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DEVELOPMENT OF RESPIRATORY CHEMOREFLEXES IN RESPONSE TO ALTERNATIONS OF FRACTIONAL INSPIRED OXYGEN IN THE NEWBORN INFANT

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SUMMARY

1. We studied the reflex respiratory response to breath-by-breath alternations of fractional inspired oxygen (F_{I,O_2}) in full-term human infants delivered either vaginally or by caesarian section at 3–10 h ($n = 6$), 12–24 h ($n = 12$), 24–48 h ($n = 18$), 3–4 days ($n = 21$) and 5–8 days ($n = 7$) postnatally.

2. Respiration was measured by inductance plethysmography (Respitrace) and respiratory variables for each breath were calculated on-line by a microcomputer. Test runs (with alternations of F_{I,O_2} between 0·21 and 0·16) and control runs (with an F_{I,O_2} of 0·21) of 50–100 breaths were carried out during quiet sleep. For each respiratory variable the magnitude of the reflex breath-by-breath alternation was compared between control and test runs.

3. There was little respiratory response during control runs at any postnatal age. However, there was a significantly greater response to test runs in all infants studied and at all ages.

4. There were no significant differences in the degree of alternation during test runs between infants of similar postnatal ages delivered by caesarean section and those born vaginally.

5. In all infants the magnitude of the respiratory response increased with postnatal age, presumably reflecting postnatal increases in the hypoxic sensitivity of the peripheral arterial chemoreceptors.

6. The results indicate that in human infants the alternate breath method can be used to detect developmental changes in peripheral chemoreflexes between birth and postnatal day 8.

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INTRODUCTION

The carotid chemoreceptors can detect changes in arterial oxygen pressure (P_{a,O_2}) to a frequency of *ca* 0·25 Hz (Kumar & Nye, 1985; Kumar, Nye & Torrance, 1988). Moreover, the oscillations produced in chemoreceptor discharge lead to reflex

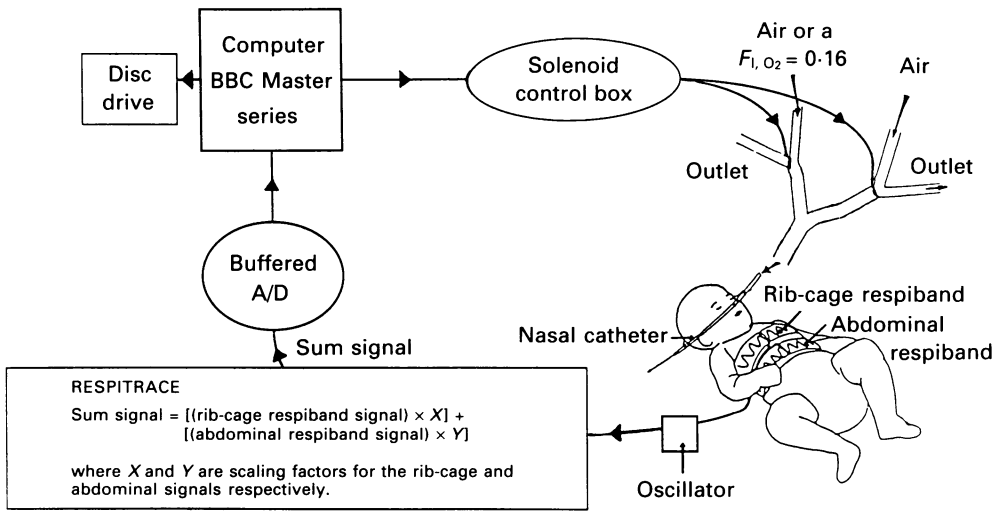

Fig. 1. The experimental set-up used for controlling the supply of inspired gas and for measuring and recording the response to alternate breaths of air and test gas (F_{I,O_2} of 0·16).

alternations in respiratory variables. Accordingly in adult man and cats, breath-by-breath changes in alveolar P_{O_2} produce a reflex alternation in at least one respiratory variable (Ward, Drysdale, Cunningham & Petersen, 1979; Kumar, Nye & Torrance, 1983).

Such reflex studies have only recently been performed in the neonate, in which developmental changes in respiratory control occur. Studies of the reflex respiratory responses to hypoxia and hyperoxia have indicated, albeit indirectly, that peripheral chemoreceptor sensitivity increases postnatally (for reviews see Haddad & Mellins, 1984; Hanson, 1986). Moreover, in the lamb direct electrophysiological recordings have shown that the range of sensitivity to P_{a,O_2} of the carotid (Blanco, Dawes, Hanson & McCooke, 1984; Hanson, Kumar & McCooke, 1986) and aortic chemoreceptors (Kumar & Hanson, 1989) resets during the first two postnatal weeks. Thus, with increasing postnatal age the hyperbolic stimulus–response curves of the peripheral chemoreceptors shift upwards and to the right. Hence, a given alternation of P_{a,O_2} would be expected to increase the mean level and the amplitude of the oscillation in chemoreceptor discharge with increasing postnatal age. These in turn should increase the size of the breath-by-breath alternation in respiratory variables.

In newborn kittens, alternations in respiratory variables have been produced by delivering alternations in F_{I,O_2} (Hanson, Kumar & Williams, 1987; Hanson, Kumar & Williams, 1989). Moreover, this respiratory response to alternations in F_{I,O_2} was

found to increase with increasing postnatal age, with a time-scale approximating to that of peripheral chemoreceptor resetting. Lambs were also found to respond to the alternate breath test and the respiratory response was abolished following bilateral section of the carotid sinus nerves, indicating that the alternations in respiratory variables were reflex in nature, arising from the carotid chemoreceptors (Williams & Hanson, 1990).

Respiratory failure is an important cause of infant morbidity and mortality yet currently there are no reliable methods for screening human infants to assess whether the chemical control of breathing is developing normally. Moreover, abnormalities in this component of respiratory control have been implicated in sudden infant death syndrome (SIDS) (for review see Bentele & Albani, 1988) and presumably, at least terminally, infants dying of SIDS show a poor respiratory response and delayed arousal from sleep during an episode of acute hypoxaemia. Therefore, as the peripheral chemoreceptors are the 'first line' in the defence of the neonate against hypoxia, a test of peripheral chemosensitivity might be important clinically for identifying infants at high risk of respiratory failure and SIDS.

We previously applied the alternate breath method to full-term infants on postnatal days 2–3 and found that they showed a respiratory response to alternations in F_{I,O_2} (Blanco, Degraeuwe, Hanson, Kumar & Williams, 1988). In the present paper we have investigated the development of the reflex respiratory response in full-term infants on postnatal days 1–8. We have also examined the possibility that mode of delivery at birth might influence the magnitude of the response.

METHODS

Local ethical committee approval and informed parental consent were obtained. Sixty-four infants born at term and aged between 3 h and 8 days were studied. Of these thirty-seven were born by vaginal delivery (vertex presentation, NV) and twenty-seven by caesarean section (CX). The number of infants born by NV and CX studied at each postnatal age were as follows: at < 12 h NV = 6, CX = 0; at 12–24 h NV = 8, CX = 4; at 24–48 h NV = 9, CX = 9, at 3–4 days NV = 10, CX = 11 and at 5–8 days NV = 4, CX = 3. Longitudinal studies were not carried out as different infants were studied at each postnatal age. Infants were studied at a mean (±S.E.M.) gestational age of 39 weeks and 5 days (±1·2 days; range 36 weeks and 5 days to 41 weeks and 3 days) and mean body weight (±S.E.M.) of 3421±70·6 g (range 2800–4850 g). All infants had an Apgar score of $\geqslant 9$ by 10 min after birth and were studied between 20 min and 2 h after a feed (mean time±S.E.M., 54·8±4·8 min).

Experimental protocol

Ambient temperature was not measured during tests. However, all tests were carried out on the wards at the maternity hospital where ambient temperature was *ca* 23 °C. Moreover, infants' bedding was standardized whenever possible to a single blanket. Nonetheless, many infants were wearing their own clothes and it is likely that there were some differences in the thermal insulation that this clothing provided. Testing was carried out only when infants were in non-rapid eye movement (N-REM) sleep as judged behaviourally. Most commonly the control and test runs (1–2 min each) were carried out in succession during a single period of N-REM (duration *ca* 20 min).

The experimental set-up is shown in Fig. 1. Breathing was measured by inductance plethysmography (Respitrace). The Respitrace was calibrated for each infant by the least squares graphical technique using a facemask (Laerdal size 2) and pneumotachometer (P. K. Morgan Ltd.) to derive scaling factors for the rib-cage and abdominal signals (Warren & Alderson, 1988; Williams & Hanson, 1990). The gains of the rib-cage and abdominal components of the Respitrace sum signal were adjusted according to the scaling factors derived.

A repeated validation of Respitrace scaling factors were carried out in six babies (during quiet wakefulness or N-REM sleep). The tidal volumes (mean ± S.E.M.) measured by pneumotachometer for twenty breaths at 5, 15 and 25 min after the Respitrace was calibrated (16·6 ± 1·9, 16·7 ± 1·4 and 16·9 ± 1·09 ml, respectively) were compared to the values measured by Respitrace. The mean

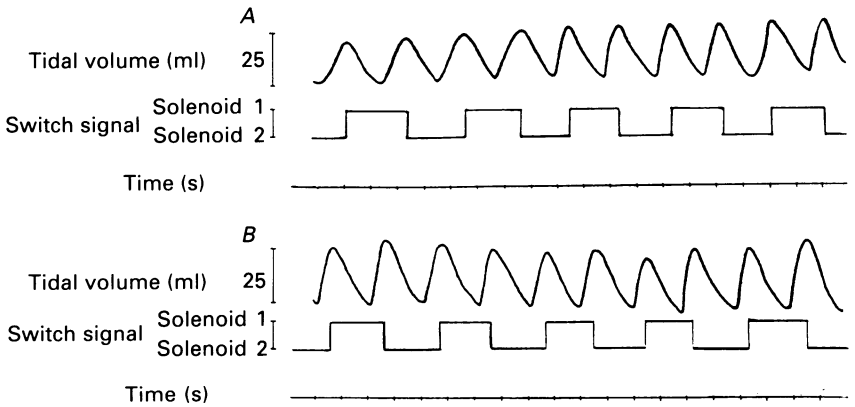

Fig. 2. Recordings made during a control run (*A*) with constant F_{I,O_2} of 0·21 and during a test run (*B*) with an alternation of F_{I,O_2} between 0·21 (via solenoid 2) and 0·16 (via solenoid 1). In each case the respitrace sum signal and switch signal are shown.

absolute error (mean ± S.E.M.) of the Respitrace from the pneumotachometer measured values was 3·05 ± 0·28 at 5, 3·07 ± 0·35 at 15 and 2·76 ± 0·26 % at 25 min. The correlation coefficients for the pneumotachometer *versus* Respitrace signals were > 0·95 for each validation run (twenty breaths). The high correlation showed that the scaling factors derived at the start of each run remained valid for at least 25 min after calibration of the Respitrace. The mean (± S.E.M.) scaling factors for the rib-cage and abdominal respibands were 0·79 ± 0·02 and 0·87 ± 0·05 respectively.

Inspired gas was humidified and supplied to the infant at 2·0–2·5 l min^{-1} via a nasal catheter (No. 1615, Salter Labs) which was attached by means of a Y-connector to two gas delivery lines. The composition of gas in each delivery line was set using rotameters connected to cylinders of medical grade air (F_{I,O_2} 0·21) and gas with an F_{I,O_2} of 0·16 (pre-calibrated; BOC Special Gases). A BBC Master microcomputer controlled two solenoid valves which switched the supply of inspired gas between the two gas delivery lines at the start of each expiration. During test runs breath-by-breath alternations of air and an F_{I,O_2} of 0·16 were delivered for fifty to one hundred breaths. For control runs air was delivered in both gas delivery lines. Two test and two control runs were carried out on each of the infants studied. Figure 2 shows recordings made during a test and a control run.

In ten infants, arterial oxygen saturation (S_{a,O_2}) was measured during test and control runs using a pulse oximeter (Invivo Research). S_{a,O_2} tended to decrease slightly (*ca* 1–2 %) during test runs but the minimum value of S_{a,O_2} observed was always above 91 %.

Data analysis

The computer also digitized the sum signal from the Respitrace, on-line, at a frequency of 40 points s^{-1}. Tidal volume (V_T), inspiratory time (T_I) and expiratory time (T_E) were measured for each breath using a peak picking routine. The frequency ($f = 1/T_I + T_E$), the instantaneous ventilation ($V_E = V_T f$), the mean inspiratory flow (or drive, $Dr = V_T/T_I$) and the inspiratory duty cycle (or timing, $Tim = T_I/(T_I + T_E)$ were then derived for each breath. The first breath of all runs was always a breath of air. The values of each of the seven respiratory variables for a breath were compared with their values in the previous breath using the method described by Hanson *et al.* (1989) and Williams & Hanson (1990). Briefly, data were normalized by expressing the differences between each respiratory variable in alternate breath pairs as a percentage change about the average of the two values. The sign (+ or −) of the alternate differences was reversed and the differences between

successive values of a variable were summed to derive a cumulative plot. Thus, for a regular alternating response, the cumulative plot was displaced sequentially from the abscissa throughout the run. However, if there was no alternation in a variable the cumulative plot was not displaced consistently from the abscissa. The average breath-by-breath percentage change in a respiratory variable was derived from the slope of the cumulative plot by regression analysis.

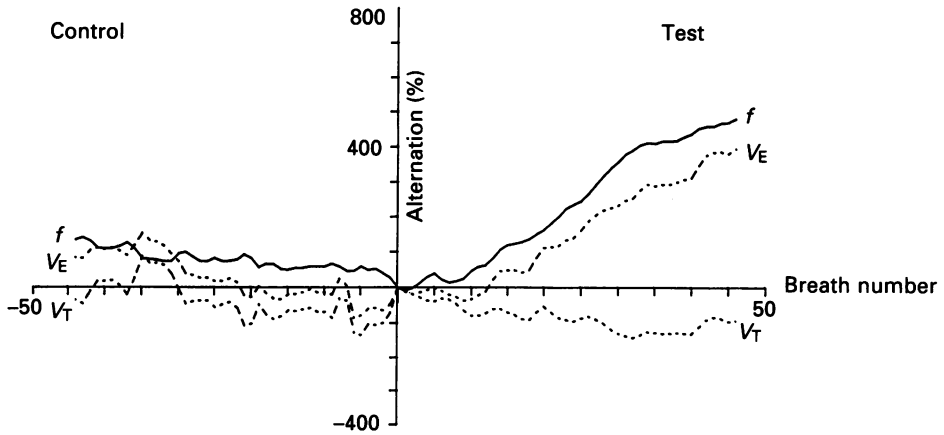

Fig. 3. The difference between each respiratory variable in alternate breath pairs was expressed as a percentage change about the average of the two values. The sign (+ or −) of the alternate differences was reversed and the differences between successive values of a variable were summed to derive a cumulative percentage alternation plot as shown for one infant on postnatal day 6. The responses to a control and test run are plotted from the origin to the left and to the right, respectively. Plots are shown for tidal volume (V_T), breath frequency (f) and instantaneous ventilation (V_E). The average breath-by-breath percentage change in a respiratory variable (slope) was derived from the slope of the cumulative plot by regression analysis. The slope and the coefficient of correlation (r) were as follows. For the control run: V_T slope = −0·79, r = 0·53; f slope = 2·0, r = 0·78 and V_E slope = 2·23, r = 0·47. For the test run: V_T slope = −2·51, r = 0·82; f slope = 12·6, r = 0·99 and V_E slope = 10·0, r = 0·98.

Statistical analysis

The respiratory response to alternations in F_{I,O_2} was evaluated by comparing the average breath-by-breath percentage alternation during test runs with that obtained during control runs, using Student's t test for paired observations. The percentage alternation for each respiratory variable was compared between age groups and between the caesarean and vaginally delivered infants at postnatal days 1–2 and 5–8 by unpaired t test. Differences were considered significant when $P <$ 0·05.

RESULTS

Figure 3 shows an example of the cumulative plots for control and test runs analysed for V_T, f and V_E from one infant at postnatal day 6. The responses to control and test runs are plotted from the origin to the left and to the right, respectively. During control runs the cumulative plot remained close to the abscissa. In contrast during test runs the cumulative plot was sequentially displaced from the abscissa. Figure 4 shows the mean (±S.E.M.) breath-by-breath alternation for V_T, f and V_E from infants born vaginally and by caesarian section at postnatal days 1–2 and 3–8. The mean responses of the two groups of infants were not different at either postnatal

age. Therefore, data from vaginally and caesarian section delivered infants were combined for each age group for determination of the postnatal development of the response.

The mean results (±S.E.M.) from all infants at 3–10 h ($n = 12$ runs from six infants), 12–24 h ($n = 24$ runs from twelve infants), 24–48 h ($n = 36$ runs from

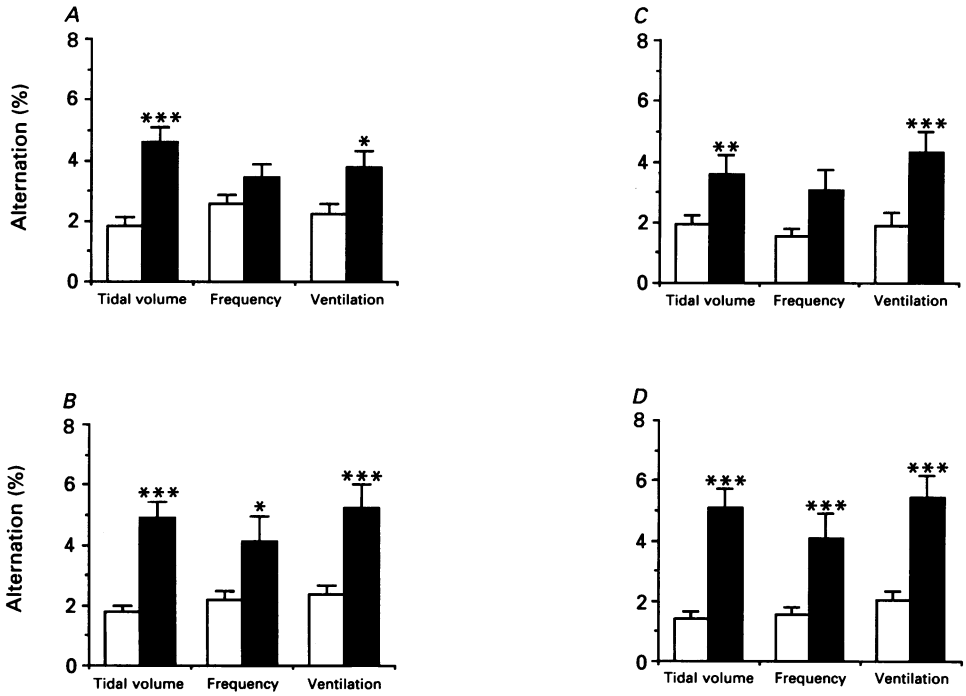

Fig. 4. The mean percentage breath-by-breath alternation for infants born by normal vaginal delivery (NV) on postnatal days 1–2 (A; $n = 17$, 34 runs) and 3–8 (B; $n = 14$, 28 runs) and for infants born by caesarian section (CX) on days 1–2 (C; $n = 13$, 26 runs) and 3–8 (D; $n = 14$, 28 runs). Mean responses (±S.E.M.) are shown during control (open bars) and test runs (filled bars) for tidal volume, breath frequency and instantaneous ventilation. Asterisks show significant differences between control and test runs; $*P < 0{\cdot}05$, $**P < 0{\cdot}02$, $***P < 0{\cdot}01$ by Student's paired t test.

eighteen infants), 3–4 days ($n = 42$ runs from twenty-one infants) and 5–8 days ($n = 14$ runs from seven infants) are shown in Fig. 5. In infants < 10 h there was a significant alternation only in V_T ($P < 0{\cdot}05$) during test as compared to control runs. However, by 12–24 h and 24–48 h a significant increase in alternation from control

Fig. 5. The mean percentage breath-by-breath alternation for infants at postnatal ages of 3–10 h (A; $n = 12$ runs from six infants), 12–24 h (B; $n = 24$ runs from twelve infants), 24–48 h (C; $n = 36$ runs from eighteen infants), 3–4 days (D; $n = 42$ runs from twenty-one infants) and 5–8 days (E; $n = 14$ runs from seven infants). Mean responses (±S.E.M.) are shown during control (open bars) and test runs (filled bars) for tidal volume, inspiratory time, expiratory time, breath frequency, inspiratory drive, timing and instantaneous ventilation. Asterisks show significant differences between control and test runs; $*P < 0{\cdot}05$, $**P < 0{\cdot}02$, $***P < 0{\cdot}01$ by Student's paired t test.

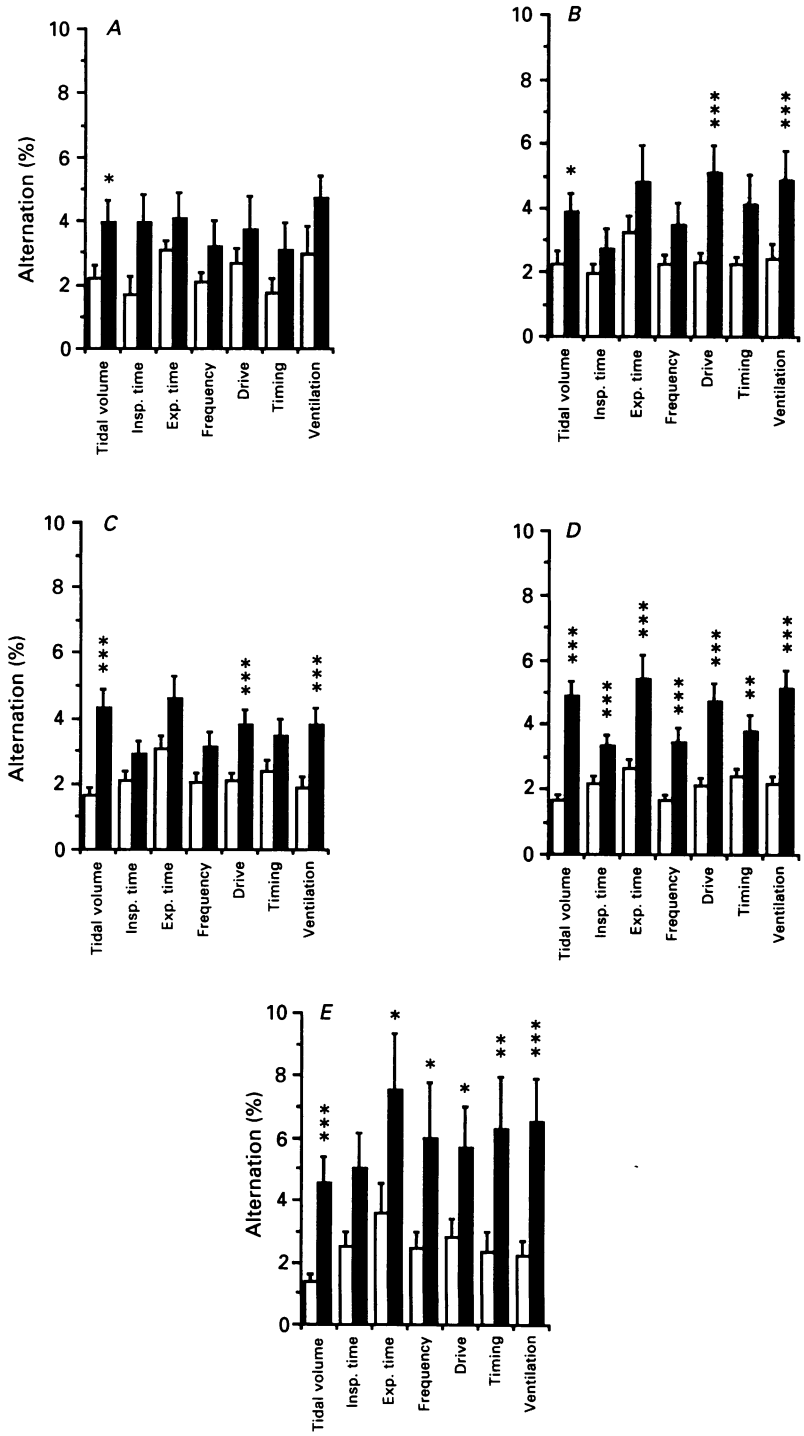

Fig. 5. For legend see facing page.

was found not only in V_T ($P < 0{\cdot}05$ and $0{\cdot}01$, respectively) but also in drive ($P < 0{\cdot}01$) and V_E ($P < 0{\cdot}01$). The mean breath-by-breath alternation was *ca* twice as great during test than during control runs. On days 3–4 significant alternation was found in all respiratory variables ($P < 0{\cdot}02$). The amplitude of response during test runs was increased further at days 5–8, when mean breath-by-breath alternations were *ca* three times as great during test than during control runs. At this age significant alternation was found in all respiratory variables ($P < 0{\cdot}05$) except T_I.

DISCUSSION

These results support our earlier observations (Blanco *et al.* 1988) that there is a reflex respiratory response to breath-by-breath alternations of F_{I,O_2} in full-term infants. As F_{I,O_2} was alternating on a breath-by-breath basis and the respiratory responses occurred at this frequency during test runs the reflex was presumably mediated by the arterial chemoreceptors. Previous reports that the response is poor in animals with reduced peripheral chemoreceptor hypoxic-sensitivity and virtually abolished by bilateral section of the carotid sinus nerves provide further support for this hypothesis (Hanson *et al.* 1989; Williams & Hanson, 1990). Therefore, our results indicate that the peripheral chemoreflex is functional, albeit relatively weak, in infants by 3–10 h after birth.

We hypothesized that mode of delivery at birth might affect the gain of the reflex respiratory response due to residual effects from caesarian sections. We therefore compared the respiratory responses in infants born vaginally with those of infants delivered by caesarian section at similar gestational ages and birth weights. At 1–2 days and at 3–8 days the respiratory responses were not significantly different between the two groups of infants. Accordingly, mode of delivery at birth does not seem to influence the magnitude of the reflex respiratory response to alternations in F_{I,O_2}. Thus, the data from both groups of infants were combined for the analysis of the effects of postnatal age on the response.

With increasing postnatal age, there was an increase in both the amplitude of the reflex breath-by-breath response and in the number of components of the response that were significantly greater during test than control runs. We examined relative changes in the response to exclude any effect of the changes in mean V_T, f and V_E with increasing postnatal age. Thus, postnatal increases in the response presumably reflect increases in the hypoxic sensitivity of the peripheral chemoreceptors, the poor response in infants less than 10 h old reflecting weak peripheral chemoreflexes.

The time course of the increase in chemoreflex response found in this study is in agreement with that suggested by several previous studies in newborn animals and infants. In animal studies, recordings made from carotid and aortic chemoreceptor afferents have shown that the hypoxic sensitivity of the peripheral chemoreceptors increases gradually between days 1 and 10 (Blanco *et al.* 1984; Hanson *et al.* 1986; Kumar & Hanson, 1989). Accordingly, in kittens and lambs, the reflex respiratory response to alternations in F_{I,O_2} increases during this time (Hanson *et al.* 1989; Williams & Hanson, 1990). In newborn infants, studies of the reduction in V_E which occurs following one or two breaths of 100% O_2 have shown that the reduction is small in infants less than 24 h old but increases in size in the early postnatal period

(Girard, Lacaisse & Dejours, 1960; Herzberg & Lagercrantz, 1987). Presumably these changes reflect greater decreases in peripheral chemoreceptor afferent activity as the sensitivity or mean discharge of the receptors increases. Moreover, changes in the compensatory respiratory response to a sigh and in the stability of oxygenation during feeding appear to reflect increases in the sensitivity of the peripheral chemoreceptors with postnatal age (Fleming, Goncalves, Levine & Woollard, 1984; Mok, McLaughlin, Pintar, Hak, Amaro-Galvez & Levison, 1986).

However, other factors may contribute to the postnatal development of the response. In early neonatal life arteriovenous shunts may remain patent and may reduce the size of the alternating stimulus reaching the peripheral chemoreceptors. Moreover, improvements in gas exchange at the lung with increasing postnatal age may result in increases in the magnitude of the alternating stimulus. Ideally the end-tidal gases should have been measured to estimate the size of the P_{a,O_2} stimulus at the peripheral chemoreceptors. However, we were unable to make this measurement because the inspired stimulus was supplied via a nasal catheter.

Our results show that the reflex respiratory response to alternate breaths of air and a mildly hypoxic gas can be used to assess the development of peripheral chemoreflexes in infants. Oxygen saturation was measured in ten infants and was found to remain above 91% during test and control runs. Therefore, it is very unlikely that the alternate breath method should lead to significant hypoxia in full-term infants. The method may have a role clinically as a simple non-invasive test of peripheral chemoreflex sensitivity. In this cross-sectional study we have defined the maturation of the response in normal full-term infants. However, further work is needed to perform a longitudinal study so that we can evaluate whether the maturation of the response can be detected in individual babies. Lastly, previous studies in kittens suggest that the method is capable of detecting pathological changes in peripheral chemoreflex sensitivity (Hanson *et al.* 1989) and we are currently applying the method to infants at high risk for SIDS.

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