PULMONARY GAS EXCHANGE AND DIAPHRAGMATIC POSITION

Effect of Tonic Phrenic Stimulation Compared with that of Increased Airway Pressure

C. P. H. HENEGHAN AND J. G. JONES

The impairment of pulmonary gas exchange observed in man during anaesthesia has been correlated significantly with the decrease in lung volume which occurs simultaneously (Hickey et al., 1973; Hewlett et al., 1974). In spite of this, gas exchange is not improved significantly by returning lung volume to its pre-anaesthetic values (Wyche et al., 1973; Heneghan, Bergman and Jones, 1984), an observation which could be seen to disprove the hypothesis that the impairment in gas exchange is causally related to the decrease in lung volume. Alternatively, the abnormality in gas exchange could be caused by a change in the shape and position of the diaphragm as a result of the effects of anaesthesia on the phasic and tonic activity of the respiratory muscles (Jones, 1977). In this case, the decrease in lung volume is a manifestation of this change, but the restoration of lung volume to pre-anaesthetic values may not restore diaphragmatic position, regional lung volume, regional lung recoil pressure, or normal regional ventilation. This theory is supported by the observation that, in supine man, anaesthesia alters diaphragmatic tone and movement, with a cephalad displacement of, and reduction of movement in, the dependent, dorsal part of the diaphragm (Froese and Bryan, 1974). Furthermore, during positive pressure ventilation, the greatest diaphragmatic excursion is shifted from the dependent, dorsal part to the upper, ventral part. This is believed to decrease the ventilation in the dependent part of the lung and worsen the matching of ventilation to perfusion, thus producing the abnormality of gas exchange associated with the induction of anaesthesia.

SUMMARY

The influence of diaphragmatic position on abnormal gas exchange has been examined to investigate the theory that the impairment in gas exchange in anaesthetized man is caused by disturbance of diaphragmatic mechanics, resulting in abnormalities of dependent lung ventilation. A gas exchange abnormality, probably caused by airway closure in the dependent regions of the lung, was induced in anaesthetized rabbits by reducing lung volume to residual volume and allowing passive re-expansion. The effects on gas exchange of increases in lung volume produced by two methods—the application of positive end-expiratory pressure (PEEP) and phrenic nerve stimulation (PNS)—were compared. Both methods were adjusted to give the same increase in lung volume. PNS was found to produce greater caudal movement of the diaphragm than PEEP, particularly in the dependent regions. PNS also improved gas exchange significantly more than PEEP. These findings support the theory that alterations in diaphragmatic mechanics during anaesthesia contribute to the gas exchange impairment in man.

The present study was designed to test this theory. The ideal approach would have been to restore tone and movement to the diaphragm, so as to regain the position and pattern seen in the awake state. However, no method has been devised to achieve this in anaesthetized animals or man. Therefore we adopted a different approach in an animal model and, after inducing an abnormal gas exchange in the dependent part of the lung, increased lung volume in two ways, one passive and one active, ensuring that the change in
lung volume was the same under both conditions. The two ways were, first, by increasing end-expiratory airway pressure and, second, by tonic stimulation of the phrenic nerves. The former was expected to displace the diaphragm predominantly in the upper, ventral part, analogous to the findings of Froese and Bryan (1974). The latter was expected to produce movement predominantly in the dependent, dorsal part. In this way, the same increase in lung volume—produced by movement of different parts of the diaphragm—would be expected to have different effects on the induced abnormality of gas exchange.

MATERIALS AND METHODS

Arterial and venous cannulae were inserted under local anaesthesia to ear vessels of five 3-kg sandy halflop rabbits and samples of arterial blood (control) were taken. All samples were drawn into heparinized 2-ml glass syringes and blood-gas analysis was carried out immediately on an IL 613 blood-gas analyser calibrated before each measurement.

Each rabbit was then anaesthetized with a fentanyl-diflunisal mixture (Hypnorm) 0.5 ml i.m. and diazepam 2.5 mg i.v. Supplementary doses of Hypnorm 0.1 ml and diazepam 1.0 mg were administered as indicated clinically. The rabbit was turned supine and remained in this position throughout the remainder of the experiment. The trachea was dissected free, and a tracheostomy tube was introduced. The lungs were ventilated mechanically with air using a Harvard small animal respirator, giving a tidal volume of 20 ml. Ventilatory frequency was adjusted to produce normocapnia. Arterial pressure and airway pressure were recorded continuously with strain gauges and displayed on a chart recorder, and tidal flow was measured using a pneumotachograph with tidal volume derived by electronic integration.

The phrenic nerves were dissected free bilaterally. The cervical nerve roots were exposed and a leash of nerves was seen to deviate caudally from the main stream. This was demonstrated by electrical stimulation to contain a mixture of fibres which moved the forequarter and others which moved the diaphragm. Further blunt dissection allowed the more medial fibres, leading to the diaphragm, to be separated from those to the forequarter, and a cotton thread to be looped around the fibres supplying the diaphragm. Once separated, these fibres made up a bundle of about 0.5 mm in diameter: this will henceforth be referred to as the phrenic nerve.

After bilateral dissection, each nerve was laid across a set of fine wire electrodes from a bipolar nerve stimulator and covered with a saline-soaked swab. The stimulating voltage was adjusted so that the increase in lung volume, measured by integration of the pneumotachograph signal, was 20 ml, the same as the tidal volume. The stimulating frequency was 20 Hz, which produced the best balance between contractile force and rate of fatigue (Edwards, 1978). At this point, radiographic screening was used to confirm that bilateral diaphragmatic movement was occurring, and the screening was repeated each time phrenic nerve stimulation (PNS) was instituted.

Positive end-expiratory pressure (PEEP) was adjusted to increase the FRC to the same volume as with PNS, that is, 20 ml. Cine-radiographs were taken to record the position and movement of the diaphragm in control conditions, and during PNS and PEEP.

Functional residual capacity (FRC) was measured by helium dilution: a 20-ml syringe containing 10% helium in air was attached to the airway (at end-expiration) via a side arm in the ventilator circuit and the lungs were ventilated with the syringe for six to eight breaths. Helium concentration was later measured by mass spectrometry.

Impairment of gas exchange was induced by applying a syringe to the airway and applying a subatmospheric pressure of 30 cm H2O; this resulted in the lungs being emptied to residual volume (RV). In a previous study this manoeuvre had been observed to produce a sustained reduction in pulmonary compliance—as shown by an increase in airway pressure.

After the induction of anaesthesia and bilateral phrenic dissection, two studies were performed, the first in four rabbits, the second in five. In the four rabbits in which both studies were performed, the order was randomized.

Study 1. To test the effects of PEEP and PNS in the absence of any prior intervention

A control series of measurements was obtained. This consisted of taking an arterial blood sample and measuring the blood-gas tensions, measuring FRC, and running the recorder at a fast rate to record dynamic airway pressure and flow traces for the subsequent measurement of tidal volume,
end-inspiratory and end-expiratory pressure, and dynamic compliance ($C_{dyn}$). $C_{dyn}$ was calculated as the tidal volume divided by the corresponding pressure change, with both measured at points of zero flow. FRC was then increased by either PEEP or PNS, the order being randomized. The series of measurements was repeated and the FRC was then allowed to return to control. The whole procedure was repeated in the same animal, once the FRC had been increased by the other method.

**Study 2. To test the effects of PEEP and PNS after induction of a gas exchange abnormality**

This experiment was a repeat of that described above, but modified to create an abnormality of gas exchange by decreasing the volume of the lungs to RV by the application of a subatmospheric pressure of 30 cm H$_2$O, and then allowing the lungs to inflate passively. This will be referred to as "the RV manoeuvre". Thus, the procedure had four extra steps: the RV manoeuvre at the beginning of each half of the investigation, and reinflation with a triple tidal volume at the end of each half. This means that there were nine steps in total: control, the RV manoeuvre, PEEP (or PNS) on, PEEP (or PNS) off, reinflation, the RV manoeuvre, PNS (or PEEP) on, PNS (or PEEP) off, reinflation. A complete series of measurements (as above) was performed at each of these nine steps.

Alveolar–arterial oxygen tension difference ($P_{A\text{O}_2} - P_{A\text{O}_2}$) was calculated using the equation:

$$P_{A\text{O}_2} = P_{I\text{O}_2} - P_{A\text{CO}_2}/R$$

$$+ F_{I\text{O}_2} \cdot P_{A\text{CO}_2} (1-R)/R$$

where the Pappenheimer convention is used throughout, and $R$ = respiratory exchange ratio, which was assumed to be 0.8. Eleven 1-ml blood samples were taken from each animal. Each sample was replaced by an equal volume of Ringer's lactate solution.

**Statistical analysis**

Comparison of data was by two-way analysis of variance, unless otherwise stated.

**RESULTS**

In every rabbit there was a decrease in ($P_{A\text{O}_2} - P_{A\text{O}_2}$) with the induction of anaesthesia, and $P_{A\text{O}_2}$ was increased: the change was statistically significant (table I).

<table>
<thead>
<tr>
<th></th>
<th>Awake</th>
<th>Anaesthetized</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{A\text{O}_2}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{A\text{CO}_2}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>($P_{A\text{O}<em>2} - P</em>{A\text{CO}_2}$)</td>
<td>14.0</td>
<td>4.2</td>
</tr>
<tr>
<td>$P_{A\text{CO}_2}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{A\text{CO}_2}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>($P_{A\text{O}<em>2} - P</em>{A\text{CO}_2}$)</td>
<td>9.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Mean</td>
<td>4.0</td>
<td>1.7</td>
</tr>
<tr>
<td>SD</td>
<td>0.9</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Position and motion of the diaphragm**

The effects of PEEP and PNS on the position and motion of the diaphragm are illustrated in figure 1, and in table II the displacements of different parts of the diaphragm which resulted from the two interventions in three animals are shown. PNS produced more movement of the diaphragm throughout its length, and this difference was statistically significant in the dorsal section.
TABLE II. Mean movement of the diaphragm caudally (mm) (SEM) resulting from PNS and PEEP, n = 3. Measurements made from tracings of lateral cine-radiographs: “ventral” = 1 cm posterior to the most anterior point of diaphragm; “middle” = midpoint of antero-posterior diameter of thorax measured from vertebral body to xiphisternum, “dorsal” = 1 cm anterior to the most posterior point of diaphragm. *P < 0.05

<table>
<thead>
<tr>
<th></th>
<th>PNS</th>
<th>PEEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral</td>
<td>7.3 (0.3)</td>
<td>5 (0)</td>
</tr>
<tr>
<td>Middle</td>
<td>12.3 (2.3)</td>
<td>10.7 (0.9)</td>
</tr>
<tr>
<td>Dorsal</td>
<td>18.7 (6.2)</td>
<td>9.7 (0.7)*</td>
</tr>
</tbody>
</table>

**Study 1**

FRC, $C_{dyn}$ and $(P_{A_O_2} - P_{A_O_2})$ at each step of the experiment are shown in figure 2. There were significant changes of both FRC and $C_{dyn}$ with both PEEP and PNS, with no change in $(P_{A_O_2} - P_{A_O_2})$.

**Study 2**

The position and motion of the diaphragm were unaffected by the RV manoeuvre. In both halves of the experiment, this produced a consistent, significant decrease in $C_{dyn}$ (fig. 3b), with no change in FRC (fig. 3a), and a consistent,

**Fig. 2.** Investigation 1: The effect of PNS and PEEP on FRC, $C_{dyn}$ and $(P_{A_O_2} - P_{A_O_2})$. Values are mean and SEM. **P < 0.01, *P < 0.05, compared with controls.

**Fig. 3.** Study 2 (mean and SEM). C = Control; PRV = post residual volume (after application of subatmospheric pressure of 30 cm H$_2$O to airway, reducing lung volume to residual volume); PNS/PEEP = during application of the relevant intervention; Post P/S = immediately after PNS or PEEP. ● = PNS; ○ = PEEP. Statistics: There were no differences between PNS and PEEP data, absolute values. A: Decrease in FRC in PRV group was not significant (0.1 > $P > 0.05$), returning to control in post P/S. B: PRV and PNS/PEEP different from all other values ($P < 0.001$), and PRV different from PNS and PEEP ($P < 0.01$). C: Each value in both runs different from previous value ($P < 0.05$); PRV v. C, PNS v. PRV, $P < 0.001$; PEEP v. PRV, $P = 0.04$.

**Fig. 4.** Comparison of change of $(P_{A_O_2} - P_{A_O_2})$, PRV to PNS and PRV to PEEP. Symbols as for figure 3. $P = 0.02$. **BRITISH JOURNAL OF ANAESTHESIA**
significant increase in $(PA_{O_2} - PA_{O_2})$ (fig. 3c). The introduction of both PNS and PEEP improved gas exchange, but the improvement with PNS was significantly greater than that with PEEP (fig. 4). There was partial recovery of compliance and gas exchange after discontinuation of both PNS and PEEP, and there was no difference between the two halves of the investigation at this stage. After a three tidal volume hyperinflation manoeuvre, gas exchange recovered to control values (fig. 3c).

Although the efficiency of gas exchange was slightly worse (i.e. slightly higher $(PA_{O_2} - PA_{O_2})$) overall in association with PNS (fig. 3c), gas exchange was better with PNS than with PEEP.

In the four animals in which arterial pressure recordings were technically adequate, there was a slightly greater decrease in mean arterial pressure during PNS (9.8 mm Hg, SEM 4.8) than during PEEP (6.2 mm Hg, SEM 4.4). This difference was not significant $(P > 0.05)$, and there was no correlation between change in arterial pressure and change in $(PA_{O_2} - PA_{O_2}) (P = 0.8$ by regression analysis).

**DISCUSSION**

There are five interesting findings in this study:

(a) Gas exchange, far from being impaired, was actually improved by the induction of anaesthesia and the supine posture in the rabbit.

(b) Neither PEEP nor PNS had any consistent effect on $(PA_{O_2} - PA_{O_2})$ if no gas exchange abnormality had been induced.

(c) There was no significant change in FRC when $C_{dyn}$ was decreased substantially (and $(PA_{O_2} - PA_{O_2})$ considerably increased) by the RV manoeuvre.

(d) After the RV manoeuvre, PNS improved $(PA_{O_2} - PA_{O_2})$ significantly more than PEEP.

(e) Diaphragmatic position was affected differently by PEEP and PNS, and, unlike in man (Froese and Bryan, 1974), movement of the diaphragm was greater in the dependent than in the upper portion in both conditions.

The failure of anaesthesia to produce a gas exchange abnormality in the rabbit means that this model is not directly comparable to the human. The reason for this difference is obscure, but may be related to the difference in diaphragmatic movement in the rabbit, producing better ventilation in the dependent parts of the lung. The absence of impairment in gas exchange led us to use the technique of inducing one by a transient reduction of lung volume to RV, and to investigate the effects of PEEP and PNS on this induced impairment. Clearly, this model may not be a precise analogue of the impairment in gas exchange induced by anaesthesia in man; however, there is compelling evidence that the RV manoeuvre induces changes predominantly in the dependent parts of the lung (Jones, 1982), so the likelihood is considerable that this is a close analogue. Second, even if this is not a closely analogous model, it does represent a model of impairment in gas exchange which is quantitatively comparable to that induced by anaesthesia in man and is, thus, a useful tool with which to investigate factors which may affect gas exchange.

The lack of any effect of PEEP or PNS on $(PA_{O_2} - PA_{O_2})$ in the first investigation is not very surprising, as there was no impairment of gas exchange and, thus, probably little maldistribution of ventilation, and little scope for improvement. Neither PEEP nor PNS worsened $(PA_{O_2} - PA_{O_2})$, so if either intervention decreased venous return and cardiac output, this did not decrease $P_{O_2}$ sufficiently to increase $(PA_{O_2} - PA_{O_2})$.

The observation that one can induce a major reduction in pulmonary compliance and produce a significant increase in $(PA_{O_2} - PA_{O_2})$ without affecting FRC significantly is difficult to interpret. The obvious mechanism for the reduced $C_{dyn}$ following the RV manoeuvre is airway closure (Jones, 1982), and the impairment of gas exchange would then be caused by the regional hypoventilation resulting from this. However, in spite of a major reduction in compliance, which should represent closure of a large proportion of the airways, FRC was not decreased, when measured either by helium dilution, or radiographically. This could be explained by measurement errors, but these are unlikely, since the increases in FRC induced by PEEP and PNS were easily detected with helium dilution and radiographically. The only other explanation which fits the data is that reducing lung volume to RV results in a greater non-linearity of the lung compliance curve than in the normal state, so that the recoil pressure at FRC is the same as at the control FRC, but above this volume the recoil pressure is considerably greater.

Next there is the observation that PNS improved $(PA_{O_2} - PA_{O_2})$ more than did PEEP. Lateral radiography revealed that, during PNS, the dorsal part of the diaphragm was more caudal at end-expiration and would, thus, exert a greater distending force on the dependent lung than during PEEP. PNS would, therefore, tend to open...
airways more than PEEP so that, although tidal diaphragmatic motion was similar in PNS and PEEP, regional ventilation would be better during PNS. The greater concurrent improvement in \((P_{\text{A}O_2} - P_{\text{a}O_2})\) during PNS supports the idea that regional ventilatory abnormalities, like the changes seen by Froese and Bryan (1974), contribute to the impairment in gas exchange seen during anaesthesia in man.

It is possible that this finding results from different changes in cardiac output during PEEP and PNS: although PEEP and PNS both increase airway pressure, PEEP increases airway pressure throughout the respiratory cycle, whereas PNS only increases the inspiratory pressure, leaving expiratory pressure at zero. If cardiac output were significantly differentially affected, this might be reflected in changes in arterial pressure. Although there was a tendency for arterial pressure to be decreased more by PNS, this did not reach statistical significance, nor was there a correlation between the change in mean arterial pressure and \((P_{\text{A}O_2} - P_{\text{a}O_2})\). Under these circumstances, it is unlikely that this mechanism is important in generating the difference between PEEP and PNS.

It is interesting to note that the motion of the rabbit diaphragm in response to the two manoeuvres was not as expected from the work of Froese and Bryan (1974). They showed that, during anaesthesia in man, mechanical ventilation moved the anterior part of the diaphragm more, while spontaneous ventilation moved the posterior part more. Therefore we expected that PEEP would affect mainly the ventral part of the diaphragm of the rabbit and PNS the dorsal part. In fact, we observed that there was always a greater movement in the dorsal part of the diaphragm, and a gradient of motion from ventral to dorsal. This difference from man was quite unexpected, and it may arise from the difference in the shape of the rabbit chest wall and diaphragm, and the stresses imposed on them by the abdominal contents. Thus in man, the chest can be described as having a modified oval cross section, while that of the rabbit, like many quadrupeds, is much more triangular, pointed ventrally. This would tend to reduce the freedom of movement of the ventral part of the diaphragm, since it is tethered laterally much more than that of man.

In conclusion, we have investigated whether two different methods of increasing lung volume during anaesthesia, increasing airway pressure and phrenic nerve stimulation, have different effects on pulmonary gas exchange. Phrenic stimulation improved \((P_{\text{A}O_2} - P_{\text{a}O_2})\) more than PEEP did, which supports the theory that abnormalities of gas exchange observed during anaesthesia may be attributable to regional alterations in chest wall motion.

ACKNOWLEDGEMENTS
The authors thank Dr R. Wilkins, Mrs B. Sandin and Mr D. Maltby, of the Division of Radiology, Clinical Research Centre, for their radiological and radiographic assistance, and the Department of Medical Illustration for preparing the diagrams.

REFERENCES