

Regulatory Mechanisms of Hemoglobin Oxygen Affinity in Acidosis and Alkalosis

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ABSTRACT The recent reports of the effect of 2,3-diphosphoglycerate (2,3-DPG) on hemoglobin affinity for oxygen suggested that this substance may play a role in man's adaptation to acidosis and alkalosis.

A study of the effect of induced acidosis and alkalosis on the oxyhemoglobin dissociation curve of normal man was therefore carried out, and the mechanisms involved in the physiological regulation of hemoglobin oxygen affinity examined.

In acute changes of plasma pH there was no alteration in red cell 2,3-DPG content. However, there were changes in hemoglobin oxygen affinity and these correlated with changes in mean corpuscular hemoglobin concentration (MCHC). With maintained acidosis and alkalosis, red cell 2,3-DPG content was altered and correlated with the changes in hemoglobin oxygen affinity. Both of these mechanisms shift the hemoglobin oxygen dissociation curve opposite to the direct pH (Bohr) effect, and providing the rate of pH change is neither too rapid nor too large, they counteract the direct pH effect and the *in vivo* hemoglobin oxygen affinity remains unchanged.

It is also shown that approximately 35% of the change in hemoglobin oxygen affinity resulting from an alteration in red cell 2,3-DPG, is explained by effect of 2,3-DPG on the red cell pH.

INTRODUCTION

Since the classical description by Bohr, Hasselbalch, and Krogh (1), the effect of pH on the hemoglobin affinity for oxygen has been well known. From this time, the position of the hemoglobin oxygen dissociation curve had been considered fixed except for the effects of pH and temperature. However, the work of Chanutin and Curnish (2), and Benesch and Benesch (3), showing

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that 2,3-diphosphoglycerate (2,3-DPG)¹ a glycolytic intermediate present in high concentrations in red cells, causes a reduction in hemoglobin oxygen affinity, led us to consider the role of 2,3-DPG in acidosis and alkalosis. In 1942, Guest (4) had shown that diabetic acidosis is associated with a low red cell 2,3-DPG. Such a reduction of 2,3-DPG would lead to a raised hemoglobin affinity for oxygen whereas the fall in pH, through the Bohr effect, would lead to a shift of the dissociation curve to the right. The purpose of this study was to investigate in detail the effect, in normal man, of both acidosis and alkalosis on the oxygen dissociation curve, and to delineate the mechanisms involved in its regulation, as well as to consider the possible effects on oxygen transport of any changes that occurred.

METHODS

Acidosis was induced acutely in four healthy normal volunteers using intravenous acetazolamide (DIAMOX)² and maintained for 1 wk with oral acetazolamide and ammonium chloride. The acidosis was then rapidly corrected and alkalosis induced using intravenous sodium bicarbonate and maintained with an oral dose.

Before induction of acidosis control values for hemoglobin oxygen affinity, red cell organic phosphates, plasma and red cell pH, blood gases, plasma electrolytes, hemoglobin, and hematocrit readings were made. Frequent serial values were obtained during acidosis and alkalosis. Arterialized blood, obtained by immersing the arm in water at 46–48°C for 5 min before venepuncture was used for all measurements. By this technique the hemoglobin oxygen saturation was 80% or greater in all samples and thereby ensured that no changes in intracellular pH occurred as a result of changes in hemoglobin oxygen saturation. Samples for determination of plasma and red cell pH and blood gases were collected anaerobically into heparinized 1 ml tuberculin syringes.

¹ *Abbreviations used in this paper:* 2,3-DPG, 2,3-diphosphoglycerate; MCHC, mean corpuscular hemoglobin concentration; pH effect, Bohr effect.

² Lederle Laboratories, division of American Cyanamid Co., Pearl River, N. Y.

TABLE I
The Mean Values for Plasma and Red Cell pH, Pco₂, and Base Excess Obtained during the Control Period and Greater than 96 hr of Acidosis or Alkalosis

	Plasma pH	Red cell pH	Pco ₂	Base excess
			<i>mm Hg</i>	<i>mEq/liter</i>
Control	7.415 ±0.015 (n = 7)	7.205 ±0.034 (n = 5)	37.3 ±4.24 (n = 5)	- 0.94 ±2.79 (n = 5)
Last phase acidosis	7.333 ±0.021 (n = 7)	7.167 ±0.040 (n = 6)	28.7 ±2.96 (n = 6)	-10.22 ±0.85 (n = 6)
Last phase alkalosis	7.460 ±0.020 (n = 8)	7.264 ±0.018 (n = 6)	39.0 ±4.81 (n = 8)	+ 3.71 ±2.81 (n = 8)

* ±1 standard deviation given for all results.

The hemoglobin affinity for oxygen was determined by the mixing technique as described by Lenfant, Ways, Aucutt, and Cruz (5). Results were expressed as the partial pressure of oxygen at half-saturation at pH 7.4 and temperature 37°C (P_{50(7.4)}). The standard deviation of the method on the same sample of blood is 0.22 mm Hg (n = 8). The in vivo hemoglobin oxygen affinity (P_{50(i.v.)}) was determined using the simultaneously measured plasma pH and the standard Bohr effect ($\Delta \log P_{50} / \Delta \text{pH}$) factor of -0.48 used by Severinghaus (6).

Red cell content of the organic phosphates was obtained by extraction and fractionation using a modification (7) of the method of Robinson, Loder, and de Gruchy (8). Total phosphate in each fraction was measured using the method of Bartlett (9). The standard deviation of the method on the same sample of blood is ±0.59 μmoles/g Hb (n = 8).

Samples for plasma pH and blood gas measurements were immediately placed on ice and measured within 15 min of sampling. For these measurements standard Radiometer electrodes (Radiometer, Copenhagen, Denmark), mounted in a microcuvette, were used. Red cell pH was measured on the lysed packed red cells by the freeze-thaw method (10). Base excess was calculated from the plasma pH and Pco₂ using the Severinghaus blood gas calculator (6).

Hemoglobin concentration was determined as cyanmethemoglobin spectrophotometrically at 540 mμ. The microhematocrit value was determined in capillary tubes at 11,500 g for 5 min. The mean corpuscular hemoglobin concentration (MCHC) in g Hb/100 ml red cells was calculated by dividing the hemoglobin concentration by the hematocrit.

RESULTS

The mean plasma pH before induction of acidosis was 7.415. There was a slow fall during the first 4 hr of acidosis and then a more rapid fall to a mean value of 7.332 at 24 hr which was maintained without significant change for the remainder of the period of acidosis. The period of alkalosis had a different pattern, the infusion of sodium bicarbonate resulted in a sharp rise in pH to a mean of 7.423 at the end of the 1st hr and then a more gradual rise to a mean of 7.450 at 24 hr which was maintained for the rest of the week. The mean plasma

pH for the control period, the last phase of acidosis and the last phase of alkalosis,³ alongside the corresponding values for red cell pH, are given in Table I. The individual values during the experiment are shown graphically in Fig. 1.

The mean control red cell 2,3-DPG was 14.52 μmoles/g Hb. There was no significant change for the first 4 hr of acidosis. It then fell progressively reaching a low point at 48 hr which was maintained for the remaining period of acidosis. The mean at the end of acidosis was 10.38 μmoles/g Hb. Similarly the onset of alkalosis did not cause any change in 2,3-DPG for about 4 hr. It then rose to above normal levels by 48 hr not significantly changing for the rest of the week, the mean at the end of alkalosis being 16.23 μmoles/g Hb. (Fig. 1).

The mean control P_{50(7.4)} was 27.2 mm Hg and during the acidosis fell gradually for 48 hr and remained at this level for the rest of the week ending with a mean value of 24.1 mm Hg. The alkalosis caused a gradual rise in P_{50(7.4)} for 48 hr; it then stayed above normal values with the maintained alkalosis. The mean value was 29.2 mm Hg at the end of alkalosis (Fig. 1).

The P_{50(i.v.)} showed no significant change throughout the period of acidosis. However, at the beginning of alkalosis there was a transient fall in P_{50(i.v.)} for about 8 hr before returning to normal values which were maintained for the remainder of the alkalotic period (Fig. 1).

The MCHC changes are shown in Fig. 2. Within the 1st hr of acidosis there was a rapid fall but as the acidosis was maintained a return to normal occurred. Changes during alkalosis were similar except that there was a rise in MCHC with induction of alkalosis and again a return to normal as alkalosis was maintained. The mean values for control, last phase of acidosis, and

³ "last phase of acidosis" or "last phase of alkalosis" refers to samples taken at or after 96 hr of the respective periods.

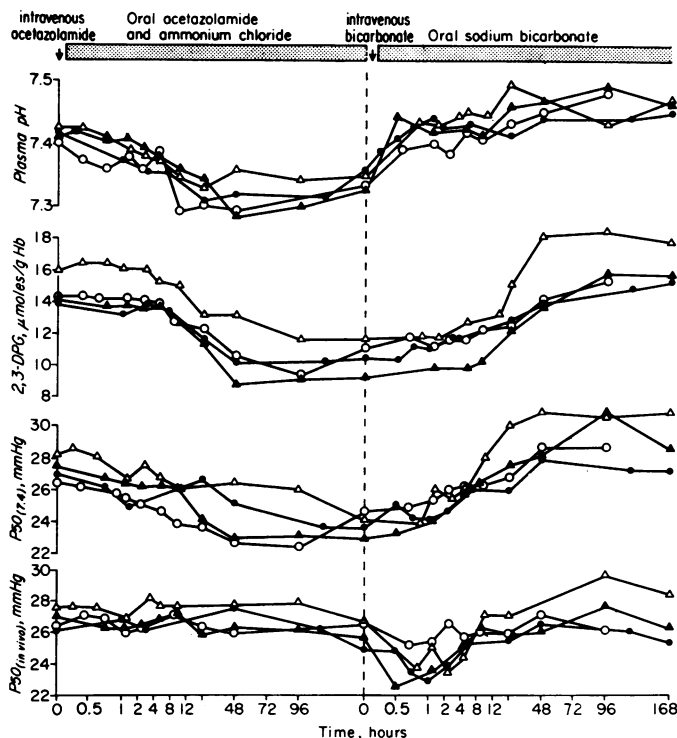


FIGURE 1 Changes in plasma pH, red cell 2,3-DPG, $P_{50(7.4)}$ and in vivo P_{50} occurring in four subjects during a week's acidosis followed by a week's alkalosis. Each subject is represented by a different symbol. Note the changing time scale.

last phase of alkalosis, were 35.31 ± 1.22 , 35.03 ± 0.69 , and 35.23 ± 1.09 , respectively. There is no significant difference between these values.

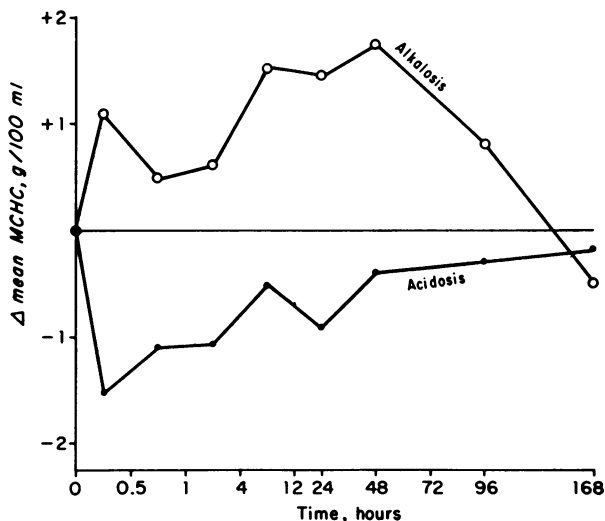


FIGURE 2 Change in mean MCHC occurring during acidosis and alkalosis. The zero value for acidosis was the control value and for alkalosis the value at the end of acidosis, immediately before the infusion of sodium bicarbonate was taken as zero.

As would be expected with a metabolic acidosis both plasma PCO_2 and base excess were reduced during acidosis and similarly both were raised during the alkalotic period (Table I).

No significant changes in red cell ATP, blood urea nitrogen, and plasma sodium occurred during the experiment.

DISCUSSION

During both acidosis and alkalosis the changes in $P_{50(7.4)}$ showed two phases (Fig. 3). In acidosis the initial phase, in which no significant change in 2,3-DPG occurred, lasted 4 hr and was associated with a fall in $P_{50(7.4)}$. In the second phase, lasting from 4 to 48 hr, there was a further fall in $P_{50(7.4)}$ but the changes paralleled the changes that occurred in 2,3-DPG in this period. In alkalosis the initial phase again lasted 4 hr and occurred without any significant change in 2,3-DPG but in this case there was a rise in $P_{50(7.4)}$. In the second phase there was a further rise in $P_{50(7.4)}$ paralleling the changes in 2,3-DPG. The most likely explanation of these independently occurring $P_{50(7.4)}$ changes would seem to be a change in red cell volume as this could act through two mechanisms. Firstly, by altering the intracellular hemoglobin concentration, as experiments

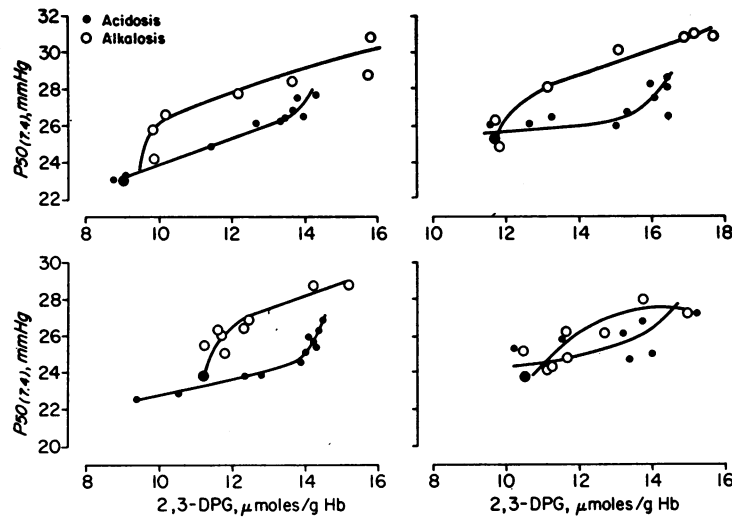


FIGURE 3 $P_{50(7.4)}$ as a function of 2,3-DPG for the entire period of acidosis and alkalosis. Each quadrant represents a different subject. ●, acidosis; ○, alkalosis.

on hemoglobin in solution both with, and stripped of, 2,3-DPG have shown that the hemoglobin affinity for O_2 decreases with increasing hemoglobin concentration (11). These findings have recently been shown to be true for hemoglobin at concentrations comparable to those in the red cell.⁴ Secondly, by altering the red cell concentration of 2,3-DPG there would be a change in the equilibrium between hemoglobin and 2,3-DPG, which is represented by the equation $[Hb][DPG] = K[HbDPG]$. As increasing the bound fraction of hemoglobin results in an increase in $P_{50(7.4)}$, shrinking of red cells would cause an increase in $P_{50(7.4)}$ both by increasing the intracellular hemoglobin concentration per se and by increasing the binding of 2,3-DPG to hemoglobin by moving the above equation to the right. The converse arguments would hold for swelling of the red cell when a decrease in $P_{50(7.4)}$ would occur.

In order to consider the possible effect of cell volume changes on hemoglobin O_2 affinity the data were examined for a relationship between $P_{50(7.4)}$ and MCHC which is the best indicator of changes in cell volume. However, it was first necessary to allow for the effect of carbamino compounds. It has been shown that increasing PCO_2 raises the P_{50} of hemoglobin solutions, maintained at constant pH, due to the formation of carbamino compounds (12). The $P_{50(7.4)}$ values were corrected using the factor $\Delta \log P_{O_2} = 0.0013 \text{ BE}$ (base excess) as determined by Naeraa, Petersen, Boye, and Severinghaus (13) on whole blood. The changes in the corrected $P_{50(7.4)}$ in the first 5 hr of acidosis and alkalosis, when significant change in 2,3-DPG had not occurred, were examined in relation to the alterations in MCHC

⁴ Radford, E. Personal communication.

during this period (Fig. 4). The highly significant correlation that exists shows that an increase in MCHC of 1.0 g/100 ml resulted in an increase in $P_{50(7.4)}$ of the order of 0.5 mm Hg. The question remains which of the two mechanisms outlined above is the main contributing factor to these changes. As the exact behavior of hemoglobin and the binding of 2,3-DPG under

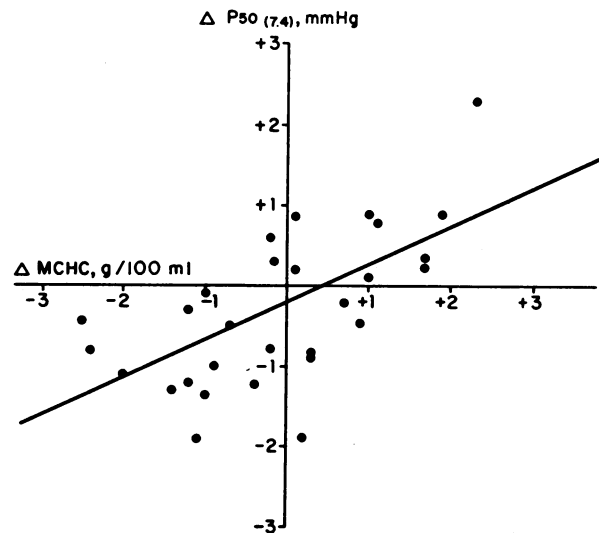


FIGURE 4 Change in $P_{50(7.4)}$ (corrected to a base excess of zero) as a function of change in MCHC during the first 5 hr of both acidosis and alkalosis. The zero value for acidosis was the base line reading, and for alkalosis the reading at the end of acidosis immediately before the infusion of sodium bicarbonate was taken as zero. The regression line, $\Delta P_{50(7.4)} = 0.471 \Delta \text{MCHC} - 0.216$, is shown ($r = 0.624$ $P < 0.001$).

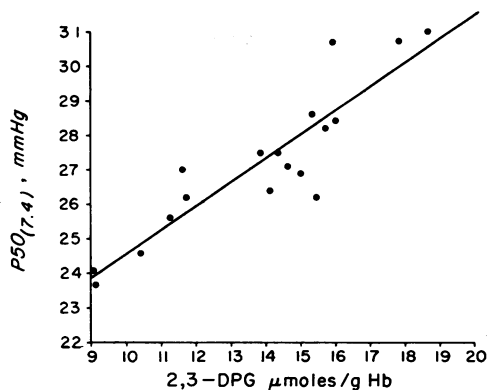


FIGURE 5 $P_{50(7.4)}$, (corrected to a base excess of zero) as a function of 2,3-DPG in samples obtained for control and last phase of both acidosis and alkalosis. The regression line, $P_{50(7.4)} = 0.694 \text{ DPG} + 17.63$, is shown ($r = 0.900$ $P < 0.001$).

conditions existing within the red cell are unknown, any answer is at present speculative. However, changes in MCHC have been shown to play a significant role in the adaptations in oxygen transport that occur at altitude⁵ and with exercise.⁶

In view of the effect of MCHC on $P_{50(7.4)}$ to determine the effect of 2,3-DPG on $P_{50(7.4)}$, it is important to eliminate any changes due to MCHC effect. As the values for MCHC in the control period and the last phase of both acidosis and alkalosis showed no significant change, the $P_{50(7.4)}$ 2,3-DPG relationship was examined in this period after the $P_{50(7.4)}$ had been corrected for changes in base excess. The highly significant correlation shows that a change of 0.69 mm Hg in $P_{50(7.4)}$ is caused by a change of 1.0 $\mu\text{moles/g Hb}$ in 2,3-DPG (Fig. 5).

It has been shown that the effect of 2,3-DPG in reducing the affinity of hemoglobin for oxygen is due to its specific binding with the hemoglobin molecule (14). However, these experiments were on hemoglobin solution and it has been suggested by Battaglia, McGaughey, Makowski, and Meschia (15) that, in vivo, 2,3-DPG has a dual role in its effect on oxygen hemoglobin dissociation by altering intracellular pH as well as its specific interaction with hemoglobin. 2,3-DPG is a highly charged anion to which the red cell is impermeable and any increase in its concentration decreases intracellular pH relative to plasma pH. Accordingly as $P_{50(7.4)}$ is measured at plasma pH 7.4, any increase in red

cell 2,3-DPG by decreasing intracellular pH will raise the $P_{50(7.4)}$ through the Bohr effect. There is a highly significant correlation between red cell 2,3-DPG and the difference between plasma and intracellular pH (ΔpH) (Fig. 6). From this relationship a change of 2,3-DPG of 10.00 $\mu\text{moles/g Hb}$ alters the ΔpH by 0.094 pH units which is very close to the theoretical value of 0.116 pH units suggested by Battaglia et al. (15). Shown in Fig. 7 is the relationship of intracellular pH to plasma pH obtained from our data both before and after correcting the ΔpH for the change in 2,3-DPG using the slope of the regression line in Fig. 6. It can be seen that the uncorrected data differ considerably from that obtained by Hilpert, Fleischmann, Kempe, and Bartels (10), but their curve was obtained in vitro on normal blood with presumably normal 2,3-DPG levels. When our data are corrected for the change in 2,3-DPG levels it is seen that the new regression line is very close to the one obtained by Hilpert et al. (10). The change in 2,3-DPG in our experiment was of the order of 6 $\mu\text{moles/g Hb}$ from the extremes of acidosis to alkalosis which would alter the ΔpH by 0.058 units. Such a change in ΔpH would cause a change in $P_{50(7.4)}$ of the order of 1.8 mm Hg. As the mean change in $P_{50(7.4)}$ from the extremes of acidosis to alkalosis was 5.1 mm Hg about 35% of the change in $P_{50(7.4)}$ due to 2,3-DPG is a result of the change in ΔpH between red cell plasma that 2,3-DPG causes.

In the light of the above observations, it is important to reconsider methods of reporting red cell 2,3-DPG. It is suggested that 2,3-DPG is reported in relation to hemoglobin (i.e. micromoles per gram of Hb) and that the coexisting MCHC is also given. As the hemoglobin content of the cell remains unchanged this mode of expression will not be affected by changes in cell volume and is therefore to be preferred when considering cell content of 2,3-DPG and the kinetics of any changes. The effect of 2,3-DPG on $P_{50(7.4)}$ can also be derived by considering any changes in MCHC together with changes in 2,3-DPG and using the factors for these two parameters that are derived above. If 2,3-DPG is expressed in relation to volume of cells (moles/liter packed cell) or in relation to apparent cell water as suggested by Hjelm (16), no account will be taken of the effect of hemoglobin concentration or altered 2,3-DPG binding on $P_{50(7.4)}$. Furthermore both of these methods will show changes in 2,3-DPG purely as a result of changes in red cell volume when no change in red cell content has occurred.

The importance of the changes in 2,3-DPG and MCHC in maintaining a normal in vivo hemoglobin affinity for oxygen is shown in fig. 1 by the lack of a significant change in $P_{50(i.v.)}$ in spite of the maintained acidosis or alkalosis. That this argument holds for more severe acidosis, as in diabetic ketoacidosis, has been

⁵ Lenfant, C., J. Torrance, and C. Reynafarje. The shift of the O_2 Hb dissociation curve at altitude: mechanism and effect. *J. Appl. Physiol.* In press.

⁶ Shappell, S. D., J. Murray, A. J. Bellingham, R. D. Woodson, J. C. Detter, and C. Lenfant. Adaptation to exercise: role of hemoglobin affinity for oxygen and 2,3-diphosphoglycerate. *J. Appl. Physiol.* In press.

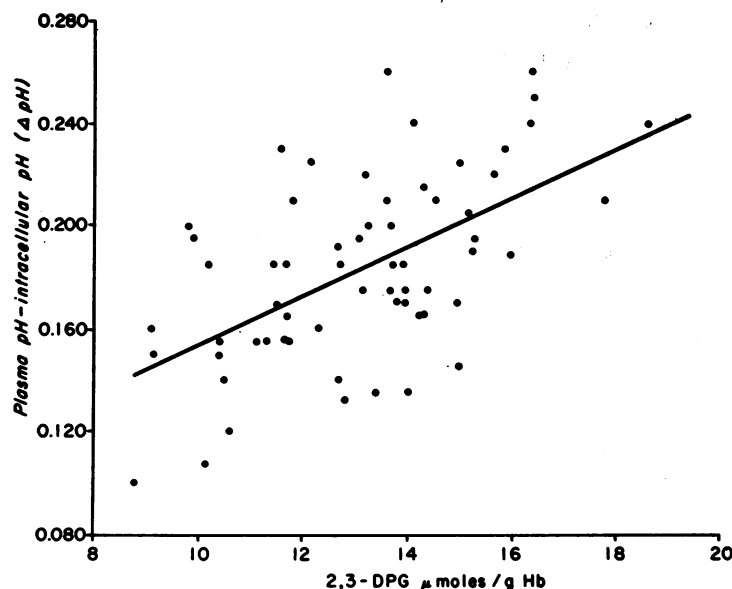


FIGURE 6 The difference between plasma pH and red cell pH (Δ pH) as a function of the 2,3-DPG throughout the experiment. The regression line, Δ pH = 0.0094 DPG + 0.060, is shown ($r = 0.567$ $P < 0.001$).

shown by Bellingham, Detter, and Lenfant (17). However the body is limited in the rate with which it can adapt to pH changes and this is shown at the beginning of alkalosis where the infusion of sodium bicarbonate caused a sharp fall in $P_{50(i.v.)}$ as a result of the sudden pH change and relatively slow response of 2,3-DPG. The importance of this sudden rise in in vivo hemoglobin oxygen affinity in clinical management of acidosis has been emphasized by Bellingham et al (17). The induction of acidosis was not so sudden and since the MCHC changes occur rapidly (Fig. 2), these insure that $P_{50(i.v.)}$ remains unchanged while the level of 2,3-DPG changes. There is obviously a limit to the change in cell volume that can occur, and the greater pH change at the induction of alkalosis is beyond the capabilities of this compensatory mechanism so the $P_{50(i.v.)}$ decreases until the 2,3-DPG mechanism can readjust.

In clinical practice when calculating hemoglobin oxygen saturation from P_{O_2} using blood gas data it is usual practice to apply corrections for pH, temperature, and base excess. Both a blood gas calculator (6) and a nomogram (18) have been devised for this. With effect of both 2,3-DPG and MCHC on hemoglobin oxygen affinity it is obviously necessary to apply correction for changes in these factors, otherwise serious errors will occur particularly in patients with acid-base disturbances of more than 24 hr standing. It is possible to combine the factors for 2,3-DPG and MCHC with those for temperature (T), pH, and base excess (BE) used in the blood gas calculator (6) to

derive the in vivo hemoglobin affinity for oxygen. This is as follows:

$$\log P_{50(i.v.)} = \log [26.6 + 0.5(\text{MCHC} - 33) + 0.69(\text{DPG} - 14.5)] + 0.0013\text{BE} + 0.48(7.4 - \text{pH}) + 0.024(\text{T} - 37).$$

The P_{50} at pH 7.4 and 37°C in normal blood with a base excess of zero is taken as 26.6 mm Hg the value used by Severinghaus (6). The normal values for 2,3-DPG and MCHC of 14.5 moles/g Hb and 33 g/100 ml respectively are the normal values in our laboratory.

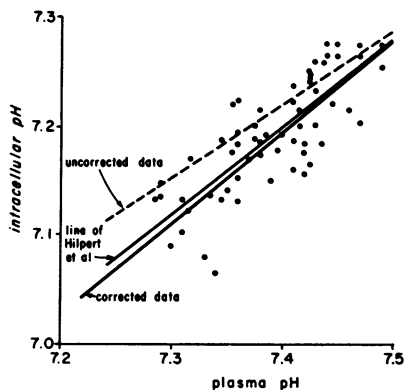


FIGURE 7 Red cell pH (pHc) as a function of plasma pH (pHp) throughout the experiment. The red cell pH values have been corrected for changes in 2,3-DPG (see text). The regression line, $\text{pHc} = 0.780 \text{ pHp} + 1.427$, is shown ($r = 0.797$ $P < 0.001$). Also shown is the regression line obtained before the pHc had been corrected for 2,3-DPG together with the line of Hilpert et al (10).

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