*J. Physiol.* (1986), **381**, *pp.* 483–495
*With 6 text-figures*
*Printed in Great Britain*



# A PSEUDO-REBREATHING TECHNIQUE FOR ASSESSING THE VENTILATORY RESPONSE TO CARBON DIOXIDE IN CATS

BY A. BERKENBOSCH, J. DEGOEDE, C. N. OLIEVIER AND J. J. SCHUITMAKER

*From the Department of Physiology and Physiological Physics, University of Leiden, P.O. Box 9604, 2300 RC Leiden, The Netherlands*

(*Received 23 October 1985*)

## SUMMARY

1. The ventilatory sensitivity to $CO_2$ obtained from a non-steady-state step-ramp $CO_2$ challenge (analogous to the Read rebreathing method) was compared with the one of the steady-state method.

2. Experiments were performed during normoxia on twenty cats anaesthetized with chloralose–urethane. In eight of these cats additional measurements were carried out during metabolic acidosis and alkalosis.

3. The slope of the non-steady-state ventilatory response curve to $CO_2$ was not significantly different from the steady-state one only if the ratio of the step-wise increase in end-tidal $P_{CO_2}$ ($P_{ET,CO_2}$) ($A$) above its resting value and the subsequent rate of rise of the $P_{ET,CO_2}$ ($R$) was equal to the time constant of the central chemoreflex pathway ($\tau_c$). This also held true during metabolic acidosis and alkalosis.

4. It is predicted that in human beings during hyperoxia the ventilatory response line obtained with Read's rebreathing method is to a fair approximation shifted to the right by a value of $A$ with respect to the steady-state response line, provided $A/R = \tau_c$.

5. We argue that Read's prescription that a $P_{ET,CO_2}$ equilibrium should be established between mixed venous blood, arterial blood and end-tidal gas has to be regarded as an experimental condition leading to stable experiments rather than dictated by physiological mechanisms.

## INTRODUCTION

Since the classical papers of Read (1967) and Read & Leigh (1967) rebreathing techniques have been frequently used to assess the ventilatory response to $CO_2$. Although originally developed for clinical use they are now also extensively applied in fundamental research. Read (1967) showed that for normal man rebreathing from a small bag (about 6 l) filled with a gas mixture of 7 % $CO_2$ in $O_2$ (from now on called Read's condition) the slope of the non-steady-state ventiliation *vs.* end-tidal $P_{CO_2}$ ($P_{ET,CO_2}$) response line did not differ significantly from the steady-state one. However, the response lines obtained by rebreathing were shifted to higher $P_{ET,CO_2}$ values with respect to the response lines obtained with the steady-state method. In a subsequent theoretical analysis Read & Leigh (1967) concluded that the initiation

of rebreathing at a $P_{ET,CO_2}$ near the $P_{CO_2}$ of brain tissue and mixed venous blood and the use of a small rebreathing bag give rise to a linear relationship between the changes in arterial $P_{CO_2}$ and brain tissue $P_{CO_2}$ with a slope close to one. With the assumption that ventilation is an instantaneous function of brain tissue $P_{CO_2}$ this consequently leads to a ventilatory $CO_2$ response line with the same slope as the steady-state one.

Rebreathing methods are also often used in other species. Furthermore, they have been applied under physiological conditions which differ appreciably from those of normal man. In this respect it is of considerable interest that in human beings during metabolic acidosis and alkalosis appreciable deviations were found between the ventilatory $CO_2$ sensitivity obtained with Read's rebreathing method and the steady-state technique (Linton, Poole-Wilson, Davies & Cameron, 1973).

To the best of our knowledge no studies have been performed in an animal preparation to investigate the conditions to be fulfilled in a non-steady-state method to obtain a linear ventiliation *vs.* $P_{ET,CO_2}$ response line with a slope equal to that of the steady-state response. In this paper we will address this question in anaesthetized cats. As $CO_2$ challenge we will use a step $A$ followed by a rate of rise $R$ in $P_{ET,CO_2}$ (referred to as a step-ramp). To formulate conditions on $A$ and $R$ we will use an empirical two-compartment model originally introduced for human beings (Bellville, Whipp, Kaufman, Swanson, Aqleh & Wiberg, 1979). We recently showed that in cats the dynamic response of ventilation following step changes in $P_{ET,CO_2}$ can be satisfactorily described by this model (DeGoede, Berkenbosch, Ward, Bellville & Olievier, 1985).

## METHODS

### *Two-compartment model*

As shown by the DeGoede *et al.* (1985) the ventilatory response of anaesthetized cats following an isoxic step increase in $P_{ET,CO_2}$ can be described by the following two-compartment model:

$$\tau_c \frac{d\dot{V}_c}{dt} + \dot{V}_c = S_c\,[P_{ET,CO_2}(t-T_c) - B], \tag{1}$$

$$\tau_p \frac{d\dot{V}_p}{dt} + \dot{V}_p = S_p\,[P_{ET,CO_2}(t-T_p) - B]. \tag{2}$$

Eqn. (1) characterizes the ventilatory response of the central chemosensitive structures ($\dot{V}_c$) with time constant $\tau_c$, $CO_2$ sensitivity $S_c$, off-set $B$ and $T_c$ the transport delay time to carry the disturbance in $P_{ET,CO_2}$ from the lungs to the central chemosensitive structures. Similarly eqn. (2) characterizes the ventilatory response of the peripheral chemoreflex loop ($\dot{V}_p$) with time constant $\tau_p$, $CO_2$ sensitivity $S_p$, off-set $B$ and transport delay time $T_p$.

The total ventilation $\dot{V}_E$ is given by

$$\dot{V}_E = \dot{V}_c + \dot{V}_p. \tag{3}$$

As input function we choose a step-ramp input. The change in $P_{ET,CO_2}$ with time is then given by

$$\Delta P_{ET,CO_2} = A + R.t, \quad (t > 0), \tag{4}$$

in which $A$ is the step increase in $P_{ET,CO_2}$ at $t = 0$ and $R$ the rate of rise. The change in ventilation $\Delta\dot{V}_E$ following this change in $P_{ET,CO_2}$ is given by:

$$\Delta\dot{V}_E = \Delta\dot{V}_c + \Delta\dot{V}_p$$

with
$$\Delta\dot{V}_c(t) = S_c[(A - R.\tau_c) + R(t-T_c) + (R.\tau_c - A)\exp\{-(t-T_c)/\tau_c\}]\,U(t-T_c) \tag{5}$$

and
$$\Delta\dot{V}_p(t) = S_p[(A - R.\tau_p) + R(t-T_p) + (R.\tau_p - A)\exp\{-(t-T_p)/\tau_p\}]\,U(t-T_p) \tag{6}$$

in which the unit step function $U(t)$ is defined by

$$U(t) = 0, \quad (t < 0)$$

and

$$U(t) = 1, \quad (t > 0).$$

The time constant $\tau_p$ and time delay $T_p$ of the peripheral chemoreflex loop is of the order of 5 s, so that after about 20 s the change in ventilation $\Delta \dot{V}_p(t)$ is to a good approximation

$$\Delta \dot{V}_p(t) = S_p[(A - R.\tau_p) + R(t - T_p)]. \tag{7}$$

Hence, introducing $r = S_p/S_c$ and $S = S_c + S_p$, using eqn. (4) we find

$$\Delta \dot{V}_E = S\left[\Delta P_{ET,CO_2} - \frac{R}{1+r}\left\{(\tau_c + T_c) + r(\tau_p + T_p) + \frac{R\tau_c - A}{R}\exp\left[-\frac{(t-T_c)}{\tau_c}\right]\right\}\right]. \tag{8}$$

If we choose the ratio of $A/R$ equal to $\tau_c$, eqn. (8) becomes

$$\Delta \dot{V}_E = S\left[\Delta P_{ET,CO_2} - \frac{R}{1+r}\{(\tau_c + T_c) + r(\tau_p + T_p)\}\right]. \tag{9}$$

The steady-state ventilatory response line is (see eqns. (1), (2) and (3))

$$\dot{V}_E = S(P_{ET,CO_2} - B). \tag{10}$$

We thus see, that if we choose $A/R$ equal to $\tau_c$, the ventilation following a step-ramp change in $P_{ET,CO_2}$ is parallel (after about 20 s) to the steady-state response line (eqn. 10) but shifted to the right along the $P_{ET,CO_2}$ axis by an amount

$$\Delta B = \frac{R}{1+r}\{(\tau_c + T_c) + r(\tau_p + T_p)\}. \tag{11}$$

Substituting $R = A/\tau_c$, eqn. (11) is equivalent to

$$\Delta B = \frac{A}{\tau_c(1+r)}\{(\tau_c + T_c) + r(\tau_p + T_p)\}. \tag{12}$$

When the contribution of the peripheral chemoreceptors is negligible, as is the case in human beings during hyperoxia, eqn. (12) reduces to

$$\Delta B = A(1 + T_c/\tau_c). \tag{13}$$

In human beings $T_c/\tau_c$ is about 0·15 (Ward & Bellville, 1983) so that $\Delta B$ is somewhat larger than $A$. In cats the contribution of the peripheral chemoreceptors is not negligible. Also in that case the shift to the right of the non-steady-state curve is close to $A$.

*Simulations*

We made a number of simulations using the above equations using the parameter set given in Table 1. Fig. 1 shows the result of two simulations with different values for $A$ and $R$ but with their ratio equal to $\tau_c$. The beginnings of these curves are curvilinear due to the influence of the peripheral chemoreceptors. This curvilinearity is negligible after 20 s (4 times the peripheral time constant). Therefore, the first 20 s of the ventilatory response were omitted in the analysis of the experimentally obtained response curves. It is clear that the slopes of the linear part of the lines are the same; the intercepts are according to eqn. (12) shifted to values slightly lower than $A$. Fig. 2 shows the results of simulations in which $A/R$ is greater and smaller than $\tau_c$. It illustrates that when $A/R$ is greater than $\tau_c$ the curve is concave towards the $P_{ET,CO_2}$ axis, the curvature being larger for higher values of $A$. A convex curve is found for $A/R$ smaller than $\tau_c$. In general when such curves are fitted with a linear function the slope will be higher than the slope of the steady-state $\dot{V}_E$–$P_{ET,CO_2}$ curve for $A/R > \tau_c$ and smaller for $A/R < \tau_c$. The deviation ($\Delta S_d(t)$) from the steady-state slope $S$ is found by differentiating eqn. (8) with respect to $\Delta P_{ET,CO_2}$ and as function of time is given by

$$\frac{\Delta S_d(t)}{S} = \frac{1 - \dfrac{A}{R\tau_c}}{1+r}\exp\left\{-\frac{(t-T_c)}{\tau_c}\right\}. \tag{14}$$

We will use this equation to estimate the magnitude of the deviation from the steady-state slope.

TABLE 1. Parameter set used for simulations

| Peripheral component | Central component |
|---|---|
| $S_p = 0{\cdot}25$ l min$^{-1}$ kPa$^{-1}$ | $S_c = 1$ l min$^{-1}$ kPa$^{-1}$ |
| $\tau_p = 5$ s | $\tau_c = 120$ s |
| $T_p = 4$ s | $T_c = 6$ s |

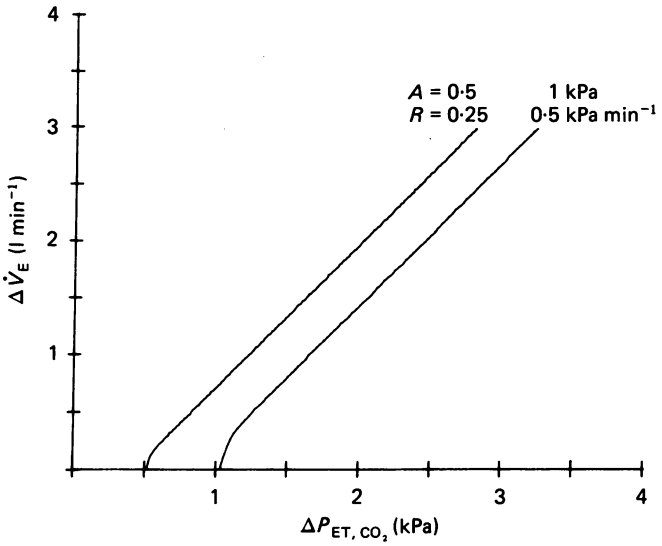


Fig. 1. Change in ventilation as a function of the change in $P_{ET,CO_2}$ obtained from simulations with the parameter set given in Table 1. $A/R$ was made equal to a central time constant $\tau_c$ of 2 min.

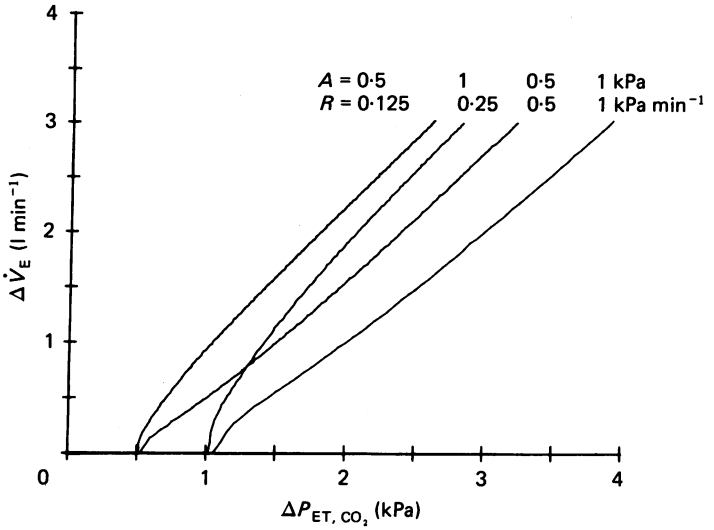


Fig. 2 Simulations of the ventilatory response to $CO_2$ for values of $A/R$ of 4 and 1 min.

*Procedures*

Experiments were performed on twenty adult cats of either sex anaesthetized with chloralose (20 mg $kg^{-1}$ I.V.) and urethane (100 mg $kg^{-1}$ I.V.) after induction with ketamine hydrochloride (15 mg $kg^{-1}$ I.M.). Anaesthesia was maintained with a continuous infusion of 1·0 mg $kg^{-1}$ $h^{-1}$ chloralose and 5 mg $kg^{-1}$ $h^{-1}$ urethane I.V. A polyethylene catheter was inserted into a femoral artery and blood pressure was monitored with a Statham transducer. In eight cats a catheter was inserted via a femoral vein into the vena cava for administration of a HCl or a $NaHCO_3$ solution. The trachea was cannulated and connected via a Fleisch No. 0 flow transducer head to a T-piece. One arm of the T-piece was receiving a gas mixture with a flow of 5 l $min^{-1}$ from a gas mixing system. The gas mixing system consists of three mass flow controllers (Type AFC-260, Advanced Semiconductor Materials, The Netherlands) by which the flows of pure $O_2$, and $N_2$ and $CO_2$ could be set at a desired level. A microcomputer (PDP LSI 11/23) delivered the steering signal for the mass flow controllers in order to adjust the composition of the inspiratory gas mixture so that the $P_{ET,CO_2}$ could be forced to follow a specific dynamic pattern in time while the end-tidal $P_{O_2}$ ($P_{ET,O_2}$) was kept constant at a normoxic value (15 kPa). This dynamic end-tidal forcing technique, which we have appropriately adjusted for use in cats, has been described in detail by Swanson & Bellville (1975).

*Measurements*

Tracheal gas flow was measured with a Fleisch No. 0 pneumotachograph connected to a differential pressure transducer (Statham). The flow signal was electronically integrated to yield a volume signal. The $CO_2$ concentration in tracheal gas was measured with a fast infra-red analyser (Gould Godart Mk2 capnograph, The Netherlands) and the $O_2$ concentration with a fast zirconium oxide cell (Jaeger $O_2$ test, F.R.G.). All signals were recorded on polygraphs, digitized (sample frequency 40 Hz) and processed by a PDP 11/23 microcomputer. Steady-state values of ventilation, $P_{ET,CO_2}$ and $P_{ET,O_2}$ were averaged over twenty breaths. For the dynamic end-tidal forcing experiments, tidal volume, ventilation and $P_{ET,CO_2}$ were determined by the microcomputer and stored on a breath-by-breath basis. Details about the measurements are described earlier (Berkenbosch, Heeringa, Olievier & Kruyt, 1979). The acid–base status of the animals was determined with a conventional sample method (Radiometer BMS2 Mk2, Denmark) in blood samples drawn at regular time intervals from a femoral artery.

*Experimental protocol*

All experiments were performed during normoxia ($P_{ET,O_2}$ 15 kPa). Every non-steady-state response was followed or preceded by a steady-state one. A comparison of these two types of responses was therefore little hampered by slow fluctuations with time of the responsiveness to $CO_2$ for example caused by slow changes in the depth of anaesthesia.

The non-steady-state ventilatory response was determined by a step-wise increase of $P_{ET,CO_2}$ followed by a constant rate of rise in $P_{ET,CO_2}$ for 3 or 4 min after which the $P_{ET,CO_2}$ was decreased to its original value. The step was varied between 0 and 1·5 kPa and the rate of rise between 0·2 and 1·5 kPa $min^{-1}$, although in the majority of the experiments (96 out of 140) $R$ was 0·27 kPa $min^{-1}$. The steady-state ventilation was determined to two levels of $P_{ET,CO_2}$ using two somewhat different methods. In the first method $P_{ET,CO_2}$ was increased step-wise from the resting level (about 4 kPa) by about 1 kPa. After 7 min when ventilation had reached a new steady-state the $P_{ET,CO_2}$ was decreased to its original value and the new steady-state ventilation was determined after 7 min. In the second method after the step-ramp $CO_2$ challenge the $P_{ET,CO_2}$ was decreased by the same amount as the step in the beginning and kept at this value till ventilation reached a new steady state. Subsequently the $P_{ET,CO_2}$ was returned to its starting value.

In all twenty cats under normal acid–base conditions 140 paired observations of the non-steady-state and the steady state were obtained. In eight of these cats after the measurements under normal acid–base conditions the acid–base balance was changed by intravenous infusion of a HCl solution (0·3 mol $l^{-1}$) or a $NaHCO_3$ solution (0·6 mol $l^{-1}$) at a rate of 0·15 mmol $min^{-1}$. After about 10 min the infusion rate was decreased to about 0·03 mmol $min^{-1}$. During metabolic acidosis (mean decrease in arterial $HCO_3^-$ concentration 6·6 mmol $l^{-1}$) thirty paired observations of the steady-state and non-steady-state ventilatory response were assessed. During metabolic alkalosis (mean increase in $HCO_3^-$ concentration 6·7 mmol $l^{-1}$) twenty-four paired observations were obtained.

*Data analysis*

The steady-state ventilatory response line was determined according to eqn. (10).

The non-steady-state ventilatory sensitivity to $CO_2$ of each run was determined by taking only the breath-by-breath ventilation together with the $P_{ET,CO_2}$ of that part of the run during which the $P_{ET,CO_2}$ linearly increased. The first 20 s after the step increase were not used. The non-steady-state ventilatory $CO_2$ sensitivity and the extrapolated $P_{ET,CO_2}$ at zero ventilation were

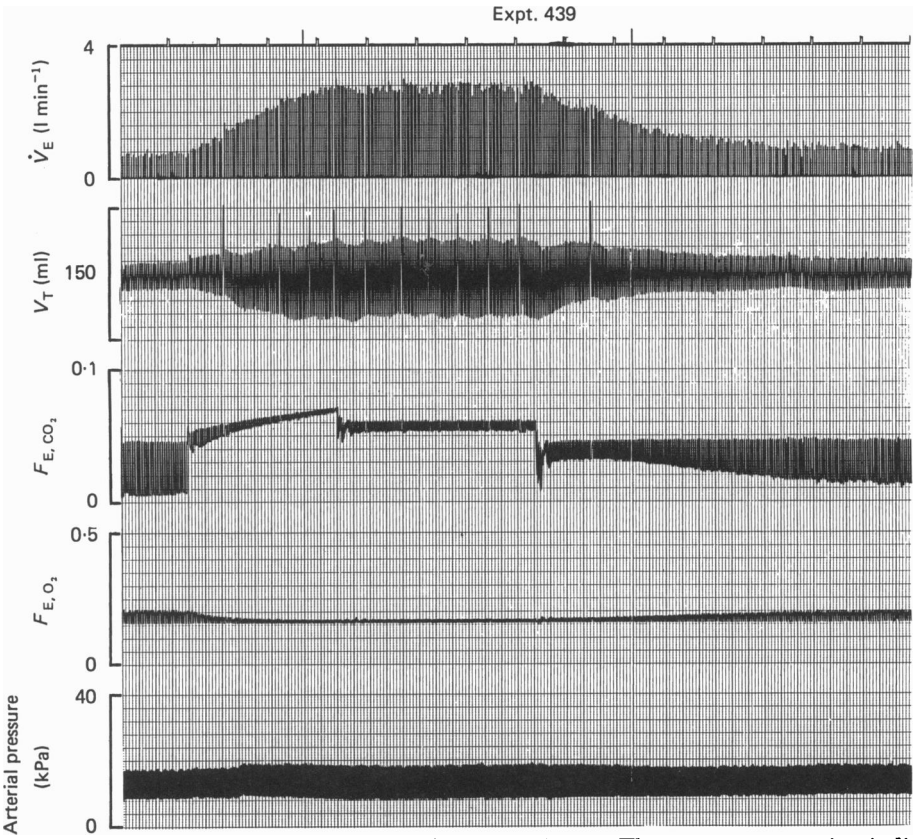


Fig. 3 Recording of a pseudo-rebreathing experiment. The uppermost tracing indicates 1 min time intervals. $F_{E,CO_2}$ and $F_{E,O_2}$ are the fraction of $CO_2$ and $O_2$ in tracheal gas. Note the immediate linear increase of breath-by-breath ventilation with time after the step-wise increase in $P_{ET,CO_2}$ followed by the ramp. Ventilation is immediately constant when the $P_{ET,CO_2}$ at the end of the ramp is decreased with the same amount as at the step increase at the beginning of the $CO_2$ challenge. A further step-wise decrease in $P_{ET,CO_2}$ leads to an exponential decay of the ventilation.

calculated by linear regression of $\dot{V}_E$ on $P_{ET,CO_2}$ (see Fig. 5). The rate of rise $R$ was calculated for each run from linear regression of $P_{ET,CO_2}$ on time. To obtain an estimate for $A$, the intercept of this line with the $P_{ET,CO_2}$ axis was diminished with the mean $P_{ET,CO_2}$ of twenty breaths preceding the step increase in $P_{ET,CO_2}$.

## RESULTS

In Fig. 3 a recording of an experiment is given in which the non-steady-state and the steady-state ventilatory response to $CO_2$ are shown together. After a steady-state level of ventilation the $P_{ET,CO_2}$ was suddenly increased with 0·8 kPa, whereafter the

$P_{ET,CO_2}$ increased with a rate of rise of 0·55 kPa $min^{-1}$. Breath-by-breath ventilation increased linearly with time. After 3 min when $\dot{V}_E$ had increased to 2·8 l $min^{-1}$ the $P_{ET,CO_2}$ was suddenly decreased by 0·8 kPa. Although ventilation appears to be constant in a few seconds the ventilation was followed for another 4 min keeping the $P_{ET,CO_2}$ constant. Thereafter the $P_{ET,CO_2}$ was decreased to its original level and ventilation decayed to its former value in about 7 min. Fig. 4 shows part of the

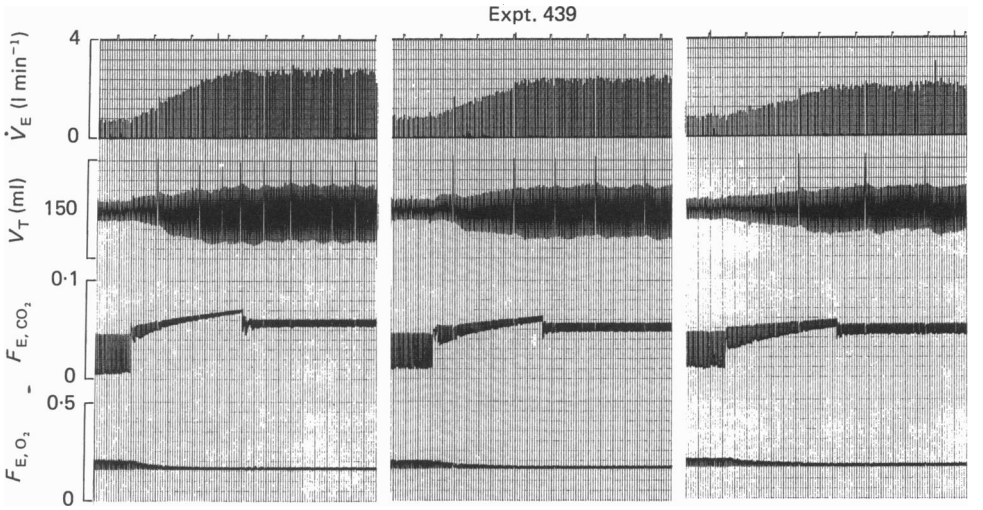


Fig. 4 Oscillographic recording of three non-steady-state responses of the same cat measured with different values of $A$ and $R$. However, $A/R$ was about the same. $F_{E,CO_2}$ and $F_{E,O_2}$ are the fractions of $CO_2$ and $O_2$ in tracheal gas.

recording of Fig. 3 together with two others obtained in the same cat. The step increase $A$ was varied with the rate of rise $R$ in such a way that $A/R$ was nearly the same in all three runs. It shows that the breath-by-breath ventilation increased linearly with time in all three cases. At the end of the ramp the $P_{ET,CO_2}$ was decreased by the value of $A$ and ventilation reached a new steady-state in a few seconds.

In Fig. 5 the non-steady-state ventilatory responses of Fig. 4 together with the steady-state data are plotted. These results illustrate that when $A$ and $R$ are such that $A/R$ has the same value, the slope of the response curves are about the same. The intercepts on the $P_{ET,CO_2}$ axis are, however, different. The intercept increases with $A$. The value of the ratio of $A$ and $R$ determines whether the slope of the non-steady-state ventilatory response is different from that of the steady-state one. This is also demonstrated in Fig. 6 where the difference in slope between the non-steady-state and the accompanying slope of the steady state ($\Delta S$) as a fraction of the steady-state slope is plotted against $A/R$ of the data of all cats. Using the data obtained under normal acid–base conditions a significant correlation between the $\Delta S/S$ and $A/R$ was found ($r = 0{\cdot}67$, $n = 140$). Linear regression analysis showed that the slope of this relation was 0·152 $min^{-1}$ with intercept −0·316. From these results it can be readily calculated that when $A/R$ is 2·1 min there is no difference between the non-steady-state and the steady-state slope of the ventilatory response to $CO_2$.

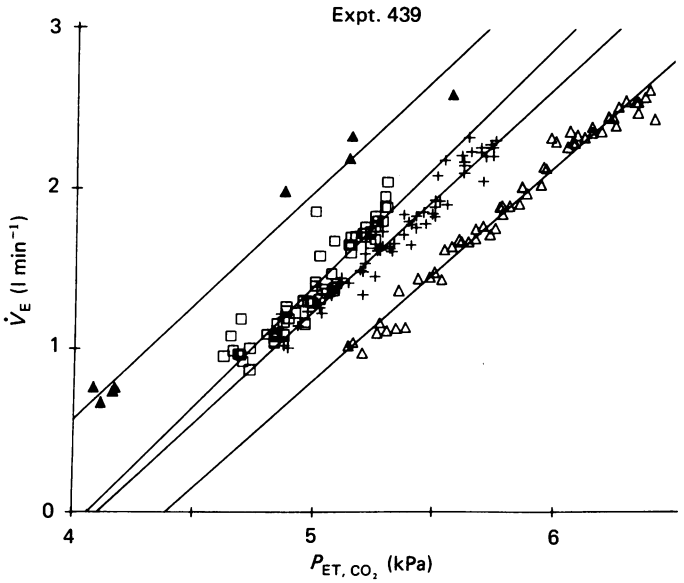


Fig. 5. Ventilatory responses obtained with the steady-state method (▲) and the pseudo-rebreathing method in the same cat. Data of the recording of Fig. 4. △, $A$ is 0·8 kPa, $R$ is 0·55 kPa min$^{-1}$; +, $A$ is 0·58 kPa, $R$ is 0·37 kPa min$^{-1}$; □, $A$ is 0·40 kPa, $R$ is 0·26 kPa min$^{-1}$.

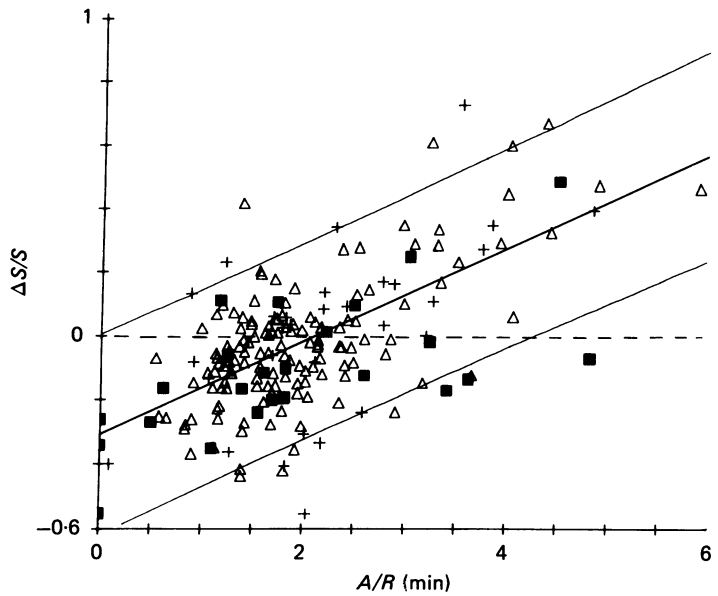


Fig. 6 Difference between slopes measured with the pseudo-rebreathing method and the steady-state method as a fraction of the steady-state slope as a function of the ratio $A/R$. The drawn lines are the regression line and the 95 % one-at-the-time prediction intervals for the data measured under normal acid–base conditions (△). + and ■, values obtained during metabolic acidosis and alkalosis respectively. Data of all cats.

At values of $A/R$ greater than 2·1 min the non-steady-state slope of the $CO_2$ response curve is larger than the steady-state slope and for values of $A/R$ smaller than 2·1 min the reverse is found. The deviation of the non-steady-state slope is 15 % for a change in $A/R$ of 1 min. In Fig. 6 the data obtained during metabolic acidosis and alkalosis are also shown. These data are not manifestly different from those obtained under normal acid–base conditions. The parameters of the regression lines

$$\Delta S/S = H \,.\, A/R + I,$$

TABLE 2. Slope ($H$) and intercept ($I$) of the relation $\Delta S/S = H \,.\, A/R + I$, during metabolic alkalosis and acidosis and normal acid–base conditions

| | Normal | Alkalosis | Acidosis |
|---|---|---|---|
| Slope ± S.D. ($min^{-1}$) | 0·152 ± 0·014 | 0·100 ± 0·026 | 0·156 ± 0·042 |
| Intercept ± S.D. | −0·316 ± 0·031 | −0·296 ± 0·061 | −0·334 ± 0·104 |
| Runs | 140 | 24 | 30 |

during metabolic acidosis and alkalosis are given in Table 2. For all the measurements where $A/R$ had a value between 1·6 and 2·4 min and $R$ was about 0·25 kPa $min^{-1}$, we calculated the difference between the extrapolated $P_{ET,CO_2}$ at zero ventilation of the non-steady-state response curve and the steady-state one and subtracted from this value the accompanying step increase $A$ in $P_{ET,CO_2}$. In forty runs a mean value (with standard deviation) of −0·14 ± 0·28 kPa was found. This value was significantly different from zero ($P = 0·004$).

## DISCUSSION

In this paper we studied the dynamics of the ventilation following a step-ramp input function. Such input functions are used in the rebreathing method of Read (1967). Our results show that in cats during normoxia and normal acid–base balance a value of $A/R$ of 2·1 min leads to the correct estimation of the steady-state $CO_2$ sensitivity. This compares favourably with the value of the central time constant of the ventilatory on-transient following a step increase in $P_{ET,CO_2}$ (DeGoede *et al.* 1985). After acute alkalosis the optimum $A/R$ was 3·0 min. Although this value is not significantly different from that during normal acid–base balance it may indicate that there is a tendency for the optimum $A/R$ value to be higher during alkalosis. During acute acidosis an optimum $A/R$ value of 2·1 min was found to be close to the value found during normal acid–base balance. From these results together with our simulations we therefore concluded that step-ramp input functions can also successfully be used in cats to estimate the steady-state ventilatory sensitivity to $CO_2$, provided that the ratio of $A/R$ equals the time constant of the central chemoreflex loop. We emphasize that this can be achieved by an infinite number of combinations of $A$ and $R$ (see Fig. 5). Due to the fast response of the peripheral chemoreceptors to $CO_2$ challenges the steady-state $CO_2$ sensitivity can also be assessed in the presence of a peripheral ventilatory component omitting about 20 s of the beginning of the response and keeping the $P_{ET,O_2}$ constant during the test. By decreasing the $P_{ET,CO_2}$ at the end of the ramp by the value of $A$ a steady-state ventilation is obtained after 20 s (Fig. 3) provided $A/R = \tau_c$, a result which follows

from our two-compartment model. This procedure can therefore be used to shorten the time necessary to obtain a new steady state after a change in $P_{ET,CO_2}$.

The coefficient of variation of the residuals of the regression line of $\Delta S/S$ *vs.* $A/R$ is about 18 %. In repeat experiments in one cat the coefficient of variation of the $CO_2$ sensitivity was roughly 15 % (DeGoede *et al.* 1985). This suggests that pooling the data of different cats does not introduce an additional scatter in this study. From eqn. (14) it is clear that the deviation $(\Delta S_d(t)/S)$ from the steady-state $CO_2$ sensitivity depends on the duration of the rebreathing. Using the same parameter values as in our simulations (Table 1), a data set which corresponds reasonably well with values normally found in cats (DeGoede *et al.* 1985) we calculate, if rebreathing is performed with a value of $A/R$ 1 min different from $\tau_c$, that after 20 s the deviation from the steady-state $CO_2$ sensitivity is 36 %. After 3 min rebreathing this value diminished to 5 %. As all the data between 20 s and 3 min are used for the determination of the slope of the curve, the deviation $(\Delta S/S)$ experimentally found will be between 5 and 36 %. We found 15 % deviation from the steady-state $CO_2$ sensitivity for a change of 1 min in $A/R$ from the optimum value.

The $P_{ET,CO_2}$ at zero ventilation found from rebreathing experiments with an optimum ratio of $A$ and $R$ is shifted to the right compared to the steady-state value. The magnitude of the shift can be calculated from eqn. (12). Using the data from Table 1 and an $A/R$ of 2 min and a $R$ of 0·25 kPa $min^{-1}$ a value of −0·15 kPa for the shift diminished with the step $A$ is calculated. Experimentally we found a mean value of −0·14 kPa with a standard error of 0·04 kPa which is in good agreement with the calculated value. Therefore a reasonable estimate of the steady-state intercept can be made by shifting the response line in the pseudo-rebreathing method by a value of $A$ to lower $P_{ET,CO_2}$ values.

*Comparison with the rebreathing method in the human*

From a theoretical analysis of the $CO_2$ exchange of medullary chemoreceptor tissue Read & Leigh (1967) concluded that when rebreathing is performed in such a way that negligible amounts of $CO_2$ exchange between blood and the hyperoxic gas mixture in the rebreathing bag, the change in brain tissue $P_{CO_2}$ is virtually equal to the change in arterial $P_{CO_2}$ and that the arterial $P_{CO_2}$ is a reasonable index of the intracranial stimulus. The ventilatory $CO_2$ sensitivity measured with the rebreathing technique should therefore be equal to the $CO_2$ sensitivity at the site of the intracranial chemoreceptors since the contribution of the peripheral chemoreceptors in human beings during hyperoxia is negligible. However, this conclusion cannot be entirely right from a physiological point of view. It is known that cerebral blood flow increases with a rise in arterial $P_{CO_2}$ $(P_{a,CO_2})$, consequently the ventilatory $CO_2$ sensitivity to a change in brain tissue $P_{CO_2}$ ought to be larger than the $CO_2$ sensitivity to a change in $P_{a,CO_2}$. Assuming that brain tissue $P_{CO_2}$ is the mean of arterial and cerebral venous $P_{CO_2}$ (Pontèn & Siesjö, 1966) it can be calculated from data of Fencl, Vale & Broch (1969) that in human beings the slope of the curve relating ventilation to brain tissue $P_{CO_2}$ is about 30 % larger than the slope of the $\dot{V}_E$–$P_{a,CO_2}$ curve. Such differences were, however, not found experimentally by Read (1967). Interestingly, the theoretical analysis of Read & Leigh (1967) also leads to the result that the sensitivity obtained from rebreathing is larger than from the steady-state method

(see their Fig. 11 *A*). Although they attributed this discrepancy to the relatively small $P_{a,CO_2}$ range (5·3–8 kPa) covered in the rebreathing experiments, we think that this discrepancy is due to the simplifying assumptions in their theoretical model. For instance, it is assumed that ventilation is an instantaneous function of tissue $P_{CO_2}$. In cats there is evidence of considerable neuronal dynamics in the ventilatory response to changes in $P_{ET,CO_2}$ (Teppema, Vis, Evers & Folgering, 1982). Our empirical two-compartment model leads to the conclusion, provided the central time constant does not change appreciably with cerebral blood flow, that in a rebreathing experiment the steady-state $CO_2$ sensitivity should be obtained. It is of considerable interest to note that Read's condition for human beings of a step increase in $P_{ET,CO_2}$ of about 8 torr and a rate of rise of about 6 torr $min^{-1}$ gives for $A/R$ a value of 1·3 min, a value which is in good agreement with a central time constant of 1·2 min reported by Ward & Bellville (1983). Therefore we think that the essence of Read's rebreathing method is the condition that $A/R$ is equal to the time constant of the central chemoreflex pathway rather than the condition that the difference between arterial and tissue $P_{CO_2}$ should be virtually zero. This view is supported by our results in cats where we showed that different combinations of $A$ and $R$ led to the steady-state slope, provided $A/R$ equals $\tau_c$. The condition realized in Read's rebreathing method, that there is negligible $CO_2$ exchange between bag and lungs is to be regarded as an experimental condition leading to stable experiments in which the rate of rise in $P_{ET,CO_2}$ is independent of the level of ventilation rather than as a condition dictated by physiological mechanisms.

An equation of the type of eqn. (1) can be derived from physiological considerations (cf. Read & Leigh, 1967). The time constant $\tau_c$ is then found to be inversely proportional to the cerebral blood flow. In our model we assumed $\tau_c$ to be constant. This may be a first approximation in our analysis as the blood flow is known to vary with $P_{CO_2}$, but the approximation seems to be reasonably good, at least in cats. The question whether the time constant $\tau_c$ depends on cerebral blood flow in the ventilatory on-transient has not been investigated. There is evidence, however, that the time constant of the on-transient is different from that of the off-transient in cats as well as in human beings. It is possible that this difference is due to the net result of changes in cerebral blood flow and neuronal dynamics.

When the rate of rise $R$ was lowered to about one-third of Read's condition an increase in $CO_2$ sensitivity (mean 45 %) was found (Read, 1967). This change was, however, of doubtful significance due to the large scatter of the data. Increasing $A/R$ by decreasing $R$ leads to an increased slope of the rebreathing curve compared to the steady-state one (see Fig. 2). The deviation from the steady-state slope depends on the duration of the rebreathing (eqn. (14)): the longer the duration of the rebreathing the smaller the deviation of the slope. From the data presented by Read (1967) it is not clear how long the rebreathing with a diminished $R$ was performed, but it can be deduced from his Fig. 3 that about the same $P_{ET,CO_2}$ range was covered, indicating that the rebreathing with diminished $R$ was performed during a considerably longer time than the normal 4 min. The deviation from the steady-state slope will then be less than when a rebreathing time of 4 min is used. Keeping in mind that the coefficient of variation of the slope is of the order of 20 % his results are therefore not in contradiction with ours.

When Read (1967) increased $A$ by hyperventilation he did not observe a significant change in slope. However, in that case he only used the linear portion of the response curves at higher levels of $P_{ET,CO_2}$ and it can be seen from our simulations that the higher the $P_{ET,CO_2}$ the less the deviation is from the steady-state slope.

Linton *et al.* (1973) observed that under conditions of metabolic acidosis and alkalosis the slope of the rebreathing curve was increased and decreased respectively compared to the steady-state curve. In the light of the above discussion their results can be readily explained qualitatively.

First of all they used the same gas mixture for the initial filling of the bag under conditions of metabolic acidosis and alkalosis. However, it can be seen from their data that the resting $P_{ET,CO_2}$ was decreased during acidosis and increased during alkalosis. This means that the value of $A$ was increased during acidosis. As the rate of rise remained about the same the value of $A/R$ was too large and consequently led to an over-estimation of the steady-state slope (see Fig. 2). During alkalosis $A$ was decreased compared to the value they used during normal acid–base balance ($R$ being again unchanged) and therefore a diminished slope may be expected just as they observed.

Secondly it is reported that cerebral blood flow increases during chronic metabolic acidosis. The reverse happens during metabolic alkalosis (Fencl *et al.* 1969). The central time constant $\tau_c$ might therefore be decreased during metabolic acidosis and increased during alkalosis. Therefore to estimate the steady-state slope using a rebreathing method it may be that $A/R$ should be decreased during acidosis and increased during alkalosis, but just the opposite was done by Linton *et al.* (1973). One can then expect an increased slope during acidosis and the reverse during alkalosis as was indeed reported by Linton *et al.* (1973). Our results also show that during metabolic acidosis and alkalosis reliable estimates of the steady-state slope can be obtained by step-ramp functions when the appropriate value of $A/R$ is used. To estimate the ventilatory $CO_2$ sensitivity under conditions where resting $P_{ET,CO_2}$, cerebral blood flow or metabolic $CO_2$ production are changed (e.g. studies on the effect of drugs on control of breathing), rebreathing methods are frequently used with the standard conditions formulated by Read (1967). However, the results should be interpreted with care because as shown in this study deviations from the steady-state $CO_2$ sensitivity have to be expected.

The technical help of Mr L. Philips is gratefully acknowledged. This research was supported by the Foundation for Medical Research FUNGO which is subsidized by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

## REFERENCES

BELLVILLE, J. W., WHIPP, B. J., KAUFMAN, R. D., SWANSON, G. D., AQLEH, K. A. & WIBERG, D. M. (1979). Central and peripheral chemoreflex loop gain in normal and carotid body-resected subjects. *Journal of Applied Physiology* **46**, 843–853.

BERKENBOSCH, A., HEERINGA, J., OLIEVIER, C. N. & KRUYT, E. W. (1979). Artificial perfusion of the ponto-medullary region of cats. A method for separation of central and peripheral effects of chemical stimulation of ventilation. *Respiration Physiology* **37**, 347–364.

DEGOEDE, J., BERKENBOSCH, A., WARD, D. S., BELLVILLE, J. W. & OLIEVIER, C. N. (1985). Comparison of chemoreflex gains obtained with two different methods in cats. *Journal of Applied Physiology* **59**, 170–179.

FENCL, V., VALE, J. R. & BROCH, J. A. (1969). Respiration and cerebral blood flow in metabolic acidosis and alkalosis in humans. *Journal of Applied Physiology* **27**, 67–76.

LINTON, R. A. F., POOLE-WILSON, P. A., DAVIES, R. J. & CAMERON, I. R. (1973). A comparison of the ventilatory response to carbon dioxide by steady-state and rebreathing methods during metabolic acidosis and alkalosis. *Clinical Science and Molecular Medicine* **45**, 239–249.

PONTÈN, U. & SIESJÖ, B. K. (1966). Gradients of $CO_2$ tension in the brain. *Acta physiologica scandinavica* **67**, 129–140.

READ, D. J. C. (1967). A clinical method for assessing the ventilatory response to carbon dioxide. *Australasian Annals of Medicine* **16**, 20–32.

READ, D. J. C. & LEIGH, J. (1967). Blood–brain tissue $P_{CO_2}$ relationships and ventilation during rebreathing. *Journal of Applied Physiology* **23**, 53–70.

SWANSON, G. D. & BELLVILLE, J. W. (1975). Step changes in end-tidal $CO_2$: methods and implications. *Journal of Applied Physiology* **39**, 377–385.

TEPPEMA, L. J., VIS, A., EVERS, J. A. M. & FOLGERING, H. TH. (1982). Dynamics of brain extracellular fluid pH and phrenic nerve activity in cats after end-tidal $CO_2$ forcing. *Respiration Physiology* **50**, 359–380.

WARD, D. S. & BELLVILLE, J. W. (1983). Effect of intravenous dopamine on hypercapnic ventilatory response in humans. *Journal of Applied Physiology* **55**, 1418–1425.