial PaO2 occurred despite the fact that the dogs were breathing 98

DISCUSSION

The factors influencing mortality following the inhalation of higher oxides of nitrogen are obscure. There is a vast difference between the concentration of the gases considered to constitute an industrial hazard, and that required to kill a dog during acute exposure under anaesthesia.

Mortality in man has been reported after latent periods varying between a few hours and one month, following the inhalation of these gases for brief periods (Lowry and Schuman, 1956). However, no previous studies have investigated the physiological changes which occur during acute exposure to lethal concentrations of these gases, nor have the factors influencing mortality been defined. Furthermore, it should be stressed that in cases of accidental human exposure, the composition and concentration of the inhaled gases have never been precisely determined.
per cent oxygen. The cause of this profound fall in arterial \( P_{O_2} \) is discussed below.

**Low arterial pH** played a strikingly important part in the causation of diminished arterial oxygen content in certain dogs in the present series (e.g., dog 6). At normal arterial \( P_{O_2} \), a moderate acidaemia does not cause significant desaturation because of the flatness of the dissociation curve in that region. However, if the arterial \( P_{O_2} \) is within the range 15–30 mm Hg where the dissociation curve is steep, a moderate acidaemia causes a marked, and possibly critical, reduction of arterial saturation and content. Had the pH of dog 6 been normal, the arterial saturation would have been 53 per cent (a non-lethal level with 12 g/100 ml active haemoglobin). However, at pH 7.00, the saturation would be 32 per cent, a level which is probably incompatible with life.

All combinations of the factors which lower the arterial \( C_{O_2} \) are to be found in this study. In most dogs, at least two factors co-exist, while in some (e.g., dogs 4 and 7) all three factors play a part.

**Causation of the low arterial \( P_{O_2} \).**

At the time of death, certain dogs had extremely low values of \( P_{O_2} \) in spite of the fact that they were breathing 98 per cent oxygen. The morbid anatomical studies separately reported in this number by Shiel (1967) show extensive flooding of the alveoli in all the dogs which succumbed to the effects of the gas. Examination of his sections showed that the pulmonary vessels were filled and the absence of pulmonary hypertension suggests that there could not have been great restriction of the pulmonary vascular bed. Assuming that perfusion to the flooded alveoli continued, this flow must have constituted a pulmonary arterial/venous shunt, since negligible gas exchange could take place in the flooded alveoli. It may be calculated that the shunt would have been of the order of 70–80 per cent of total pulmonary blood flow in the worst affected dogs.

No sections showed oedema of the alveolar/capillary membrane without flooding of the alveoli. There was thus no histological evidence to suggest a “diffusion” defect which would in any case be unlikely to be important during the inhalation of 98 per cent oxygen.

**Causation of the pulmonary oedema.**

The action of the nitrogen dioxide on the alveolar lining fluid would be to form nitric and nitrous acids. Ionization would release hydrogen ions in sufficient quantity to account for the high acidity of the pulmonary oedema fluid and also the “metabolic” acidosis. The acid insult to the lung tissue would be sufficient to cause denaturation of proteins, rupture of lysosomes and the development of a chemical pneumonitis similar to that which occurs after aspiration of gastric contents (Mendelson, 1946). According to Shiel (1967) the fluid in the alveoli gave the appearance of having a protein content intermediate between that of a typical transudate and that of a typical exudate. Although pulmonary hypertension was not seen, in view of the congestion of pulmonary capillaries reported by Shiel, the causation of the alveolar flooding may be related to both passive transudation and the cellular response to acid. There is no obvious explanation for the patchy distribution of the oedema described by Shiel.

**The disorder of respiratory mechanics.**

Although resistance and compliance were measured in only one dog, there was a very serious hindrance to ventilation in a number of other dogs. This resulted in gross hypercapnia in some dogs breathing spontaneously and, even with vigorous artificial ventilation, it was not possible to maintain a normal alveolar ventilation in all dogs. The arterial \( P_{CO_2} \) rose to no less than 91 mm Hg in dog 7 despite our best efforts to ventilate the dog. A similar increased hindrance to ventilation occurred in the patient described by Clutton-Brock (1967). Clearly, under these conditions of alveolar hypoventilation, any reduction of the concentration of oxygen in the inspired gas would have added alveolar hypoxia to the other difficulties in the maintenance of arterial oxygenation.

**The importance of circulatory factors.**

Arterial hypotension was a constant feature of exposure of the dogs to either higher oxide of nitrogen. This is probably the direct effect of nitrite ion on vascular smooth muscle (Krantz, et al., 1940). It is uncertain to what extent the nitrate ion contributes to this effect when not combined with an alkyl radical.
In severe exposure, hypotension progressed and finally merged with the terminal decline of blood pressure. It is possible that in such cases circulatory factors would have influenced the perfusion of important organs and contributed a "stagnant" factor to the "anoxic" and "anaemic" components of hypoxia.

The important measurement would have been blood flow, both cardiac output, and regional perfusion of more susceptible organs. It is regretted that these measurements could not be made, although it may be noted that there was no evidence of the intestinal haemorrhages which are characteristically seen in dogs which have died from hypoperfusion due to hypovolaemia.

Species difference.

Previous studies in experimental animals have suggested that a species difference in susceptibility exists between dogs and rats, cats, guineapigs, mice and rabbits (Gray, 1959). It is more difficult to compare canine and human data since the latter are seldom supported by quantitative analysis of the inspired gas mixture, or even precise duration of exposure.

Nevertheless, in the cases described in this number by Clutton-Brock (1967) the rapid development of methaemoglobinemia would require the patients to have been exposed to concentrations of the order of those used in this study. The clinical picture was similar except that those dogs in the present study which survived the first 2 hours after exposure showed sustained improvement while the condition of the patient described by Clutton-Brock deteriorated steadily for some hours after exposure.

It should be noted that in contrast to man, compensatory increases in circulating blood haemoglobin concentration may occur in dogs in extremis due to contraction of the spleen. Such changes were seen in the present study and there is no doubt that they afforded a useful compensatory mechanism under the conditions of these studies, although it is doubtful if they could exert more than a marginal effect.

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REFERENCES


BOOK REVIEW


Haemoglobin is never far from the minds of anaesthetists. Apart from its role as a colour-indicator for hypoxia and as the vehicle for oxygen transport, a knowledge of its properties is essential for the anaesthetic management of patients with a wide range of conditions including sickle-cell anaemia and methaemoglobinemia. This book collects in one place an astonishingly wide range of information and, with its guide to further reading, should be an admirable starting point for a study of haemoglobin.

The main burden of the book is to trace the formation of haemoglobin from genetic code, through protein synthesis to the detailed structure and function of the molecule. On this basis a wide range of abnormalities becomes easy to understand and the reader is shown the application to the study of evolution and population dynamics. The book is a model of lucidity and the authors succeed in making a difficult subject appear easy.

The book has one strange omission. It contains almost nothing on the quantitative aspect of carriage of oxygen and carbon dioxide by haemoglobin. There are no data on dissociation curves, oxygen-combining capacity, or on velocity constants of reaction and dissociation. However, this information is frequently available to the anaesthetist elsewhere and Man's Haemoglobins is complementary to standard texts of physiology, supplying almost all the remaining information required to give a complete picture of this "second most interesting substance in the world".

This is a book for the library of anaesthetic departments, where it will serve as a reference work on the structure of haemoglobin and the haemoglobinopathies. However, it is so well written and tells such a fascinating story that many will wish to have their own copy.

J. F. Nunn