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THE IMPRECISION ARISING FROM THE APPLICATION OF THE HENDERSON–HASSELBALCH RELATIONSHIP TO THE BLOOD OF ANAESTHETIZED DOGS

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SUMMARY

1. Arterial blood samples were obtained from anaesthetized dogs in which either a respiratory alkalaemia, a respiratory acidaemia or a non-respiratory acidaemia was produced and from animals in which no deliberate changes in acid–base balance were produced. The samples were analysed for pH, carbon dioxide tension and concentration, oxygen tension and concentration and haematocrit. From these determinations the plasma carbon dioxide concentration, the plasma bicarbonate ion concentration and pK′ were calculated.

2. The value of pK′ lay within the range 5·86–6·27. The temperatures of the animals studied were between 36·75 and 39·25° C but there was no significant relationship between pK′ and temperature.

3. The values of pK′ increased with an alkalaemia and decreased with an acidaemia.

4. In addition to the variation of pK′ with pH there was a considerable residual scatter of pK′ values at any one pH value.

5. It was concluded that in these experimental conditions the use of the Henderson–Hasselbalch equation in a form not dealing with activities was unacceptable and could lead to much larger errors than has hitherto been thought.

INTRODUCTION

The Henderson–Hasselbalch equation was developed originally to show that the acidity of a fluid such as plasma was determined by the ratio of the concentrations of carbonic acid and bicarbonate ions present (Henderson,

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1908; Hasselbalch, 1916). An exact thermodynamic expression of the relationship is

$$a_{H}+ = k \frac{a_{H_2CO}}{a_{HCO_3^-}}, \quad (1)$$

where a represents the activity of the individual reactant and k is the dissociation constant.

In order to use measurable quantities this exact equation is often transformed to

$$pH = pK' + \log \frac{[HCO_3^-]_p}{S.P_{CO_2}}, \quad (2)$$

or $pH = pK' + \log[R]$,

where pK' = the apparent dissociation constant,

$[HCO_3^-]_p$ = the plasma bicarbonate ion concentration (m-equiv/l.),

P_{CO_2} = the tension of carbon dioxide (mm Hg),

S = the plasma carbon dioxide solubility coefficient (m-mole/mm Hg),

and $[R] = \frac{[HCO_3^-]_p}{S.P_{CO_2}}$.

In such a form the equation is often used to calculate one of the three main variables from measurements of the other two. But there are a number of assumptions involved in the transformation of the exact eqn. (1) to the more practically useful form (2). For example, it is assumed that the expression $S.P_{CO_2}$ indicates the plasma carbonic acid concentration and the plasma bicarbonate ion concentration is usually determined as the difference between the total plasma carbon dioxide concentration and the estimated carbonic acid concentration, i.e.

$$[HCO_3^-]_p = C_{p,CO_2} - S.P_{CO_2}. \quad (3)$$

This last assumption ignores the carriage of carbon dioxide in the plasma in forms other than these two. The evidence that these assumptions do not lead to significant errors depends on the demonstration that pK′ is a constant. When blood was examined *in vitro* Robinson, Price & Cullen (1934) found that pK′ was constant. Recent studies by Severinghaus, Stupfel & Bradley (1956) and Siggaard–Andersen (1962) have found pK′ to vary slightly, with pK′ decreasing as the pH of blood was increased.

In experiments designed to determine the effects of changes in the arterial blood carbon dioxide tension on acid–base balance in dogs we found that pK′ was not constant and, in contrast with the *in vitro* results, pK′ *increased* as pH rose (Norman & Linden, 1965; Linden & Norman, 1966). Experiments in other dogs showed a similar pattern of change with a non-respiratory acidaemia. Therefore we analysed all the determinations

obtained from blood samples taken from anaesthetized dogs to determine firstly the variation of pK′ and secondly the variation of pK′ with pH.

pK′ was determined as the difference between the pH of a blood sample and the logarithm of the ratio of the concentrations of bicarbonate ions and carbonic acid

$$pK' = pH - \log[R]. \quad (4)$$

Thus any consideration of the variation of pK′ with pH involved considering a dependent variable of which the independent variable formed a large part. An additional analysis was therefore made to determine the relationship between $[R]$ and pH to see how well a determination of $[R]$ would predict the pH of any blood sample. This analysis allowed an estimate of the validity of the original application of the Henderson–Hasselbalch equation – to show that the acidity of plasma depends on the ratio of the carbonic acid–bicarbonate ion concentrations.

The results of these analyses show that in the anaesthetized dog, pK′ is not constant and that the use of an arbitrary value for pK′ may be associated with a much greater error than has hitherto been thought.

METHODS

Experimental procedures

Blood samples were obtained from forty-eight dogs weighing between 9·5 and 29·1 kg. These animals were being used in investigations of the effects of anaesthesia on acid–base balance (Ledsome, Linden & Norman, 1971), in studying the effects of changes in the arterial carbon dioxide tension on acid–base balance (Norman & Linden, 1965; Linden & Norman, 1966), in studies of the effects of acidaemia on the responses of the heart to autonomic nerve stimulation (Linden & Norman, 1969), in a study of reflexes arising from the left atrium (Ledsome & Linden, 1967) and a study of the use of propranolol (Ledsome, Linden & Norman, 1965).

Each animal was given a subcutaneous injection of morphine sulphate (dose 8 mg) one hour before induction of anaesthesia. In twenty-five dogs anaesthesia was induced with an intravenous infusion of chloralose (British Drug Houses; dose 10 ml./kg of a warm solution containing 10 mg/ml. dissolved in a solution of either sodium chloride (150 mM) or sodium chloride (125 mM) and sodium bicarbonate (25 mM)). A steady state of light anaesthesia was maintained by further injections of chloralose (about 10 mg/kg) every 15 min. Twenty-three dogs were anaesthetized with an intravenous injection of pentobarbitone sodium (Abbott Laboratories; dose 20 mg/kg) with additional injections (of about 2 mg/kg) being given every 45 min to maintain the required depth of anaesthesia. A tracheal cannula was inserted. Eleven dogs breathed air spontaneously. The remaining thirty-seven were artificially ventilated with air (ten dogs), a mixture of 40 % oxygen and 60 % nitrogen (twenty-two dogs) or 100 % oxygen (five dogs), humidified at room temperature and supplied using an anaesthetic machine incorporating a Starling 'Ideal' pump (Ledsome, Linden & Norman, 1967). In thirteen artificially ventilated dogs muscle relaxation was obtained using suxamethonium chloride ('Anectine', Burroughs Wellcome & Co., dose 0·375 mg/min) given intravenously using a constant-rate infusion pump (Unita II*b*, Braun, Frankfurt, Germany).

The animal temperature was measured using either a mercury-in-glass thermometer inserted in the rectum or by a thermistor (probe 401, Telethermometer 43TA, Yellow Springs Instrument Co. Inc., Ohio, U.S.A.) inserted in the oesophagus. Both thermometers were calibrated using an N.P.L.-certified thermometer. The animal temperature was maintained at 38° C ± 2° C by adjusting heating lamps above and beneath the animal table.

In twenty-six animals no deliberate changes in acid–base balance were produced. In nine animals an alkalaemia was produced by overventilation for periods of between 2 and 4 hr. In five dogs carbon dioxide was added to the inspired gases for between 1 and 3 hr to produce an acidaemia. In six dogs a molar solution of hydrochloric acid was infused intravenously or intra-arterially at rates of between 0·2 and 2 ml./min to produce an acidaemia. The total amount of acid infused varied from 70 to 168 ml. and was given over periods from 73 to 200 min. One dog was subjected first to overventilation and later to the inhalation of carbon dioxide and in a second dog an acidaemia was produced first by the infusion of hydrochloric acid and later by carbon dioxide. In those dogs in which hydrochloric acid was infused the acidaemia was subsequently abolished by the infusion of a molar solution of sodium bicarbonate at rates between 0·75 and 3·75 ml./min using volumes of between 90 and 174 ml.

Blood sampling and analysis

Arterial blood samples were obtained anaerobically from a nylon cannula inserted in a femoral artery. Each sample was taken in a 10 ml. glass syringe in which the dead space was filled with a heparin solution (Pularin, Evans Medical Ltd, strength 100 i.u./ml. 0·9 % sodium chloride solution). The samples were analysed immediately for pH, carbon dioxide and oxygen tension and haematocrit using a system described elsewhere (Norman, Ledsome & Linden, 1965). The carbon dioxide and oxygen concentrations in the blood were determined at the same time using the methods of Linden, Ledsome & Norman (1965). The carbon dioxide tension and concentration, the oxygen concentration and the haematocrit values were corrected for their dilution by the volume of heparin in the dead-space of the syringe. The pH and the carbon dioxide and oxygen tensions were corrected for any difference in temperature between the animal and the measuring electrode system using the factors of Rosenthal (1948) and Bradley, Stupfel & Severinghaus (1956).

After taken each blood sample an equal volume of a dextran solution was given to the animal ('Dextraven' Benger Laboratories or 'Dextran 80', Pharmacia (Great Britain) Ltd). In some experiments during the preparation of the animal an additional volume of dextran was infused in a total dose not exceeding 8 % of the estimated blood volume (i.e. 100 ml. dextran for a 13 kg dog).

Calculation of the plasma bicarbonate ion concentration and pK'

The plasma carbon dioxide concentration was calculated from the blood concentration using the nomogram of Van Slyke & Sendroy (1928). This nomogram requires measurements of the pH, oxygen-carrying capacity and the percentage saturation with oxygen of the blood. Where the oxygen tension exceeded 150 mm Hg it was assumed that the blood was fully saturated and that the measured oxygen concentration was the same as the capacity. Where the tension was less than 150 mm Hg the oxygen-carrying capacity was estimated by multiplying the capacity of a fully saturated sample by the ratio of the haematocrits of the unsaturated and saturated samples. The percentage saturation was then calculated from the measured oxygen concentration and the estimated capacity. If no blood sample was obtained with an

oxygen tension greater than 150 mm Hg an additional sample was taken, saturated with oxygen by equilibrating it with room air at ambient temperature and the oxygen concentration and haematocrit then being measured. It was assumed that this sample was fully saturated with oxygen. When using the Van Slyke–Sendroy nomogram the errors introduced by ignoring the percentage saturation are small with a change from saturation to complete desaturation rarely changing the calculated plasma carbon dioxide concentration by more than 1 mM.

Van Slyke & Sendroy derived their nomogram from measurements made using human, ox and horse blood. To determine the applicability of the nomogram to dog blood fifteen blood samples were obtained from two dogs and the plasma carbon dioxide concentration was calculated as described. Plasma from part of each sample was obtained by centrifugation under anaerobic conditions and the plasma carbon dioxide concentration was measured directly using the method of Linden *et al.* (1965). The measured concentrations lay within the range 9·1–31·2 mM and the average difference between the measured and calculated concentration was 0·09 mM (S.E. of the mean, ± 0·16 mM; range, ± 0·8 mM).

The plasma bicarbonate ion concentration was calculated from eqn. (3) using the appropriate solubility coefficient for carbon dioxide (Austin, Lacombe, Rand & Chatterjee, 1963). pK′ was calculated from eqn. (4).

Statistical procedures

Mean values, standard deviations of observations and standard errors of means were calculated using conventional formulae. Correlation analysis of some of the data was performed using the methods described by Goldstein (1964). The 95% confidence and tolerance limits for points given by regression equations were calculated using formulae previously described (Linden *et al.* 1965). The tolerance limits include 95% of the population of the data used to determine the regression equation.

Accuracy of measurements and calculations

The 95% tolerance limits for the measured quantities were: pH, ± 0·01 units carbon dioxide tension and concentration, ± 2·5% of the measured value (i.e. ± 1 mm Hg at a P_{CO_2} of 40 mm Hg and ± 0·5 mM at a measured $C_{b.CO_2}$ of 20 mM); oxygen tension, ± 2·5 mm Hg; oxygen concentration, 0·3 ml./100 ml.; and haematocrit, ± 1% (Norman *et al.* 1965; Linden *et al.* 1965).

Van Slyke & Sendroy (1928) claimed that their nomogram should predict the plasma carbon dioxide concentration with an error of not more than ± 1 mM. Our results obtained in dogs (see above) agree with this figure.

The accuracy with which the log[*R*] factor could be calculated was estimated by combining the possible errors of the measurements together with those of the plasma carbon dioxide concentration measurement. Table 1 gives these results. It is apparent that if the plasma carbon dioxide concentration did not fall below 20 mM the log[*R*] could be calculated with an error not exceeding ± 0·04 units. The accuracy of the pK′ calculation must include the tolerance limits for the measurement of pH (± 0·01 units). The accuracy of the calculated pK′ was therefore ± 0·05 units unless the plasma carbon dioxide concentration was 10 mM when the accuracy was only ± 0·075 units.

TABLE 1. The accuracy of the determination of log $[HCO_3^-]_p/S.P_{CO_2}$

C_{p,CO_2} (± 1 mM)	P_{CO_2} (± 2·5 % of the measured value) 20 mm Hg	40 mm Hg	100 mm Hg
10 mM	1·193 (1·133–1·249)	0·864 (0·799–0·923)	—
20 mM	1·508 (1·474–1·541)	1·193 (1·158–1·228)	0·752 (0·713–0·789)
30 mM	—	1·379 (1·352–1·405)	0·953 (0·924–0·981)

The values given are the logarithm of the ratio and in brackets the range of values for the ratio corresponding to the limits for the determinations of the carbon dioxide tension and the plasma carbon dioxide concentration. Values were calculated only for the experimental conditions observed.

RESULTS

In forty-eight animals where the temperatures were within the range 36·75–39·25° C a total of 318 arterial blood samples were obtained. The pH values are between 6·77 and 7·76; the carbon dioxide tensions between 13·5 and 138 mm Hg and the plasma carbon dioxide concentrations between 8·4 and 33·0 mM. The values for pK′ lay between 5·86 and 6·27.

pK′

pK′ in anaesthetized animals. In any of the twenty-six dogs in which deliberate changes in acid–base balance were not produced pK′ did not vary greatly. The results obtained in one dog anaesthetized with pentobarbitone and breathing spontaneously for 8 hr are shown in Fig. 1*A*. In this animal there were small progressive falls in pH and the plasma carbon dioxide concentration with the carbon dioxide tension showing no real change; the values of pK′ lay within the range 6·07–6·13. The largest range of pK′ found in any one dog was 0·13 units and the average range for the twenty-six animals was 0·07 units (S.D. ± 0·027 units). There were larger variations in the pK′ values obtained for all the animals, the overall range being from 5·99 to 6·21. Fig. 2 shows a frequency distribution of the results for pK′ from all 26 dogs. The mean value for pK′ for these 162 estimations was 6·084 (S.D. of the observation, ± 0·030 units).

The effects of overventilation on pK′. Overventilation and the consequent alkalaemia was associated with an increase in the value of pK′. The results obtained in one dog before, during and after a 4-hr period of overventilation are shown in Fig. 1*B*. With the reduction of the arterial carbon dioxide tension from 40 to 16 mm Hg, pH rose from 7·40 to 7·76. On resuming control levels of ventilation these changes were reversed. Initially in the control period before ventilation the pK′ values were 6·11 and 6·15; with overventilation the values rose to lie between 6·23 and

6·27; when ventilation was returned to the initial level pK′ fell to 6·15. In five of the dogs blood samples were taken before, during and after a 4-hr period of overventilation. Table 2 summarizes the results found in these dogs. The mean values for pK′ in the initial and final control periods were

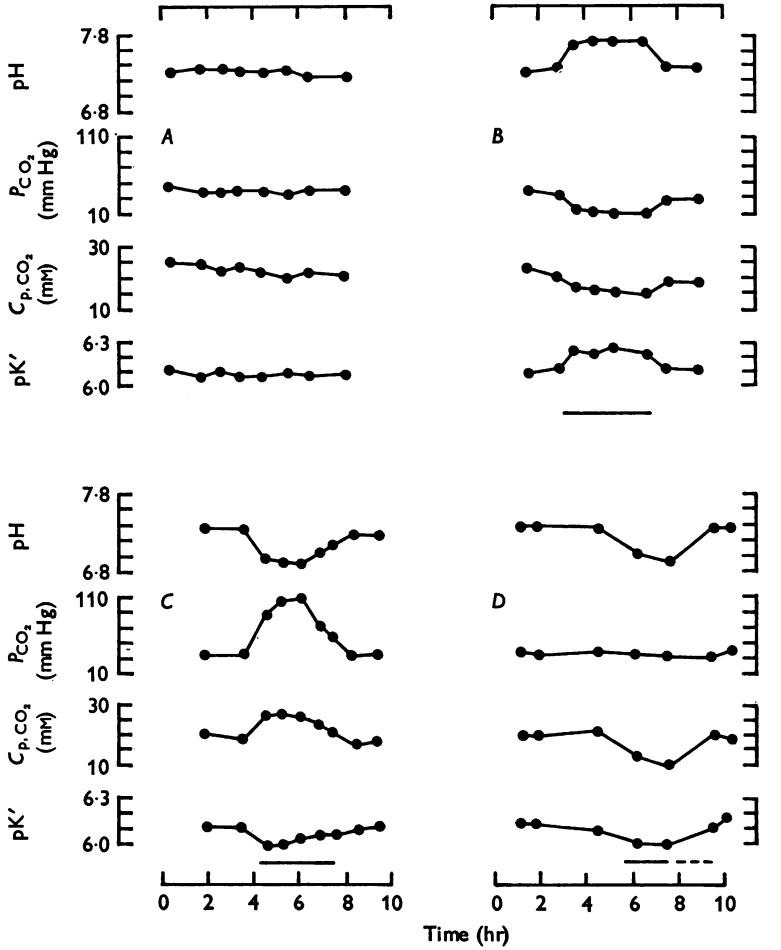

Fig. 1. Changes in pK′ in individual animals. Ordinate, from above downwards: pH, carbon dioxide tension, plasma carbon dioxide concentration and pK′ in arterial blood samples. Abscissa: time (hr) after induction of anaesthesia. Panel A: pentobarbitone anaesthesia, spontaneous ventilation. Panel *B*: pentobarbitone anaesthesia, artificial ventilation. The bar shows the period during which the animal was over-ventilated. Panel *C*: chloralose anaesthesia, suxamethonium, artificial ventilation. Carbon dioxide administered during the period shown by the bar. Panel *D*: chloralose anaesthesia, suxamethonium, artificial ventilation. Hydrochloric acid infused during the period shown by the continuous line and sodium bicarbonate during the period shown by the dashed line.

6·137 and 6·131; these mean values did not differ significantly (t = 0·43, P > 0·05). The mean value for pK′ obtained during overventilation was 6·197 and was significantly greater than those for either control period (t = 4·09 and 3·90; P < 0·005).

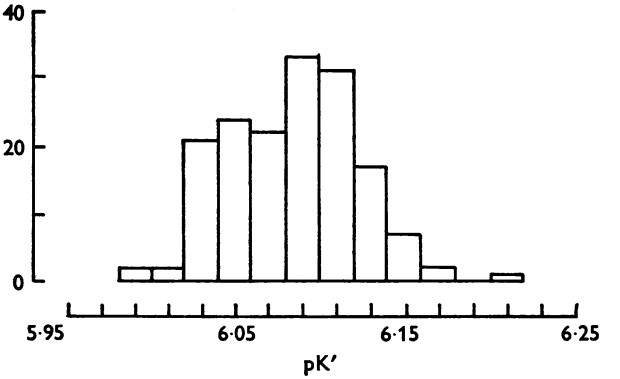

Fig. 2. Frequency distribution of pK′ values for samples taken from dogs in which no deliberate changes in acid–base balance were produced. Ordinate, frequency; abscissa, pK′.

TABLE 2. Effects of overventilation on acid–base balance. Five dogs, pentobarbitone anaesthesia, artificial ventilation. Blood samples obtained during a 2-hr period before over-ventilation, between ⅔ and 4 hr after commencing overventilation and between ⅔ and 2 hr after stopping overventilation

	Before overventilation	During overventilation	After overventilation
No. of samples	9	19	10
pH	7·393 ± 0·013 (7·37–7·41)	7·683 ± 0·052 (7·58–7·76)	7·401 ± 0·056 (7·31–7·51)
P_{CO_2} (mm Hg)	42·7 ± 4·0 (38·8–49·7)	19·8 ± 3·7 (13·5–29·5)	37·8 ± 4·7 (30·9–47·4)
$C_{p,\,CO_2}$ (mM)	24·1 ± 1·7 (21·8–26·7)	18·7 ± 2·4 (15·8–23·6)	22·1 ± 3·2 (19·4–24·6)
pK′	6·137 ± 0·022 (6·10–6·16)	6·197 ± 0·049 (6·09–6·27)	6·131 ± 0·030 (6·08–6·17)

Values are: mean ± 1 S.D. (range).

The effects of hypercarbia on pK′. When a respiratory acidaemia was produced by adding carbon dioxide to the inspired gases the decrease in pH was accompanied by a decrease in pK′. In one dog the fall in pH to 6·93 with a rise in the plasma carbon dioxide concentration to 28·5 mM was accompanied by a fall in pK′ from 6·14 to 6·03; pK′ returned to 6·15 when the administration of carbon dioxide ceased (Fig. 1*C*). Table 3 summarizes the results of a series of similar experiments in five dogs. In the initial and final control periods when carbon dioxide was not given the

mean values for pK′ were 6·148 and 6·146; these values do not differ significantly ($t = 0{\cdot}12$, $P > 0{\cdot}05$). During the acidaemia the mean pK′ value was 6·067 which was significantly lower than either mean value found in the control periods ($t = 4{\cdot}17$ and $4{\cdot}03$, $P < 0{\cdot}001$).

TABLE 3. Effects of hypercarbia on acid–base balance. Five dogs, chloralose anaesthesia, suxamethonium, artificial respiration. Blood samples obtained during a 2-hr period before the administration of carbon dioxide, between 1 and 2 hr after the start of the administration and between 1 and 2 hr after the cessation of the administration of carbon dioxide

	Before hypercarbia	During hypercarbia	After hypercarbia
No. of samples	10	10	9
pH	7·378 ± 0·051 (7·28–7·46)	6·933 ± 0·027 (6·87–6·96)	7·320 ± 0·029 (7·30–7·38)
P_{CO_2} (mm Hg)	39·7 ± 4·1 (31·0–45·3)	115 ± 13 (96–138)	42·3 ± 5·7 (33·7–49·9)
$C_{p,\,CO_2}$ (mM)	21·6 ± 1·2 (20·2–23·8)	28·8 ± 1·4 (26·1–31·0)	20·2 ± 1·3 (18·3–22·3)
pK′	6·148 ± 0·037 (6·09–6·20)	6·067 ± 0·049 (5·98–6·17)	6·146 ± 0·035 (6·10–6·20)

Values are: mean + 1 S.D. (range).

TABLE 4. Effects of hydrochloric acid on acid–base balance. Five dogs, chloralose anaesthesia, suxamethonium, artificial ventilation. Blood samples taken during a 2-hr period before, between 1 and 2 hr after commencing the administration of hydrochloric acid and between 1 and 2 hr after the administration of sodium bicarbonate to abolish the acidaemia

	Before acidaemia	During acidaemia	After acidaemia
No. of samples	10	9	9
pH	7·391 ± 0·052 (7·29–7·44)	6·959 ± 0·084 (6·77–7·06)	7·348 ± 0·039 (7·30–7·40)
P_{CO_2} (mm Hg)	38·2 ± 4·2 (32·2–46·3)	36·7 ± 5·0 (31·7–47·3)	41·0 ± 5·1 (36·1–52·4)
$C_{p,\,CO_2}$ (mM)	20·6 ± 4·4 (16·2–22·7)	10·9 ± 1·8 (8·4–13·9)	19·8 ± 2·4 (15·5–22·6)
pK′	6·161 ± 0·022 (6·11–6·19)	6·018 ± 0·108 (5·86–6·20)	6·169 ± 0·052 (6·06–6·23)

Values are: mean ± 1 S.D. (range).

The effects of hydrochloric acid on pK′. As in the dogs in which a respiratory acidaemia was produced, the infusion of hydrochloric acid produced an acidaemia in which the fall in pH was accompanied by a fall in pK′. The results obtained in one dog are shown in Fig. 1*D* where, as pH fell from 7·40 to 6·95, pK′ fell from 6·16 to 6·01. After the administration of

sodium bicarbonate the pH rose to 7·35 and pK′ to 6·20. Table 4 summarizes the results of similar experiments performed in five dogs. Before and after the period of acidaemia the mean values for pK′ were 6·161 and 6·169; these values do not differ significantly ($t = 0{\cdot}43$, $P > 0{\cdot}05$). During the period of acidaemia the mean value of 6·018 was significantly less than the mean values obtained in the initial and final control periods ($t = 4{\cdot}09$ and $3{\cdot}89$, $P < 0{\cdot}005$).

pK′ and temperature

For any given pH it may be expected that pK′ will decrease as the temperature increases. Severinghaus *et al.* (1956) found a temperature coefficient of −0·0054 pK′ units/° C rise at a pH of 7·40. In ninety-eight blood samples the pH values were within the range 7·35–7·45 units and the temperatures of the animals were within the range 36·75–39·25° C. There was no significant correlation between the pK′ values and the temperature of the animal ($r = +0{\cdot}02$, $P > 0{\cdot}1$). This absence of any significant effect of temperature probably results from the small range of temperatures encountered. Further analysis of the variation in pK′ was made using all the data from the 318 samples obtained in this relatively narrow temperature range.

Relationship between pK′ and pH

In the experiments described above an increase in pK′ accompanied a respiratory alkalaemia and decreases in pK′ were observed during a respiratory and a non-respiratory acidaemia (Fig. 3). pK′ seemed to be a function of pH rather than of the carbon dioxide tension or the plasma bicarbonate ion concentration. The results for all 318 blood samples were analysed to determine if any significant relationship existed between pK′ and pH. Linear regression analysis showed the line of best fit to be given by

$$\mathrm{pK'} = 6{\cdot}1135 + 0{\cdot}240\ (\mathrm{pH} - 7{\cdot}355). \qquad (5)$$

The correlation coefficient r was $+0{\cdot}654$ and was highly significant ($P < 0{\cdot}001$). The 95% confidence interval for the points given by this equation is shown in Fig. 4 together with lines showing the 95% tolerance limits. These tolerance limits include a zone 0·19 units wide within which lay 95% of the 318 estimations of pK′ and pH. Also shown in Fig. 4 is the line derived from the *in vitro* relationship between pK′ and pH given by Severinghaus (1965). The slope of the *in vitro* line is less than and of opposite sign to that obtained from our results. The difference between the slope values was highly significant (+0·240 pK′ units/pH unit for the *in vivo* results; −0·046 pK′ units/pH unit for the *in vitro* results ($t = 18{\cdot}2$, $P < 0{\cdot}0005$).

Relationship between log[R] and pH

The results presented above show that pK′ was not constant and that even when the relationship between pK′ and pH was considered there was a residual scatter forming a band 0·19 pK′ units wide for 95% of the results. The results obtained were also examined to see how well a determination of [*R*] would predict the pH of any blood sample.

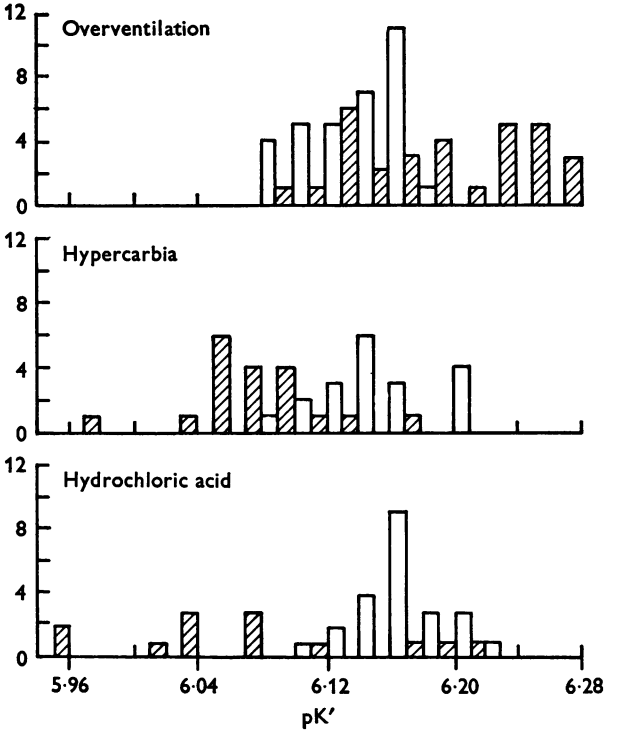

Fig. 3. Frequency distributions of pK′ in animals subjected to overventilation (upper panel), to hypercarbia (middle panel) and to hydrochloric acid (lower panel). The open columns show the values obtained during the initial and final control periods and the hatched columns the values obtained during the period of disturbance of acid–base balance. Ordinate, frequency; abscissa, pK′.

In the five dogs subjected to overventilation, whose results are summarized in Table 2, the line of best fit for the relationship between pH and log[*R*] was

$$\text{pH} = 7{\cdot}508 + 1{\cdot}21\ (\log[R] - 1{\cdot}34). \qquad (6)$$

In the five dogs subjected to hypercarbia the line of best fit was

$$\text{pH} = 7{\cdot}174 + 1{\cdot}20\ (\log[R] - 1{\cdot}06) \qquad (7)$$

and in the dogs given hydrochloric acid the line of best fit was

$$\text{pH} = 7{\cdot}241 + 1{\cdot}27\ (\log[R] - 1{\cdot}12). \tag{8}$$

For each of these three groups the regression coefficients r lay between $+0{\cdot}91$ and $+0{\cdot}97$; each value being highly significant ($P < 0{\cdot}001$). Thus, as would be expected, the pH was directly related to the logarithm of the ratio of the bicarbonate ion–carbonic acid concentrations.

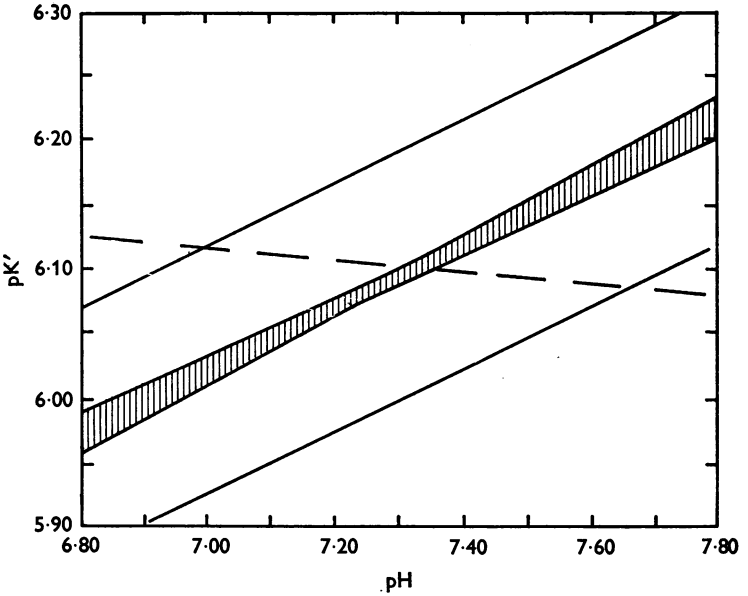

Fig. 4. The relationship between pK′ and pH for 318 blood samples. Ordinate, pK′; abscissa, pH. The hatched zone shows the 95 % confidence interval for the points given by the calculated regression equation (see text) and is surrounded by the lines showing the 95 % tolerance intervals. The dashed line is that given by the data of Severinghaus (1965) based on *in vitro* analysis.

The results obtained in all samples were analysed in the same way and the equation for the line of best fit was

$$\text{pH} = 7{\cdot}335 + 1{\cdot}159\ (\log[R] - 1{\cdot}221). \tag{9}$$

This equation, based on 318 determinations, also shows a highly significant correlation coefficient ($r = +0{\cdot}938$, $P < 0{\cdot}001$). In addition to showing that pH increases as $[R]$ increases, the equation demonstrates that for a unit increase in $\log[R]$ there was an increase in pH of 1·159 units. The Henderson–Hasselbalch equation (eqn. (2)) predicts that the increase should be only 1 unit; the difference between the value given by eqn. (9) and 1·00 is highly significant ($t = 6{\cdot}65$, $P < 0{\cdot}0005$). Fig. 5 shows the

95% confidence interval for points given by eqn. (9) together with the line expected if the increase was 1·00 pH unit for a 1·00 unit change in log[*R*]. The 95% tolerance limits for points given by eqn. (9) are also shown and form a band approximately 0·23 units wide. Thus, even allowing

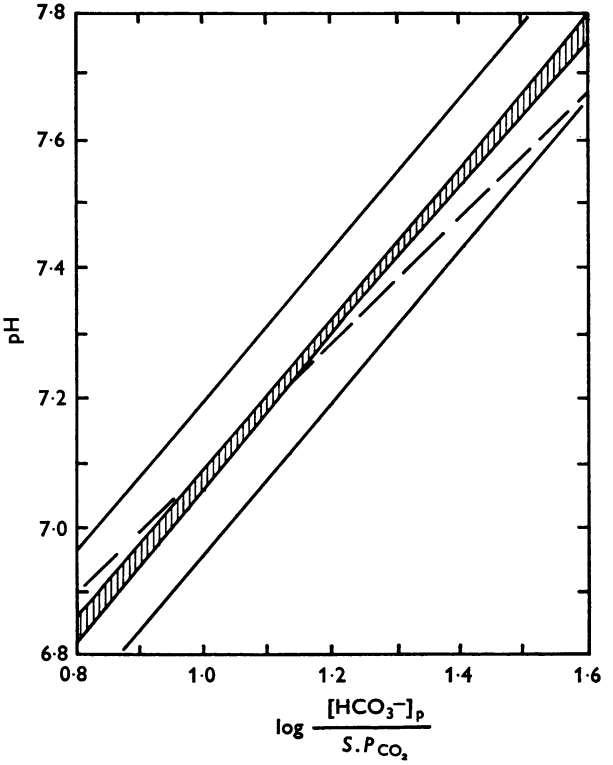

Fig. 5. The relationship between the logarithm of the ratio of the concentrations of bicarbonate ions and carbonic acid (log[*R*]) and pH. Ordinate, pH; abscissa, log[*R*]. Based on the analyses of 318 samples. The hatched zone shows the 95% confidence interval for the relationships between log[*R*] and pH and is surrounded by the 95% tolerance interval. The dashed line shows the expected relationship if pK′ is a constant.

for the departure from the Henderson–Hasselbalch equation shown in eqn. (9), for any given [*R*] value the actual pH of any one blood sample could lie within ± 0·115 units of the value predicted in 95% of the blood samples examined.

DISCUSSION

The results presented in this paper were based on analyses of blood taken from anaesthetized dogs in a variety of conditions including some deliberate changes in acid–base balance. The investigations were carried out

during relatively steady states in that samples were not analysed until some ½–4 hr after inducing a change in acid–base balance. The results show that as the ratio of the concentrations of the bicarbonate ions and carbonic acid in plasma increases (i.e. as $[R]$ increases) so does the pH but the change in pH for a given change in $\log[R]$ was greater than that predicted by the Henderson–Hasselbalch equation. As a consequence, pK′, calculated as the difference between pH and $\log[R]$, was found to be inconstant with pK′ values increasing as pH increased. There was a large range of pK′ values for any given pH value with the 95% tolerance interval being a band some 0·19 pK′ units wide. These results differ markedly from those described when blood has been examined *in vitro*. For example, Robinson *et al.* (1934) found a mean value for pK′ of 6·089 for 138 estimations; Dill, Daly & Forbes (1937) found a mean value of 6·112 for eighteen estimations and Brewin, Gould, Nashat & Neil (1955) found a mean value of 6·11 for six estimations: in these three series the range of pK′ values was from 6·07 to 6·14. Studies by Severinghaus *et al.* (1956) and Siggaard–Andersen (1962) showed that pK′ was not always constant and that pK′ decreased as pH rose. But all these investigators examined blood in tonometers. Trenchard, Noble & Guz (1967) determined pK′ in blood taken from patients in a variety of clinical states in which changes in acid–base balance occurred. They reported values for pK′ varying from 5·87 to 6·17. Their estimations did not involve tonometry.

The differences between the *in vitro* studies and the results reported here demand some explanation although our data do not allow definite conclusions. It seems possible that the explanation may lie in the assumptions used in transforming the equilibrium equation in a form dealing with activities (eqn. 1) to the more practical form (eqn. (2)). Some of these assumptions may be considered. First, pH, as measured with a glass electrode, can only be an approximate estimate of the hydrogen ion activity of the plasma for when blood is introduced into the measuring cell changes in potentials may occur at other boundaries in addition to that occurring at the glass-blood boundary. Secondly, although the carbon dioxide tension denotes the activity of the dissolved carbon dioxide it may not necessarily be a measure of the activity of the carbonic acid in the plasma. Thirdly, we have used a series of values for the solubility coefficient for carbon dioxide based on *in vitro* estimations (Austin *et al.* 1963). In 1928, Van Slyke, Sendroy, Hastings & Neill found that the solubility coefficient could change if there were changes in the fat content of plasma. Further, the plasma bicarbonate ion concentration, as estimated by us, was the amount of carbon dioxide carried in plasma in forms other than as dissolved carbon dioxide. As such it includes any carbonate ions present and any carbon dioxide carried attached to the plasma proteins. Finally,

the concentration used was expressed as a *molarity* whereas a better estimate of the activity would be to express the concentration as a *molality* which would necessitate the measurement of the plasma water concentration. Thus, if measurements were made of the water, fat and protein concentrations in the plasma it is possible that some of the wide scatter of pK′ values might disappear.

The assumptions made in using eqn. (2) apply equally well to measurements made *in vivo* and *in vitro*. But in such *in vitro* estimations where the acid–base balance of blood is changed the alterations are confined to the

TABLE 5. The calculation of P_{CO_2} or $[HCO_3^-]_p$ using the Henderson–Hasselbalch equation

		pK′ from these experiments		
	pK′ from Severinghaus (1965)	Mean	95 % confidence limits	95 % tolerance limits
Example 1: pH = 7·40, $[HCO_3^-]_p$ = 24·0 m-equiv/l. temp. = 38° C				
pK′	6·095	6·129	6·123–6·135	6·034–6·224
P_{CO_2} (mm Hg)	39·5	42·7	42·1–43·3	34·4–53·2
Example 2: pH = 7·00, $[HCO_3^-]_p$ = 30·0 m-equiv/l. temp. = 38°C.				
pK′	6·109	6·033	6·021–6·045	5·938–6·128
P_{CO_2} (mm Hg)	128	107·5	105–110	86·4–134
Example 3: pH = 7·40, P_{CO_2} = 40 mm Hg, temp. = 38° C				
pK′	6·095	6·129	6·123–6·135	6·034–6·224
$[HCO_3^-]_p$ (m-equiv/l.)	24·3	22·5	22·8–22·2	28·0–18·1
Example 4: pH = 7·60, P_{CO_2} = 40 mm Hg, temp. = 38° C				
pK′	6·085	6·177	6·166–6·188	6·082–6·272
$[HCO_3^-]_p$ (m-equiv/l.)	39·4	31·9	32·7–31·1	39·7–25·6

blood. When similar changes are induced in the whole animal, exchanges can occur with the other tissues of the body and the exchanges, whether of water, fat, protein, carbon dioxide or bicarbonate ions might be great enough to cause the discrepancies we have found. Trenchard *et al.* (1967) thought that some of their differences might be explained because they examined blood from patients in unsteady states. It seems less likely that the observations in this investigation were due to taking blood samples in unsteady states: the samples were never taken earlier than 30 min following the production of a change in acid–base balance.

Whatever the explanation of the differences, when considering experimental circumstances similar to those described here, it seems that the use of the Henderson–Hasselbalch equation to calculate any one main variable from measurements of the other two will be associated with an error greater than hitherto thought. When Ludbrook (1959) examined the problem he concluded on the evidence then available that such a use was

permissible although he noted that recent studies of pK′ in abnormal acid–base states were not available. It proved of interest to calculate the limits with which any one variable could be estimated from the other two using pK′ values suggested by Severinghaus (1965) and those reported here (eqn. 5) together with the 95% confidence and tolerance intervals for the estimation of pK′. The results of some examples are given in Table 5 and show a large discrepancy especially where the pH is far from normal values. Thus at a pH of 7·00 and a plasma bicarbonate ion concentration of 30 m-equiv/l. with the pK′ value suggested by Severinghaus (1965) the carbon dioxide tension would be 128 mm Hg whereas the results presented here would suggest a tension of 107·5 mm Hg with a 95% confidence range from 105 to 110 mm Hg. Although this 95% confidence range is small it seems that where an individual prediction is being made the 95% tolerance range would be more appropriate; for these conditions the range is from 86 to 134 mm Hg.

We would suggest, in conditions such as those described here in anaesthetized dogs, that the use of the Henderson–Hasselbalch equation to calculate one factor from measurements of the other two is neither necessary nor valuable especially now when rapid, accurate determinations of all three factors are possible (e.g. Linden *et al.* 1965; Norman *et al.* 1965).

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REFERENCES

AUSTIN, W. H., LACOMBE, E., RAND, P. W. & CHATTERJEE, M. (1963). Solubility of carbon dioxide in serum from 15–38° C. *J. appl. Physiol.* **18**, 301–304.

BRADLEY, A. F., STUPFEL, M. & SEVERINGHAUS, J. W. (1956). Effect of temperature on P_{CO_2} and P_{O_2} of blood *in vitro*. *J. appl. Physiol.* **9**, 201–204.

BREWIN, E. G., GOULD, R. P., NASHAT, F. S. & NEIL, E. (1955). An investigation of problems of acid-base equilibria in hypothermia. *Guy's Hosp. Rep.* **104**, 177–214.

DILL, D. B., DALY, C. & FORBES, W. H. (1937). The pK′ of serum and red cells. *J. biol. Chem.* **117**, 569–579.

GOLDSTEIN, A. (1964). *Biostatistics: an Introductory Text.* London: Collier–Macmillan Ltd.

HASSELBALCH, K. A. (1916). Die Berechnung der Wasserstoffzahl des Blutes aus der freien und gebundenen Kohlensäure desselben, und die Sauerstoffbindung des Blutes als Funktion der Wasserstoffzahl. *Biochem. Z.* **78**, 112–144.

HENDERSON, L. J. (1908). The theory of neutrality regulation in the animal organism. *Am. J. Physiol.* **21**, 427–448.

LEDSOME, J. R. & LINDEN, R. J. (1967). The effect of distending a pouch of left atrium on the heart rate. *J. Physiol.* **193**, 121–129.

LEDSOME, J. R., LINDEN, R. J. & NORMAN, J. (1965). The use of sympathetic β-receptor blocking agents in the investigation of reflex changes in heart rate. *Br. J. Pharmac. Chemother.* **24**, 781–788.

LEDSOME, J. R., LINDEN, R. J. & NORMAN, J. (1967). An anaesthetic machine for dogs. *J. Physiol.* **191**, 61–62*P*.

LEDSOME, J. R., LINDEN, R. J. & NORMAN, J. (1971). The effects of light chloralose and pentobarbitone anaesthesia on the acid–base state and oxygenation of arterial blood in dogs. *J. Physiol.* **212**, 611–627.

LINDEN, R. J., LEDSOME, J. R. & NORMAN, J. (1965). Simple methods for the determination of the concentrations of carbon dioxide and oxygen in blood. *Br. J. Anaesth.* **37**, 77–88.

LINDEN, R. J. & NORMAN, J. (1966). The effect of increases in P_{CO_2} on acid-base balance. *J. Physiol.* **185**, 75–76*P*.

LINDEN, R. J. & NORMAN, J. (1969). The effect of acidaemia on the response to stimulation of the autonomic nerves to the heart. *J. Physiol.* **200**, 51–71.

LUDBROOK, J. (1959). Estimation of P_{CO_2} by means of the Henderson–Hasselbalch equation. In *A Symposium on pH and Blood Gas Measurement*, pp. 34–44, ed. WOOLMER, R. F. London: Churchill.

NORMAN, J., LEDSOME, J. R. & LINDEN, R. J. (1965). A system for the measurement of respiratory and acid–base parameters in blood. *Br. J. Anaesth.* **37**, 466–479.

NORMAN, J. & LINDEN, R. J. (1965). Hyperventilation and acid–base balance. *Br. J. Anaesth.* **37**, 290–291.

ROBINSON, H. W., PRICE, J. W. & CULLEN, G. E. (1934). Studies of the acid–base condition of the blood. III. The value for pK′ in the Henderson–Hasselbalch equation for human and dog sera, determined with the Simms electrode. *J. biol. Chem.* **106**, 7–27.

ROSENTHAL, T. B. (1948). The effect of temperature on the pH of blood and plasma in vitro. *J. biol. Chem.* **173**, 25–30.

SEVERINGHAUS, J. W. (1965). Blood gas concentrations. In *Handbook of Physiology*, section 3: Respiration, vol. 2, pp. 1475–1487, ed. FENN, W. O. & RAHN, H. Washington: American Physiological Society.

SEVERINGHAUS, J. W., STUPFEL, M. & BRADLEY, A. F. (1956). Variations of serum carbonic acid pK′ with pH and temperature. *J. appl. Physiol.* **9**, 197–200.

SIGGAARD-ANDERSEN, O. (1962). The first dissociation exponent of carbonic acid as a function of pH. *Scand. J. clin. Lab. Invest.* **14**, 587–597.

TRENCHARD, D., NOBLE, M. I. M. & GUZ, A. (1967). Serum carbonic acid pK'_1 abnormalities in patients with acid–base disturbances. *Clin. Sci.* **32**, 189–200.

VAN SLYKE, D. D. & SENDROY, J. (1928). Studies of gas and electrolyte equilibria in blood. XV. Line charts for graphic calculations by the Henderson–Hasselbalch equation, and for calculating plasma carbon dioxide content from whole blood content. *J. biol. Chem.* **79**, 781–798.

VAN SLYKE, D. D., SENDROY, J., HASTINGS, A. B. & NEILL, J. M. (1928). Studies of gas and electrolyte equilibria in blood. X. The solubility of carbon dioxide at 38° C. in water, salt solution, serum and blood cells. *J. biol. Chem.* **78**, 765–799.