J. Physiol. (1982), **329**, *pp.* 57–73
With 6 text-figures
Printed in Great Britain

THE pH OSCILLATIONS IN ARTERIAL BLOOD DURING EXERCISE; A POTENTIAL SIGNAL FOR THE VENTILATORY RESPONSE IN THE DOG

BY BRENDA A. CROSS, A. DAVEY, A. GUZ, P. G. KATONA*, M. MACLEAN, K. MURPHY, S. J. G. SEMPLE, AND R. STIDWILL

From the Departments of Medicine and Anaesthesia, Charing Cross Hospital Medical School, London, W.6, and the Department of Medicine, Middlesex Hospital Medical School, London, W.1

(*Received 14 August 1981*)

SUMMARY

1. The effect of electrically induced 'exercise' on the respiratory oscillation of arterial pH was studied in chloralose-anaesthetized dogs with spinal cord transection at T8/9 (dermatome level T6/7).

2. Respiratory oscillations of arterial pH (presumed to be due to oscillations of arterial P_{CO_2}) were sensed with a fast-responding electrode in one carotid artery. Breath-by-breath estimates of the maximum rate of change of pH of the downstroke of the pH oscillation (dpH/dt↓max) were obtained by differentiating the pH signal.

3. Consistent with the findings of the previous paper (Cross *et al.* 1982), the ventilatory response to exercise could not be explained on the basis of sensitivity to CO_2; the $\Delta \dot{V}_I/\Delta P_{a,CO_2}$ was significantly greater for 'exercise' than for CO_2 inhalation.

4. On average, the amplitude of the pH oscillations decreased during 'exercise'. The change in the phase relationship (ϕ) between respiratory and pH cycles, although significant from the second breath onwards, was not thought to be responsible for the increased ventilation $\dot{V}_I$; the direction of the change was opposite to that previously found to increase $\dot{V}_I$.

5. Inspiratory duration (t_i), expiratory duration (t_e), $\dot{V}_I$ and the dpH/dt↓max changed significantly by the third breath of 'exercise'. A significantly linear relationship was obtained between t_e and dpH/dt↓max during the on-transient (first ten breaths) of 'exercise'. This relationship was maintained throughout 'exercise'. $\dot{V}_I$ and dpH/dt↓max were also linearly related during the on-transient, although the same relationship did not hold true throughout 'exercise'.

6. The dpH/dt↓max was related to CO_2 production ($\dot{V}_{CO_2}$) lending support to the prediction that the slope of the downstroke of the pH oscillation is a function of $\dot{V}_{CO_2}$.

7. It was concluded that the dpH/dt↓max (dpCO_2/dt↑max) is a potential humoral signal in 'exercise' and could account totally for the shortening of t_e. Since there was a late rise in $\dot{V}_I$ (due to an increase in tidal volume V_T) in the absence of a change in dpH/dt↓max, it was considered unlikely that the dpH/dt↓max was the only humoral signal present during 'exercise'.

* Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH, U.S.A.

INTRODUCTION

In the previous paper (Cross, Davey, Guz, Katona, MacLean, Murphy, Semple & Stidwill, 1982) it was shown that the close link between ventilation and CO_2 production during electrically induced 'exercise' was not dependent on an intact spinal cord. A humoral signal must therefore be found to account for this link between pulmonary ventilation, and carbon dioxide output ($\dot{V}_{CO_2}$) in exercise, whether this be on the venous or arterial side of the circulation. Evidence for and against the presence of venous chemoreceptors is conflicting (Cropp & Comroe, 1961; Storey & Butler, 1963; Hildebrandt, Winn & Hildebrandt, 1979; Levine, 1979; Sheldon & Green, 1981), and it is therefore reasonable at this stage to establish whether there is a potential humoral signal in the arterial blood where the presence and characteristics of chemoreceptors has long been established.

There are many possible signals, but this study is concerned with two. Firstly there could be a very small change in the mean level of the gas tensions or pH, undetected by measurement on arterial blood samples because of the moment-to-moment variation in ventilation and the errors of measurement of pH, blood gases and ventilation. Secondly, the signal could be due to a change in the pattern of the arterial P_{CO_2} or pH oscillations in arterial blood (Yamamoto, 1960; Saunders, 1980) or alteration in their temporal relationships to the ventilatory cycle (Cross, Grant, Guz, Jones, Semple & Stidwill, 1979). Both the amplitude and the rate of change of P_{CO_2} on the upstroke of the oscillation (or rate of change of pH on the downstroke of the oscillation) are largely determined by $\dot{V}_{CO_2}$ and are therefore potential humoral signals linking ventilation and $\dot{V}_{CO_2}$ in exercise (Yamamoto, 1960; Band, McClelland, Phillips, Saunders & Wolff, 1978; Saunders, 1980; Cross, Jones, Leaver, Semple & Stidwill, 1981). The postulated humoral signals enumerated above could only be detected with certainty by a rapidly responding electrode system within the arterial blood. Such a system for measuring arterial pH has been used in the experiments described here (Band & Semple, 1967). Electrically induced 'exercise' has been carried out with the cord transected. Justification for using this preparation is that it leaves the respiratory response to exercise essentially intact and avoids the effects of stimulation of afferent fibres which may have no role in the physiological response to exercise (see Discussion in preceding paper). In this way it is possible to investigate the humoral control of the respiratory response to exercise alone. A preliminary communication of these findings has been given elsewhere (Cross, Guz, Katona, MacLean, Murphy, Semple & Stidwill, 1980).

METHODS

Experiments were performed on six dogs weighing between 11 and 18 kg. Anaesthesia, surgery, blood pressure and arterial blood gas measurements were carried out as in the preceding paper (Cross *et al.* 1982).

All experiments were done in animals with spinal cord transection performed as in the preceding paper (Cross *et al.* 1982). Following cord transection the animals were returned to the supine position and given heparin (I.V. 2000 u.) before insertion of a polyethylene loop (internal diameter 1·5 mm) into the right common carotid artery. Whole body heparinization was maintained with administration of 1000 u./hr of heparin. The loop had a side arm into which a needle could be inserted containing a fast response pH electrode. This technique for continuous recording of arterial pH has been

described elsewhere (Band & Semple, 1967; Cowell, Band & Semple, 1967). The glass electrode fitted closely into the lumen of a short length of needle tubing. Blood flowed through a narrow annular space between the electrode and the tubing, through a side arm on the needle, then past a saturated calomel reference electrode and returned into the right jugular vein via another polyethylene catheter.

The experimental arrangement, as well as the procedures and statistical analyses, were those outlined in the preceding paper (Cross *et al.* 1982).

Before starting the experimental procedure, any metabolic acidaemia was corrected with an 8·5 % solution of sodium bicarbonate. Further doses were given during the experiment as required. An initial period of not less than 30 min was allowed for an animal to reach a steady state before any experimental manoeuvres were carried out.

'Exercise' runs

Currents ranging from 6 to 30 mA were employed to produce varying degrees of electrically induced 'exercise'. 'Exercise' was initiated during expiration and was maintained for 4 min. Each exercise period was bounded by control periods of not less than 5 min. Recovery was usually achieved within 3–5 min. Expired gas was collected for 1 min during the control period and during the last minute of 'exercise'. Steady-state measurements of $\dot{V}_I$, P_{ET,CO_2}, P_{a,CO_2}, P_{a,O_2} and pH were made over the same intervals of time. A run was excluded from analysis if the ventilatory variables, recorded at 5 min after 'exercise' ceased, had not returned close to the values obtained immediately before 'exercise'.

Additional measurements were also made on each of the ten control breaths immediately before 'exercise', the first ten to twenty breaths of 'exercise' and the last ten breaths of 'exercise'. The following variables were measured: inspiratory duration (t_i); expiratory duration (t_e); breath duration (t_{tot}); tidal volume (V_T); ventilation ($\dot{V}_I$); end-tidal CO_2 (P_{ET,CO_2}); the circulation time (c.t.) from lung to pH electrode (measured from the start of inspiration to the start of the corresponding alkaline swing of pH); the duration of the pH cycle (pH_{tot}); the duration of the pH cycle up to the point of peak inspiration (pH_t); the phase relationship between respiratory and pH cycles ($\phi = pH_t/pH_{tot}$; see Fig. 1, Cross *et al.*, 1982 and Fig. 4; Cross *et al.* 1979), the amplitude of the pH cycle (pH amp); the maximum rate of fall of pH obtained from the first differential of the pH signal ($dpH/dt\downarrow max$); and the CO_2 production ($\dot{V}_{CO_2}$). The method used for computation of breath-by-breath $\dot{V}_{CO_2}$ and its validation are given in the preceding paper (Cross *et al.* 1982). Measurements of $dpH/dt\downarrow max$ were obtained by first passing the pH signal through a 2 Hz low pass filter to minimize noise resulting from recording the signal originally on analogue tape. The filtered signal was then amplified and electronically differentiated using an operational amplifier (Fig. 1). The act of filtering produced a 100 msec phase shift in the signal which although affecting the upstroke did not modify the downstroke ($dpH/dt\downarrow$). This differential was calibrated in each exercise run from the linear portion of the downstroke of the pH signal on the original record. We were able to read the dpH/dt trace to 0·25 mm, and this gave an average measurement error of 5 %.

RESULTS

Steady state

The effect of electrically induced 'exercise' was measured in twelve 'exercise' runs on six dogs with cord cut. Table 1 records blood pressure, heart rate, acid–base status of blood, P_{a,CO_2}–P_{ET,CO_2} gradient and rectal temperature before and during the fourth minute of 'exercise'. Blood pressure fell in nine of the runs and was unchanged in three; the drop in mean B.P. was significant ($P < 0·01$). The rise in mean heart rate just failed to reach statistical significance although it rose in nine runs, fell in one run and was unchanged in the remaining two. There was no significant change in any of the mean values for the other variables included in Table 1.

TABLE 1. Various circulatory and acid–base variables in arterial blood measured before and during the fourth minute of 'exercise'

	B.P. (mmHg)	Heart rate (min^{-1})	P_{a,CO_2} (mmHg)	P_{ET,CO_2} (mmHg)	$P_{a,CO_2}-P_{ET,CO_2}$ (mmHg)	P_{a,O_2} (mmHg)	pHa (u.)	Blood temp. (°C)	Rectal temp. (°C)	Base excess (m-mole $l.^{-1}$)
Control	100 (17)	181 (28)	39·0 (4·2)	35·1 (5·3)	3·9 (2·3)	89 (9)	7·423 (0·018)	37·3 (0·3)	37·4 (0·4)	−0·4 (1·7)
Exercise	91 (22)	188 (24)	38·8 (4·9)	34·6 (5·7)	4·2 (1·8)	90 (15)	7·424 (0·028)	37·3 (0·4)	37·3 (0·4)	−0·4 (1·7)

The mean results are shown (±1 S.D.) for twelve exercise runs in six dogs.

Measurement of the response to 'exercise' in the steady state

$\dot{V}_I$ and $\dot{V}_{CO_2}$ were measured over 1 min both before and during the fourth minute of 'exercise'; $\dot{V}_{CO_2}$ was obtained by gas collection. $\dot{V}_I$ and $\dot{V}_{CO_2}$ at rest were 4·44 l. min^{-1} (S.D., 0·69) and 80·2 ml. min^{-1} (S.D., 1·6) respectively, whilst the corresponding values on 'exercise' were 6·11 l. min^{-1} (S.D., 1·58) and 126·5 ml. min^{-1} (S.D., 37·5). The ventilatory equivalent for the change in CO_2 production ($\Delta\dot{V}_I/\Delta\dot{V}_{CO_2}$) was 36·9 (S.D., 10·5). The increase in $\dot{V}_I$ is very similar to that recorded in the preceding paper following cord transection but the rise in $\dot{V}_{CO_2}$ is somewhat less and hence $\Delta\dot{V}_I/\Delta\dot{V}_{CO_2}$ is greater.

In the twelve runs none of the conventional chemical stimuli could consistently account for the ventilatory response to 'exercise'. P_{a,CO_2} rose in five runs, fell in five runs and was unchanged in two whilst pH fell in seven runs, rose in four and was unchanged in one. There was no metabolic disturbance produced by 'exercise', in so far as it is possible to determine this, from the calculation of 'Base excess' (Table 1).

A more detailed analysis of the changes in ventilation together with the effects of 'exercise' on P_{ET,CO_2} and the pH oscillations are shown in Table 2. All the data in the steady state in this table are the means derived from ten breaths and are slightly different from the values obtained during the 1 min of gas collection which are quoted above.

In late exercise 52 % of the increase in $\dot{V}_I$ was due to the rise in V_T. The fall in t_{tot} was due to a decrease in t_i and t_e but the change in t_e was approximately twice that of t_i when both are expressed as a proportion of their control values. The increase in $dpH/dt\downarrow max$, the change in ϕ and the decrease in pH were both statistically significant but this was not so for the mean changes in P_{ET,CO_2} or c.t.

Ventilatory response to inhaled CO_2

This response was determined in five of the six dogs. $\Delta\dot{V}_I/\Delta P_{a,CO_2}$ ranged from 0·130 to 0·728 l. min^{-1} $mmHg^{-1}$ with a mean value of 0·505 l. min^{-1} $mmHg^{-1}$ (S.D., 0·248). In these five dogs, nine exercise runs were performed. P_{a,CO_2} rose in four runs by 2·2, 1·2, 1·2 and 0·6 mmHg respectively; in the remaining runs P_{a,CO_2} fell or was unchanged. In those runs in which P_{a,CO_2} rose the 'apparent sensitivity' to CO_2 was calculted in the manner described in the preceding paper. In only one run where P_{a,CO_2} rose by 2·2 mmHg, were the apparent and true sensitivities equal; in the other three, 'apparent sensitivities' were 15·8, 1·8 and 1·6 times the true sensitivity.

Measurement of the response to 'exercise' during the on-transient

During the on transient (first ten breaths of exercise) B.P. fell in all exercise runs on either the second or third breath; the mean B.P. over the fourth to the tenth breath was 90 mmHg (S.D., 20). Heart rate rose in seven runs, remained the same in four and fell in one; mean heart rate measured over the same period as the B.P. was 188 (S.D., 20). The changes in mean B.P. and heart rate during the on transient of exercise are therefore no different from those in the steady state (Table 1).

A record of the on-transient of one exercise run is shown in Fig. 1 together with the late exercise period for comparison. The first effect is a shortening of t_e followed later by a rise in V_T. There is an alkaline swing of pH which coincides with a drop

TABLE 2. Measurements of ventilation, pH oscillation, P_{ET,CO_2} and circulation time (c.t.) before (control), during the first ten breaths of 'exercise' (on-transient) and during the fourth minute of exercise (late exercise). The breath numbers upon which there was a significant change in the variables during the on-transient from control are shown in column 3 together with the mean value of the first significant change in column 4. The final column records whether the change in late 'exercise' was significantly different from control

	Control	On-transient		Late exercise	
Variable	Mean (S.D.)	Breath nos. of significant change	Mean value of first significant change	Mean (S.D.)	Significance of change from control
t_i (sec)	1·09 (0·25)	3, 5, 7–10	1·02	0·97 (0·23)	$0·01 < P < 0·05$
t_e (sec)	2·75 (0·59)	3–10	2·56	2·24 (0·44)	$P < 0·001$
t_{tot} (sec)	3·84 (0·53)	3–10	3·58	3·22 (0·55)	$P < 0·001$
$\dot{V}_T$ (ml.)	282 (45)	6–10	292	315 (58)	$P < 0·001$
$\dot{V}_I$ (l. min^{-1})	4·455 (0·748)	3–10	4·760	6·013 (1·436)	$P < 0·001$
$\dot{V}_{CO_2}$ (ml. min^{-1})	89 (29)	6, 7, 10	101	124 (38)	$P < 0·001$
P_{ET,CO_2} (mmHg)	34·1 (4·3)	5	33·7	33·9 (4·8)	n.s.
pH amp (u.)	0·0176 (0·0076)	4–6, 8–10	0·0162	0·0156 (0·0066)	$0·01 < P < 0·05$
ϕ	0·585 (0·150)	3–10	0·552	0·455 (0·169)	$P < 0·001$
$dpH/dt\downarrow max$ u. sec$^{-1} \times 10^{-3}$	10·3 (4·2)	2–10	10·9	12·0 (4·5)	$P < 0·001$
c.t. (sec)	2·62 (0·67)	—	—	2·67 (0·67)	n.s.

The mean values for the control and late 'exercise' periods are obtained from the average of ten breaths in the twelve runs ($n = 120$). The mean values for the on-transient are an average of twelve breaths for each breath number ($n = 12$). n.s., not significant.

in P_{ET,CO_2} (not shown). The dpH/dt↓max has increased by the fourth breath and this persists through to late exercise. The pH amp is initially unchanged but has decreased by late exercise.

The changes in the variables illustrated in Fig. 1 have been analysed for all twelve 'exercise' runs and the mean results with their statistical analysis are shown in Table 2 together with other variables measured or derived. There was a significant

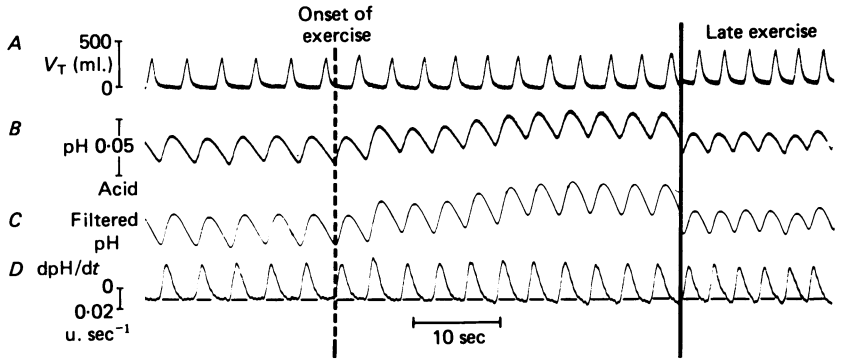

Fig. 1. Effect of 'exercise' on ventilation, arterial pH and the first differential of the pH signal. From the top the traces are: (*A*) tidal volume (V_T), (*B*) oscillations of arterial pH (unfiltered) and (*C*) after filtering with a 2 Hz filter and (*D*) the differentiated filtered pH signal (dpH/dt). The onset of 'exercise' is indicated by the dashed vertical line. The traces shown to the right of the continuous vertical line were obtained during the last minute of the 4 min 'exercise' period. The pH trace shows an alkaline deflexion during the on-transient of 'exercise' and a diminution of amplitude during late 'exercise'. The mean maximum rate of fall of pH (dpH/dt↓max) recorded during the ten breaths prior to exercise is indicated by bars on the dpH/dt trace. A fall in the signal below the bars indicates an increase in dpH/dt↓max.

increase in $\dot{V}_I$ and decrease in t_i, t_e, t_{tot} and ϕ on the third breath. The dpH/dt↓max had increased significantly by the second oscillation of the on-transient. This oscillation is sensed at the electrode during the third breath (see Fig. 2). Thus $\dot{V}_I$, t_e, t_{tot}, ϕ and dpH/dt↓max all changed during the third breath and those changes were maintained throughout the on-transient and were in the same direction as those observed in late 'exercise' (see Table 2). Mean V_T and $\dot{V}_{CO_2}$ did not increase significantly until breath six. Mean P_{ET,CO_2} fell during the on-transient but the fall was only significant on the fifth breath. There was a small late fall in pH amp.

Arterial blood samples were not taken during the on-transient but there was a continuous record of arterial pH and P_{ET,CO_2}. In six of the twelve runs pH rose on the third or fourth breath with a drop in P_{ET,CO_2}. In six runs there was no change initially in pH or P_{ET,CO_2} but in three of these runs there was a small fall in pH and rise in P_{ET,CO_2} starting on the seventh breath.

Relationship between the ventilatory response to 'exercise' and $\dot{V}_{CO_2}$, dpH/dt↓max and ϕ

To obtain a more certain and precise assessment of the relationship between these variables over a longer period of 'exercise' the analysis of the on-transient has been extended to twenty breaths. In examining the correlation between $\dot{V}_I$ and $\dot{V}_{CO_2}$,

between $\dot{V}_I$ and dpH/d$t\downarrow$max and between t_e and dpH/d$t\downarrow$max the method of analysis for the on-transient has been changed to take account of the circulatory delay between lung and electrode. This change is illustrated schematically in Fig. 2. As described in the Methods, 'exercise' was started in expiration and the breath of which it was part, referred to as 'breath 1' of the on-transient. This breath gives rise to oscillation 1' which, due to circulatory delay, coincides with 'breath 2'. The electrode

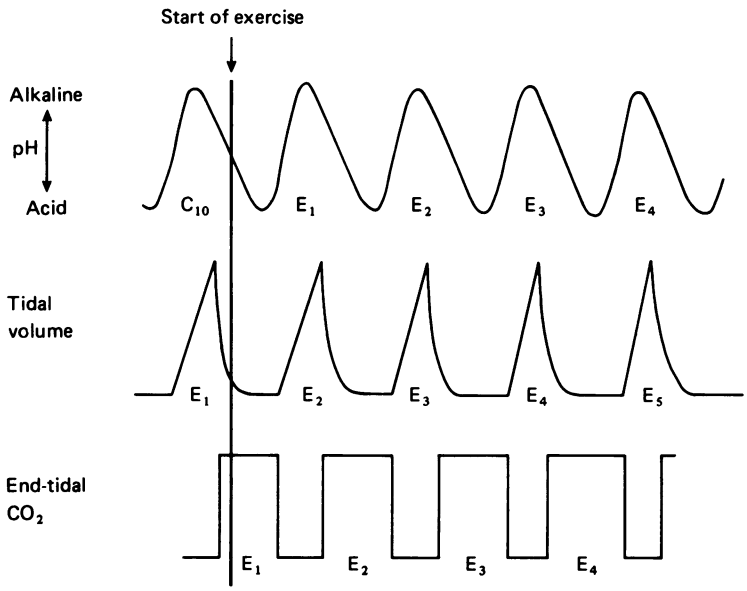

Fig. 2. Schematic diagram to illustrate the method of analysis employed. For simplicity only three signals are depicted: oscillation of arterial pH, tidal volume and end-tidal CO_2. 'Exercise' was initiated during expiration and the breath of which it was part was referred to as the first breath of 'exercise' (E_1). Due to a circulatory delay between lung and pH electrode, the oscillation E_1 generated by breath E_1 does not occur simultaneously. For reasons explained in the text two different methods of analysis were used. For Fig. 3, the CO_2 production ($\dot{V}_{CO_2}$) of a particular breath calculated from the V_T and P_{ET,CO_2} of that breath, was related to the oscillation generated by that breath, i.e. $\dot{V}_{CO_2}$ of E_1 (from V_T and P_{ET,CO_2} of E_1) related to oscillation E_1, etc. For Figs. 5 and 6 the oscillations are related to the breaths during which they occur and not to the breath from which they are generated, i.e. oscillation C_{10} related to breath E_1, oscillation E_1 related to breath E_2, etc. Similarly for Fig. 4, the $\dot{V}_{CO_2}$ of C_{10} calculated from V_T of C_{10} and P_{ET,CO_2} of C_{10} is related to breath E_1, $\dot{V}_{CO_2}$ of E_1 related to breath E_2, etc. For further explanation see text.

is close to the carotid body and from previous experiments it is known that the delay between a change in pH at the electrode and the corresponding response in phrenic nerve discharge is 1·1 sec (S.D., 0·3) (Cross *et al.* 1979). When assessing whether a particular oscillation is affecting a particular breath, rather than being determined by it, it is more appropriate to allow for the circulatory delay and relate 'oscillation 10' from the control period to 'breath 1' of the on-transient, 'oscillation 1' to 'breath 2' 'oscillation 2' to 'breath 3' etc. In correlating $\dot{V}_I$ with dpH/d$t\downarrow$max and t_e with dpH/d$t\downarrow$max therefore, the oscillations are assigned the same number as the number of the breath with which they coincide. Thus oscillation E_1 (see Fig. 2) will be

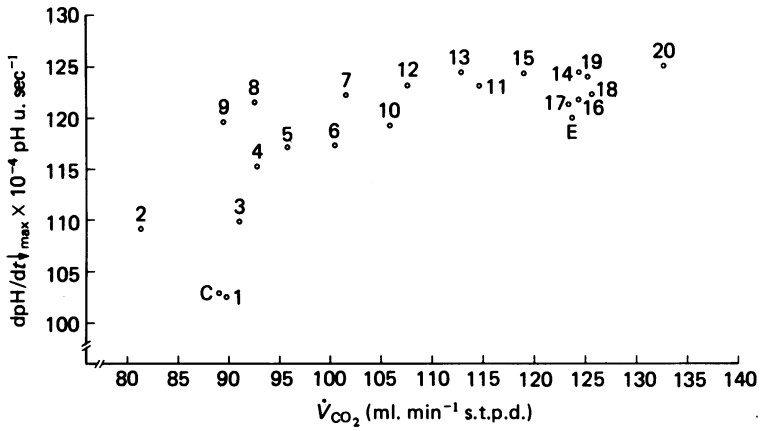

Fig. 3. Relationship between the maximum rate of change of pH on the downstroke of the oscillation (dpH/dt↓max) and CO_2 production ($\dot{V}_{CO_2}$) during the on-transient of 'exercise'. Each breath of the on-transient (numbered 1 to 20) is the mean of twelve measurements. Points marked 'C' and 'E' refer to the control and late exercise values and represent the means of 120 measurements. Note, in this Figure the numbering of the breaths is as shown in Fig. 2, i.e. 1 refers to oscillation E_1, and $\dot{V}_{CO_2}$ of breath E_1, 2 refers to oscillation and $\dot{V}_{CO_2}$ of breath E_2.

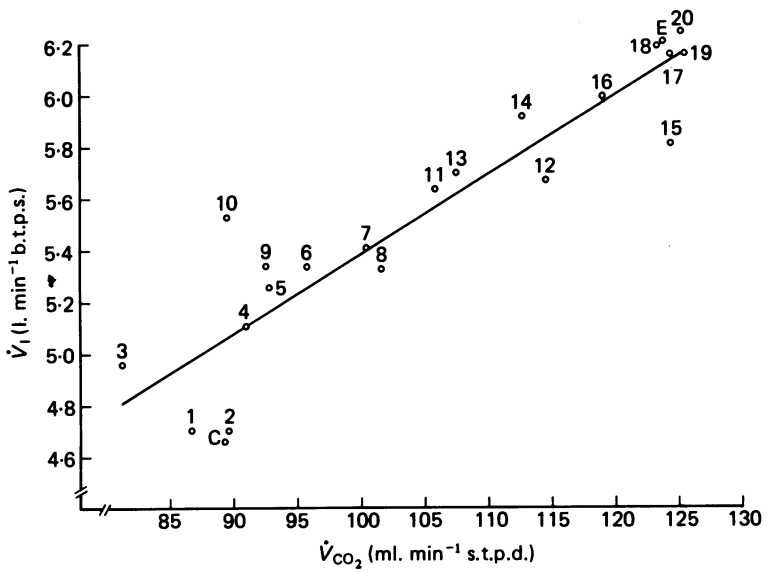

Fig. 4. Relationship between ventilation ($\dot{V}_I$) and CO_2 production ($\dot{V}_{CO_2}$) during the on-transient of 'exercise'. Individual breaths of the on-transient number 1–20. Each is the mean of twelve measurements. Control (C) and late exercise (E) points are the means of 120 measurements. Equation of the line: $y = 0{\cdot}031x + 2{\cdot}096$ ($r = 0{\cdot}92$). *Note:* 1 = $\dot{V}_I$ of breath E_1 and $\dot{V}_{CO_2}$ of breath C_{10}, 2 = $\dot{V}_I$ of breath E_2 and $\dot{V}_{CO_2}$ of breath E_1, etc. (see Fig. 2).

renumbered E_2 and correlated with breath E_2 etc. Similarly allowance is made for circulatory delay when correlating $\dot{V}_I$ with $\dot{V}_{CO_2}$ since the effect on blood pH, of the CO_2 eliminated by a particular breath, does not reach the electrode and carotid body until the following breath. No change in the method of analysis is required when

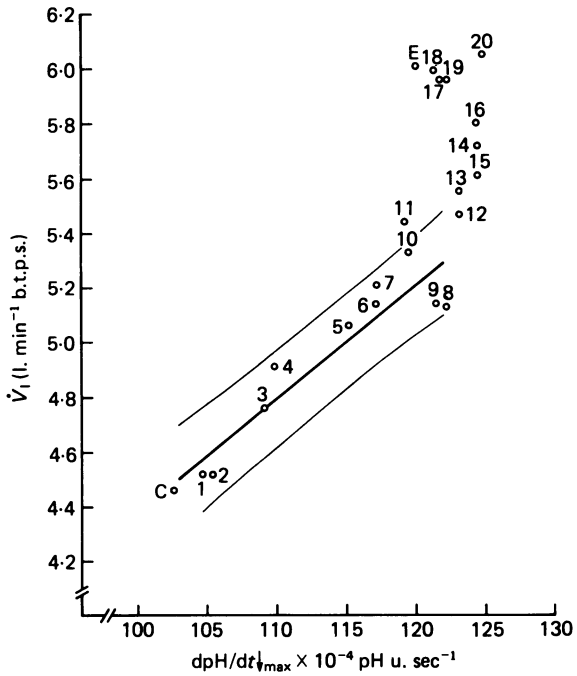

Fig. 5. Relationship between ventilation ($\dot{V}_I$) and the maximum rate of change of pH on the downstroke of the oscillation (dpH/d$t\downarrow$max) during the on-transient of 'exercise'. Numbered points are each the mean of twelve measurements. 1 refers to $\dot{V}_I$ of breath E_1 and dpH/d$t\downarrow$max of C_{10}, 2 refers to $\dot{V}_I$ of breath E_2 and dpH/d$t\downarrow$max of breath E_1, etc. (see Fig. 2). Control (C) and late exercise (E) values are the means of 120 measurements. The heavy line shows the relationship between the two variables during the first ten breaths of 'exercise'. (Equation: $y = 0{\cdot}041x + 0{\cdot}232$; $r = 0{\cdot}94$). Lighter lines represent the tolerance limits. From these it can be deduced that the relationship during the latter part of the on-transient deviates from that obtained earlier in 'exercise'.

relating $\dot{V}_{CO_2}$ to dpH/d$t\downarrow$max because the $\dot{V}_{CO_2}$ of a particular breath must be associated with the downstroke of the oscillation generated by that breath. Thus the $\dot{V}_T$ and P_{ET,CO_2} of breath E_2 (see Fig. 2) used to calculate the $\dot{V}_{CO_2}$ of that breath is correlated with the downstroke of oscillation E_2.

There was a significant drop in ϕ beginning on the third breath and persisting to late 'exercise' (Table 2). The change in ϕ was small and confined to one side of the oscillation (downstroke); from previous experiments it can be anticipated that such changes in ϕ would produce either no change or minimal increases (not decreases) in t_e (Cross *et al.* 1979).

The dpH/d$t\downarrow$max was related to $\dot{V}_{CO_2}$ (Fig. 3). Analysis of the entire relationship shows that 53 % of it ($r^2 = 0{\cdot}53$) can be accounted for by linear regression ($r = 0{\cdot}73$, dpH/d$t\downarrow$max $= 0{\cdot}31\ \dot{V}_{CO_2} + 85{\cdot}5$, $P < 0{\cdot}001$). Visual inspection of Fig. 3 suggests

that the relationship could be either curvilinear or that above a $\dot{V}_{CO_2}$ of 115 ml. min^{-1}, there is no further rise of dpH/d$t\downarrow$max. Fitting a quadratic polynomial to the entire data gave a small, but non-significant, rise in the correlation co-efficient to 0·78.

A linear correlation was found between $\dot{V}_I$ and $\dot{V}_{CO_2}$ for the on-transient and late exercise (Fig. 4). The equation given in the legend to Fig. 4 includes all data points

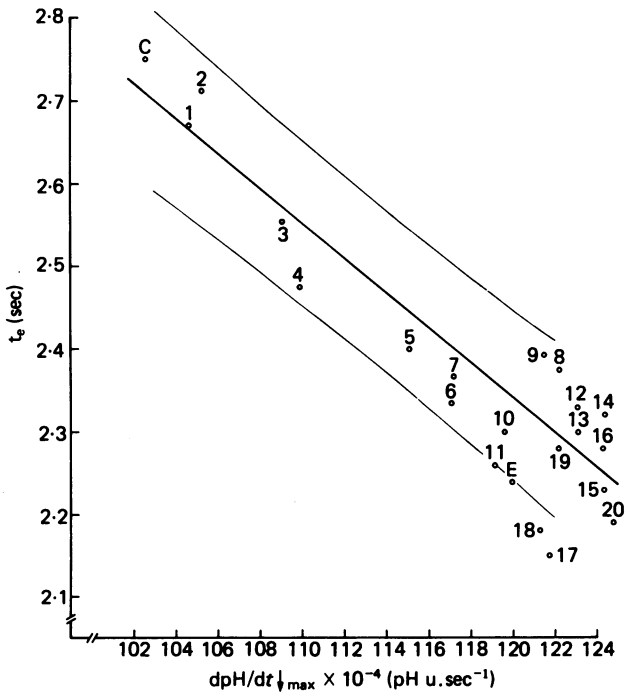

Fig. 6. Relationship between expiratory duration (t_e) and the maximum rate of change of pH on the downstroke of the oscillation (dpH/d$t\downarrow$max) during the on-transient of 'exercise'. Breath numbers during the on-transient are indicated 1 = t_e of breath E_1 and dpH/d$t\downarrow$max of breath C_{10}, 2 = t_e of breath E_2 and dpH/d$t\downarrow$max of breath E_1, etc. (see Fig. 2). Each numbered point is the mean of 12 measurements. Control (C) and late 'exercise' (E) points are the means of 120 measurements. The regression line (heavy central line) through the first ten breaths of 'exercise' has the equation: $y = 4{\cdot}854 - 0{\cdot}021x$; $r = -0{\cdot}93$. The lines bordering the regression line show the tolerance limits. From this it can be deduced that the same relationship holds true for the remainder of the on-transient through to late 'exercise'.

shown in the Figure, i.e. control, the first twenty breaths of exercise and late exercise. However regression analysis of the control and first ten breaths alone gives a regression line of identical slope and intercept. Thus the linear relationship between $\dot{V}_I$ and $\dot{V}_{CO_2}$ established in these experiments is applicable both to the on-transient and the steady state of exercise.

The relationship between dpH/d$t\downarrow$max and $\dot{V}_I$ and t_e for the steady-state and breath-by-breath results are shown in Figs. 5 and 6. The regression lines and their tolerance limits are calculated for the first ten breaths only. This was done to determine if any relationship established early in 'exercise' was maintained after

the first ten breaths as occurred with $\dot{V}_I$ and $\dot{V}_{CO_2}$ (Fig. 4). The closest relationship is between t_e and dpH/d$t\downarrow$max; t_e has fallen to the steady-state value by breath ten and thereafter all points lie close to or within the tolerance limits of the regression line calculated for the first ten breaths alone. There was also a linear relationship between $\dot{V}_I$ and dpH/dt$\downarrow$max but after breath fourteen the points lie outside the tolerance limits of the regression line due to a continuing rise in $\dot{V}_I$ with no increase in dpH/d$t\downarrow$max. The rise in $\dot{V}_I$ beyond breath 10 was predominantly due to an increase in V_T.

It can be anticipated that there will be a linear relationship between the variables shown in Fig. 5. This is because there is a linear relationship between dpH/d$t\downarrow$max and $\dot{V}_{CO_2}$, as well as between $\dot{V}_I$ and $\dot{V}_{CO_2}$. Although a regression line can be fitted to all the data points in Fig. 5 ($r = 0{\cdot}86$, $P < 0{\cdot}001$), we have not done this because there is plainly a change in the relationship between $\dot{V}_I$ and dpH/d$t\downarrow$max after breath ten.

DISCUSSION

There are many potential chemical stimuli to ventilation in exercise but as CO_2 production is so perfectly matched to ventilation it is logical to consider CO_2 as the humoral signal. Catecholamines and other chemical or hormones may be the humoral signal, but if so then they are produced in a quantity which so accurately regulates ventilation to $\dot{V}_{CO_2}$ that there is no change in arterial P_{CO_2}. Such a regulatory system is possible but is indirect and unlikely, so it is appropriate to investigate CO_2 as the humoral signal in exercise in the first instance. To achieve this end the present study includes, for the first time, a continuous record of arterial pH both during the on-transient and the steady state of exercise.

The breath-by-breath oscillations in pH recorded by the electrode are presumed to be due to fluctuations in P_{a,CO_2}, for the amplitude of the pH oscillation is compatible with their arising from fluctuations of alveolar P_{CO_2} (Dubois, Britt & Fenn, 1952). In addition Plaas-Link, Mueller, Luttman, Mückenhoff & Loeschcke (1977) have recorded simultaneously P_{a,CO_2} and pH oscillations with appropriate electrodes and shown that breath-by-breath oscillations of pH can be solely accounted for by changes in P_{a,CO_2}. The assumption is made in this discussion, therefore, that the oscillations of pH are due to P_{CO_2} but that the mean level of the oscillations is determined by alveolar ventilation and the production of metabolic acids and carbon dioxide.

Three methods were used in the present experiments to measure a chemical stimulus to ventilation in exercise: analysis of arterial blood samples, continuous recording of arterial pH and breath-by-breath measurement of tracheal CO_2. Each method is open to criticism although the defects of each method are not the same. In spite of these defects, each method yielded the same result in the steady state, namely that there was no important or consistent change in mean arterial pH or P_{CO_2}. Where P_{a,CO_2} did rise, this could not account for the rise in $\dot{V}_I$, on the basis of the prevailing ventilatory sensitivity to CO_2. Likewise, during the first breaths of the on-transient there is no evidence that P_{a,CO_2} rose or arterial pH fell; indeed in some 'exercise' runs the opposite occurred. Only in two runs did pH fall and this occurred late in the on-transient *after* the first increase in $\dot{V}_I$. The simultaneous record of

P_{ET,CO_2} and arterial pH excludes a change in arterial to alveolar gradient of P_{CO_2} masking a rise in P_{a,CO_2} during the on-transient.

The present experiments demonstrate that there are increases in the slope of the downstroke of the pH oscillation (upstroke of the P_{CO_2} oscillation) in exercise which over a certain range are related to the rises in CO_2 production. The close correlation between the changes in slope and ventilation, both for the steady state and on-transient, establish these slope changes as a potential humoral signal between CO_2 production and ventilation in exercise. This link between CO_2 production, $dpH/dt\downarrow max$ and ventilation in exercise has been postulated by Saunders (1980) and has also been shown experimentally to operate when CO_2 production is changed by methods other than exercise (Cross *et al.* 1981). Unlike other features of the oscillation (such as their amplitude) the slope of the downstroke is independent of the ventilatory response to a change in $\dot{V}_{CO_2}$ providing there is no important alteration in functional residual capacity. This independence of a chemical signal, from the response of the control system, gives it certain operating advantages that signals subject to feed-back do not have.

Comparison of the present experiments with other studies

The studies of Tibes (1977) and Weissman, Wasserman, Huntsman & Whipp, (1979) are similar in design and purpose to the present experiment. Firstly, all three studies were concerned with the response to electrically induced 'exercise' in the anaesthetized dog both during the on-transient and in the steady state. Secondly, the purpose of the experiments was to elucidate the neurohumoral control of breathing in 'exercise' and the method chosen to achieve this was to eliminate afferent stimuli from the exercising muscles. Many of the contrasts and similarities between the three studies are shown in the data included in Table 3.

Before attempting to separate the neural from the chemical drive to breathing in exercise it is important to demonstrate that the animals ventilatory response to exercise and other stimuli (such as hypercapnia) is satisfactory. The ventilatory response to exercise of the dogs in the present experiments, as assessed by $\Delta\dot{V}_I/\Delta\dot{V}_{CO_2}$, was 36·9. This is probably close to that pertaining in the experiments reported by Tibes (1977) and Weissman *et al.* (1979), though a direct comparison is not possible as individual values for $\Delta\dot{V}_I/\Delta\dot{V}_{CO_2}$ were not reported in the latter two studies. Tibes (1977) found a ventilatory equivalent for O_2 of 35 which is close to our result providing the R.Q. did not differ substantially from 1. $\dot{V}_E/\dot{V}_{CO_2}$ calculated from the mean results of Weissman *et al.* (1979) is 29·9. The increases of $\dot{V}_{CO_2}$ achieved by Tibes (1977), Weissman *et al.* (1979) and Kao, Schlig & Brooks (1955) were on average greater than in the present experiments although there is overlap of individual results. In spite of the difference in the $\dot{V}_{CO_2}$ achieved, the similarity in the ventilatory equivalent for a unit increase in $\dot{V}_{CO_2}$ is remarkable.

The CO_2 sensitivity to inhaled CO_2 in the present studies is substantially less than that observed by Weissman *et al.* (1979), although the method and calculation used for determining sensitivity were not the same. However, this difference is probably not relevant to the present studies, for if mean P_{CO_2} was an important stimulus to breathing in exercise, then the present studies should have shown a smaller ventilatory equivalent for CO_2; also a rise in P_{a,CO_2} would be more likely in the present

TABLE 3. Summary from similar experiments to the present studies, of (*a*) anaesthesia; (*b*) methods of inducing exercise; (*c*) afferent neural status; ventilatory responses to (*d*) exercise and (*e*) CO_2, (*f*) metabolic change with exercise and (*g*) resting acid–base status

Source	(*a*) Anaesthetic	(*b*) Method of inducing exercise	(*c*) Afferent neural status	(*d*) $\Delta\dot{V}_I/\Delta\dot{V}_{O_2}$ or $\Delta\dot{V}_I/\Delta\dot{V}_{CO_2}$	(*e*) $\Delta\dot{V}_I/\Delta P_{a,CO_2}$	(*f*) $\Delta\dot{V}_{O_2}$ or $\Delta\dot{V}_{CO_2}$	(*g*) Resting acid–base status P_{a,O_2}	P_{a,CO_2}	pHa
Kao *et al.* (1955)	None	Voluntary	Intact	30·3* (1·6)					
	None (decerebrate)	Muscle stimulation	Intact	33·3* (3·1)		126·7 (49·3) 54–251		37·2 (9·1)	7·37 (0·07)
	Barbiturate 30 mg kg^{-1}	Muscle stimulation	Intact	31·0* (1·4)					
	Nembutal 30 mg kg^{-1}	Muscle stimulation	Intact	34·7* (2·6)					
	Nembutal 30 mg kg^{-1}	Motor root stimulation	Intact	31·3* (2·6)					
	Choralose 1·6% Urethane 16% 3 ml kg^{-1}	Muscle stimulation	Intact	25·5* (1·0)					
Tibes (1977)	Sodium hexobarbital 20–50 mg kg^{-1} and 70–75% N_2O	Sciatic nerve stimulation	With and without cold block of intact nerves	35†	—	95†	94 (17)	36·3 (6·5)	7·38 (0·04)
Levine (1979)	Induction with methoxyflurane then chloralose 60 mg kg^{-1}	Muscle stimulation	With and without cord transection at L1	30*†	—	130*†	—	44·0 (9·0)	7·38 (0·04)
Weissman *et al.* (1979)	Induction with ketamine hydrochloride 10 mg kg^{-1} then chloralose 64 mg kg^{-1} and urethane 45 mg kg^{-1}	Sciatic and femoral nerve stimulation	Cut sciatic and femoral nerves	29·9†	2·0‡	147†	72 (31)	29·3 (4·0)	7·37 (0·03)
Cross *et al.* (1982)	Induction and surgery sodium thiopentone 25 mg kg^{-1} and 1–1·5% halothane in N_2O and O_2 Then chloralose 40 mg kg^{-1}	Muscle stimulation	Cord intact	30·2 (4·4)	0·46 (0·27)	151·9 (96·0) 44·4–336·8	78 (10)	47·7 (5·4)	7·35 (0·03)
			Cord cut at T8/9	27·9 (3·8)	0·39 (0·24)	87·1 (65·3) 16·5–234	80 (15)	44·7 (5·6)	7·38 (0·04)
Cross *et al.* (present study)	As above for Cross *et al.* (1982)	Muscle stimulation	Cord cut at T/8/9	36·9 (10·5)	0·51 (0·25)	46·3 (31·7) 16·3–131·9	89 (9)	39 (4·2)	7·42 (0·02)

Results expressed as mean and where possible (±S.D.) and range.

* $\Delta\dot{V}_I/\Delta\dot{V}_{O_2}$ in column 5 and $\Delta\dot{V}_{O_2}$ in column 7. † Derived from mean values of $\Delta\dot{V}_I$ and $\Delta\dot{V}_{O_2}$ or $\Delta\dot{V}_{CO_2}$. ‡ Rebreathing CO_2 method.

studies than in the experiments of Weissman *et al.* (1979). In fact we found no consistent rise in P_{a,CO_2} in the steady state and no evidence for any rise at all at the start of the on-transient.

Our results for the steady state of exercise are consistent with the overwhelming evidence from the literature, namely that changes in mean arterial pH, P_{a,CO_2} or P_{a,O_2} cannot account for the ventilatory response to exercise (see review by Wasserman, Whipp & Davis, 1981). This, Weissman *et al.* (1979) and ourselves, find to be true when afferent neural stimuli from the exercising muscles are absent. This contrasts with the finding of Tibes (1977) who recorded a rise in P_{a,CO_2} and fall in P_{a,O_2} during exercise when afferent stimuli were stopped from reaching the central nervous system by cold block of the femoral nerves. These changes in arterial gas tensions did not occur when afferent traffic in the nerves was not blocked, P_{a,CO_2}, P_{a,O_2} and pH being virtually unchanged. None of the data in Table 3 suggest an explanation for the different findings of Tibes; the only difference in experimental design is the method for blocking afferent stimuli from the exercising muscles.

The absence of any change in mean P_{a,CO_2} and pH in the steady state of exercise has been extended by the present studies to the on-transient. This result is at variance with the studies of Weissman *et al.* (1979) who found a rise in P_{a,CO_2} at the start of exercise, which, on the basis of the ventilatory response to inhaled CO_2, could have accounted for the increase of $\dot{V}_I$ at the on-transient. Tibes did not report on the changes of P_{ET,CO_2} or P_{a,CO_2} during the on-transient.

An alternative explanation for the rise in P_{a,CO_2} at the start of exercise, found by Weissman *et al.* (1979), is that the change in ventilation did not match the delivery of CO_2 to the lung. This implies that the increase in P_{a,CO_2} was a consequence of the ventilatory response rather than the cause of it. The low mean P_{a,O_2} with the wide S.D. of this mean in the experiments reported by Weissman *et al.* (1979) suggests that some of the dogs were under a hypoxic drive accounting for the low mean P_{a,CO_2} (see Table 3). This low mean P_{a,O_2} can only be accounted for by areas of low ventilation–perfusion ratios within the lung. This disturbance of gas exchange may have been responsible for the small rise in P_{a,CO_2} observed by Weissman *et al.* (1979) during the on-transient.

What is the humoral signal in exercise?

Yamamoto (1960) and Yamamoto & Edwards (1960) first put forward the hypothesis that temporal fluctuations in arterial P_{CO_2}, at a constant mean level, could contain sufficient information to constitute a chemical signal in the control of pulmonary ventilation. Many have assumed that Yamamoto was concerned only with breath-by-breath respiratory fluctuations of P_{CO_2}, i.e. 'the oscillation hypothesis'. This assumption places a constraint on the hypothesis which was not intended, for temporal fluctuations included variation of P_{a,CO_2} over a longer time period than the frequency of respiration. It is some twenty years after the hypothesis of Yamamoto was put forward that fluctuations in P_{a,CO_2} have been measured in exercise (Brewer, Cross, Davey, Guz, Jones, Katona, Maclean, Murphy, Semple, Solomon & Stidwill, 1979; Band, Wolff, Ward, Cochrane & Prior, 1980). One surprise emerging from these measurements is that the respiratory oscillations are not always increased in amplitude. Thus in our experiments the amplitude was unchanged or decreased due to the rise in respiratory frequency.

Although an alteration in phase relationship between respiratory and pH cycles can effect breathing, in the absence of alterations in mean pH and P_{a,CO_2} (Cross *et al.* 1979), there was no evidence that this operated in the present studies. $\Delta dpH/dt\downarrow max$ is a potential humoral signal in exercise and could account completely for the shortening of t_e (Fig. 6). It is unlikely that it is the complete humoral signal for there is a late rise in $\dot{V}_I$, due to an increase in V_T, which appears to be independent of $\Delta dpH/dt\downarrow max$ (Fig. 5). Nevertheless $\Delta dpH/dt\downarrow max$ is related to $\Delta\dot{V}_{CO_2}$ (Fig. 3) and could be a link between CO_2 production and ventilation in exercise. The link between $\Delta dpH/dt\downarrow max$ and $\dot{V}_{CO_2}$ have also been demonstrated in the absence of exercise (Cross *et al.* 1981).

If $dpH/dt\downarrow max$ is a humoral signal then the sensor must have a rate of change component in its sensitivity to CO_2. The evidence that the carotid body has such sensitivity is suggestive but not conclusive (Black, McCloskey & Torrance, 1971; Band *et al.* 1978; Plaas-Link, Luttmann & Mückenhoff, 1980). We favour the carotid bodies as the potential sensors of the humoral signal, for in the previous experiments we found that changes in temporal relationship between respiratory and pH cycles acted through these bodies (Cross *et al.* 1979). However, Levine (1979) found no change in the respiratory response to exercise following section of the carotid sinus nerves although his experiments did provide evidence for a humoral signal in exercise acting at a receptor site(s) which was extracranial.

Separation of the humoral and neural control of breathing in exercise

The separation of the humoral and neural control of breathing in exercise has usually been made on the basis of the initial rapid rise in breathing at the start of exercise when the assumption has been that no humoral signal has reached the arterial chemoreceptors. When the first increase of breathing occurs around the third breath this assumption is no longer tenable, for $dpH/dt\downarrow max$ is a potential humoral signal at this time. Band *et al.* (1980) have recorded a change in dpH/dt on the downstroke of the pH oscillation in the brachial artery of man by the second breath of exercise. This rapid development of a chemical signal in the arterial blood is presumably due to an abrupt increase in cardiac output at the start of exercise. Whatever the mechanism it is clear that separation of chemical and neural signals in the future require a continuous record of changes in blood acid–base status with rapidly responding electrodes.

We would like to thank Dr K. MaCrae, Senior Lecturer in Medical Statistics, Charing Cross Hospital Medical School for his invaluable advice. The Fogarty International Centre is thanked for a Senior International Fellowship to P. G. Katona. The Wellcome Trust is thanked for financial support to Brenda A. Cross. A Brewer and R. Furneaux of the Department of Medical Electronics, Charing Cross Hospital are thanked for constructing and maintaining the stimulating equipment.

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