Pediat. Res. *5:* 523–538 (1971)

acid-base homeostasis
blood volume
body composition
extracellular fluid
hypercapnia

Influence of Body Composition on the *In Vivo* Response to Acute Hypercapnia

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Extract

Changes in the acid-base status of blood *in vivo* have been investigated in anesthetized, nephrectomized, artificially ventilated dogs during acute hypercapnia before and after induced changes in body composition. The experiments were designed to study factors influencing the redistribution of bicarbonate generated by buffers in the blood compartments and the interstitial fluid volume.

The first series of experiments was designed to determine the best linear fit to data collected *in vivo* in dogs with acute steady state hypercapnia (the so-called *in vivo* CO_2 equilibration curve). Three groups were studied: a control group, a group with expanded extracellular fluid (ECF), and a group with expanded blood volume. A linear relation was found between pH-log P_{CO_2} and $[H^+]$-P_{CO_2}, the correlation coefficients being 0.98 for both pairs of variables in all three groups. The pH-log P_{CO_2} coordinate system was adopted. The linear relation between pH and log P_{CO_2} allows the slope ($\Delta \log P_{CO_2}/\Delta pH$) of the *in vivo* CO_2 equilibration curve to be determined by measuring only two points on the line.

In the second series of experiments, the slope of the *in vivo* CO_2 equilibration curve was determined before and after body composition was altered so that each dog served as his own control. Six groups of dogs were studied: *group 1* (six dogs) was control and had no change in body composition; *groups 2* and *3* (six dogs each), ECF volume was increased by infusing 100 and 200 ml/kg, respectively, of a mock ECF solution; *group 4* (eight dogs), blood volume was increased by infusing 50 ml/kg fresh, heparinized whole blood; *group 5* (eight dogs), hemoglobin concentration was decreased by replacing blood with plasma; and *group 6* (four dogs), hemoglobin concentration was increased by infusing 25–30 ml/kg packed erythrocytes. Statistical analysis of the changes in slopes caused by these changes in body composition (Table V) showed the following results: slope increased significantly as plasma bicarbonate concentration fell; slope decreased significantly as ECF volume increased; slope increased significantly as blood volume increased; slope did not change significantly with acute changes in hemoglobin concentration.

Speculation

This study indicates that the slope of the *in vivo* CO_2 equilibration curve is dependent upon certain variables of body composition, especially the volume of the extracellular fluid (ECF) and blood. Thus, correct interpretation of blood acid-base data in acute hypercapnia must take account of the redistribution of bicarbonate as a function of the body composition. For example, an infant born prematurely presenting with the

respiratory distress syndrome (RDS) and acute hypercapnia would be expected to have a lower blood base excess and plasma bicarbonate concentration at any given plasma P_{CO_2} than would adults with a similar degree of hypercapnia, a result of the premature infant's larger ECF volume and larger blood volume. This would not necessarily represent the development of a concomitant metabolic acidosis but rather the redistribution of bicarbonate in a larger ECF volume.

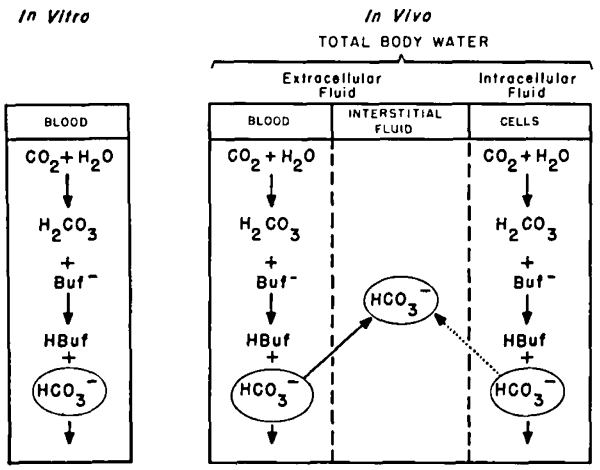

Fig. 1. Models of the *in vitro* and *in vivo* buffering of CO_2 illustrating the redistribution of HCO_3^- possible *in vivo*.

Introduction

A quantitative understanding of the buffer responses to acute hypercapnia is important for the interpretation of blood acid-base data in patients with this disorder. An abrupt elevation of Pa_{CO_2} is buffered by the conjugate bases of the nonbicarbonate buffers (Buf^-) of blood and of cells to produce bicarbonate and the weak acid of the nonbicarbonate buffers (HBuf) as shown by the following general reaction:

$$CO_2 + H_2O \rightarrow H_2CO_3 + Buf^- \rightarrow HBuf + HCO_3^-$$

Since the interstitial fluid has a small nonbicarbonate buffer capacity [5], little bicarbonate is produced in this compartment. Thus bicarbonate produced by the above reaction in blood and in cells enters the interstitial fluid. This redistribution of bicarbonate occurs rapidly, since it is produced multifocally throughout the circulating blood volume, and a new equilibrium is rapidly achieved. Consequently, blood *in vivo* at the same elevated value for P_{CO_2} shows a smaller rise in plasma bicarbonate concentration and a fall in whole blood base excess compared with blood *in vitro* where bicarbonate redistribution is not possible. Since bicarbonate does not rise as much *in vivo* as it does *in vitro* at the same plasma P_{CO_2}, blood pH will fall farther *in vivo* [20].

There are a variety of graphic methods by which these acid-base changes occurring in blood *in vivo* or *in vitro* can be represented. These vary according to which two of the several possible variables (blood pH, blood $[H^+]$, plasma bicarbonate concentration, whole blood bicarbonate concentration, plasma total CO_2 content, whole blood total CO_2 content, whole blood base excess, plasma P_{CO_2}) are plotted against each other. All such representations are variously referred to as CO_2 "titration" curves, CO_2 absorption curves, whole body titration curves, and the like. None of these terms is entirely satisfactory from a semantic point of view. In this paper such curves are called CO_2 equilibration curves, since this seems to be the least confusing terminology.

A qualitative understanding of the important influence of the volumes of the body fluid compartments and the concentration of the nonbicarbonate buffers on the degree of bicarbonate redistribution can be obtained by considering Figure 1, which illustrates a model of the system both *in vivo* and *in vitro*. Conceptually, the extracellular fluid (ECF) compartment is the acute volume of distribution of bicarbonate and is subdivided into blood volume and interstitial fluid, while the intracellular fluid compartment is all of the nonextracellular portion of the total body water. Four variables which influence the slope of the CO_2 equilibration curve can be deduced from this model: (*1*) the volume of the interstitial fluid compartment, (*2*) the volume of the blood compartment, (*3*) the concentration of the nonbicarbonate buffers of the blood, and (*4*) the amount of bicarbonate donated to the ECF by the intracellular fluid.

If the volume of the interstitial fluid compartment were increased, a larger "sink" would be produced for the bicarbonate generated by the buffer reactions occurring in the blood compartment. Hence, at any given elevated P_{CO_2} value more bicarbonate would leave the blood and a smaller rise in plasma bicarbonate concentration would result. Whole blood base ex-

cess, the change in sum of the conjugate bases of whole blood, would fall because the movement of bicarbonate into the interstitial fluid would lead to a decrease in conjugate base concentration of whole blood. Thus the net effect of an increase in the interstitial fluid volume would be a smaller rise in plasma bicarbonate and a greater fall in whole blood base excess when P_{CO_2} is raised. This smaller rise in plasma bicarbonate at the same P_{CO_2} would in turn cause a greater fall in blood pH.

When the blood volume is increased, the *amount* but not the *concentration* of the nonbicarbonate buffers of the ECF is increased so that, as P_{CO_2} rises, more bicarbonate is produced and diffuses into the interstitial fluid compartment. Thus as the blood volume increases plasma bicarbonate rises further and base excess falls less.

If nonbicarbonate buffer concentration is increased, *e.g.*, by increasing the hemoglobin concentration, however, more bicarbonate would be produced but at a higher concentration; therefore, more would diffuse into the interstitial fluid to establish equilibrium. This increased production and loss of bicarbonate would in turn consume more Buf^-, so that although the final bicarbonate concentration would be higher the base excess would be lower.

In all of the above situations, the new steady state plasma bicarbonate concentration would be influenced not only by the redistribution of bicarbonate between blood and interstitial fluid volumes but also by the amount of bicarbonate generated in the intracellular compartment and transferred to the extracellular compartment. In effect, such a transcellular bicarbonate transfer would "spare" bicarbonate generated by the nonbicarbonate buffers of the blood.

The influence of body composition upon the redistribution of bicarbonate is important to pediatricians who deal with patients having different body fluid compartment volumes. Thus the infant born prematurely, exhibiting acute hypercapnia secondary to the respiratory distress syndrome (RDS), would be expected to have different blood acid-base data than an older child or an adult with the same elevation of Pa_{CO_2} because the premature has a larger interstitial fluid volume and a larger blood volume. Accordingly, the influence of body composition upon blood acid-base data in acute hypercapnia has been studied in an animal model.

The present series of experiments was designed to evaluate the influence of acute expansion of the interstitial fluid volume, acute expansion of the blood volume, and acute increases and decreases in hemoglobin concentration on the slope of the *in vivo* CO_2 equilibration curve in mongrel dogs. A first consideration in such experiments was to study the various methods of portraying the *in vivo* response to hypercapnia both in normal dogs and in dogs with induced abnormalities of body composition. Ideally, the simplest coordinate system for portrayal of such an *in vivo* reponse would be one that would yield a straight line, since if this were the case the entire curve could be defined from the experimental determination of only two points. Accordingly, the initial series of experiments was designed with this in mind. They showed that the *in vivo* CO_2 equilibration curve was linear in a pH-log P_{CO_2} coordinate system over the range of interest. Therefore, in the second portion of the study the slope of the *in vivo* CO_2 equilibration curve was determined in each dog by exposing the animal to a normal (approximately 35 mm Hg) and a high (140–180 mm Hg) P_{CO_2} before and after the acute alteration in body composition.

Materials and Methods

Animals

Dogs weighing 9–14 kg were anesthetized with 30 mg/kg body weight of sodium pentobarbital, intubated, given succinylcholine (1 mg/kg body weight initially with doses repeated as necessary to maintain paralysis of the respiratory muscles), and ventilated with a positive pressure respiratory pump [25]. The end tidal P_{CO_2} was continuously monitored by an infrared CO_2 analyzer, and appropriate adjustments of the respirator were made to keep the end tidal P_{CO_2} constant. The sample cell of the infrared analyzer was connected to the endotracheal cannula by a needle with a constricted orifice, thereby decreasing the gas concentration in the cell so that high values for CO_2 could be measured [18]. A catheter was placed in the femoral artery for collection of blood for acid-base determination; a second catheter was placed in the femoral vein for the administration of isotopes and fluids. The abdomen was opened through a midline incision, and ligatures were placed around the splenic and both renal pedicles.

After completion of the surgery 100 μCi of tritiated water, for measurement of total body water, and 20 μCi ^{36}Cl, for measurement of extracellular fluid volume, were injected intravenously by a syringe pipette. Both isotopes were diluted in saline and a volume

exactly equal to that injected was taken for preparation of standards. Preliminary experiments showed that equilibration, judged by steady state blood concentration of the isotopes, was complete by 90 min; therefore, all samples for body composition were drawn after this time. Blood volume was determined by the volume of distribution of ^{51}Cr-tagged erythrocytes which were injected at least 20 min before the first sample was drawn. The erythrocytes were tagged with 125 μCi of a commercially available ^{51}Cr-chromate solution [26] by incubating 5 ml cells, suspended in 5 ml saline, for 0.5 hr at room temperature. The cells were then washed three times in equal volumes of normal saline and suspended in 4 ml saline for injection.

Two series of experiments were performed: *series 1,* linearity experiments, to determine the best linear fit to the data collected in dogs with acute hypercapnia; and *series 2,* to explore the effect of body composition upon the slope of the *in vivo* CO_2 equilibration curve.

Linearity Experiments

In this series of experiments, four points on the *in vivo* CO_2 equilibration curve were determined as follows: during the initial period, the end tidal CO_2 concentration was kept at 5%, and toward the end of this period three arterial blood samples were drawn at 10-min intervals for acid-base determination. End tidal CO_2 concentration was then abruptly raised to 10% by increasing the concentration of CO_2 in the inspired air. After a 20-min period for equilibration at the new P_{CO_2}, three more blood samples for acid-base determination were collected at 10-min intervals so that the third acid-base study was carried out 40 min after the end tidal P_{CO_2} had been increased. Two further experimental periods exactly similar in design to this one were carried out with end tidal CO_2 concentrations being held at 15 and 22%.

Three groups of dogs were studied. In *group 1,* six dogs served as normal controls; in *group 2* (six dogs), a nearly 100% increase in the size of ECF was produced by the intravenous administration of approximately 200 ml/kg of an artificial extracellular fluid containing Na^+, 150 mEq/liter; K^+, 4 mEq/liter; Mg^{++}, 2 mEq/liter; Ca^{++}, 2.5 mEq/liter; HCO_3^-, 26 mEq/liter; Cl^-, 130 mEq/liter; inorganic phosphate, 0.5 mmole/liter; $SO_4^=$, 2 mEq/liter. *Group 3* (three dogs) had a nearly 100% increase in blood volume induced by the administration of approximately 50 ml/kg whole dog blood.

Body Composition Studies

The experimental design for this series of experiments involved determining the slope of the *in vivo* CO_2 equilibration curve before and after the body composition of the animal was modified. Both slopes were determined by the measurement of the arterial acid-base status at two CO_2 tensions, one at a normal P_{CO_2} of 30–40 mm Hg and the other at a high P_{CO_2} of 140–180 mm Hg. All points were determined in triplicate, by averaging the three sets of acid-base data obtained at 10-min intervals at any given CO_2 tension. Thus, for the low point on the titration, carried out before body composition was altered, the acid-base status of arterial blood was determined at 70, 80, and 90 min after the injection of the isotopes, during which time the end tidal CO_2 concentration was maintained at 5%. The end tidal CO_2 concentration was then abruptly increased to 22% by increasing the CO_2 concentration of the inspired gas mixture. After a 20-min period for equilibration at the new P_{CO_2} three arterial blood samples were collected at 10-min intervals for acid-base determination so that the last sample was drawn 40 min after the P_{CO_2} had been increased. The end tidal CO_2 concentration was then returned to 5% and the body composition of the animal was altered.

The period of infusion was 0.5–1 hr. A 0.5-hr period for equilibration was allowed following which the slope of the *in vivo* CO_2 equilibration curve was redetermined in a manner exactly similar to that used for the determination of the preinfusion slope.

Six groups of animals were studied. In *group 1,* six animals served as normal controls to check on the reproducibility of the before and after equilibrations when no alteration of body composition occurred. *Groups 2* and *3,* six dogs each, were infused with approximately 100 and 200 ml/kg, respectively, of the mock ECF solution. The blood volume was expanded in *group 4,* eight dogs, by infusing approximately 50 ml/kg fresh heparinized whole blood. The hemoglobin concentration was varied in *groups 5* and *6.* In *group 5,* eight dogs were bled acutely by 20–25 ml/kg and equal amounts of plasma, from other dogs, were infused immediately after the bleeding. In *group 6,* four dogs were infused with 25–30 ml/kg fresh packed cells.

Chemical and Isotopic Methods

Ten milliliters of heparinized venous blood samples, for the measurement of tritiated water, ^{36}Cl, ^{51}Cr, PCV, Na^+, K^+, and Cl^-, and 1-ml arterial samples for the measurement of acid-base status and lactate were

drawn at the same time that the third acid-base sample was drawn during each steady state plateau. Acid-base determinations were performed on arterial blood in duplicate or triplicate by the equilibration method [22]. The *in vitro* CO_2 equilibration line is concave downward in a pH-log P_{CO_2} coordinate system so extrapolation of the line far beyond the upper equilibration point (approximately 80 mm Hg) could result in a considerable overestimation of the P_{CO_2} of the sample drawn from the dogs while they are exposed to high P_{CO_2} (approximately 150 mm Hg). To correct for this curvature of the *in vitro* equilibration line, samples were equilibrated with two high known P_{CO_2} tensions so that the unknown P_{CO_2} was bracketed. Thus acid-base determination of a blood sample with a high P_{CO_2} involved measurement of the actual pH and the pH of the sample when equilibrated with four known CO_2 tensions, the lower two for determination of base excess and the upper two for determination of P_{CO_2}. Plasma Na^+ and K^+ were determined by flame photometry, plasma Cl^- by electrometric titration, and whole blood lactate by a modified enzymatic procedure [9].

For the determination of ^{51}Cr, a 1-ml aliquot of well mixed whole blood was diluted up to 5 ml with distilled water. The hemolyzed blood was counted for ^{51}Cr in a well type crystal scintillation counter for 20–30 min so that a total of 100,000 counts were accumulated. The number of counts injected into the animal was determined by diluting up to 100 ml with distilled water a volume of the labeled cells exactly equal to the volume injected into the dog and then accumulating 200,000 counts. The blood volume was then calculated by a modification of the standard volume of dilution equation:

$$\text{Blood volume} = \text{counts injected} \div (\text{counts/ml whole blood} \times 0.88)$$

The constant 0.88 is the *F* cells factor for a splenectomized dog, a factor which corrects the packed cell volume of venous blood for the difference between large and small vessel packed cell volume [13]. The counts injected were continuously decremented by the amount of isotope removed by blood sampling. Preliminary experiments showed that the amount of ^{36}Cl present in the sample was too low to affect the counting rate in the crystal scintillation detector.

The plasma samples for tritium and ^{36}Cl were prepared for liquid scintillation counting by adding 1 ml 10% trichloroacetic acid (TCA) to 1 ml plasma [23] and then mixing 1 ml supernatant with 15 ml Bray's solution [3] in a glass liquid scintillation vial. Each sample was prepared in duplicate. The final mixture had a 15% counting efficiency for tritium and a 50% counting efficiency for ^{36}Cl. Sample to sample quenching for tritium was controlled by an internal standardization technique in which 25 μl of a 10 μCi/ml solution of tritiated methanol were added to each vial and the samples were recounted. This amount of tritiated methanol was sufficient to cause a 70,000–90,000 cpm increase in the counting rate of the sample. Quenching between samples never differed by more than 8% and was usually 2–3%. In preliminary experiments it was found that the difference in quenching of the ^{36}Cl counts was negligible even if a moderate amount of hemolysis was present in the original sample; therefore, no quench correction was made for the ^{36}Cl counts. Each sample was counted three times before and after the addition of the tritiated methanol.

The number of counts injected into the animal was determined by diluting exactly the same volume of stock isotope as was injected into the animal, up to 10 ml for tritiated water and to 5 ml for ^{36}Cl. Duplicate 50-μl aliquots of each of these solutions were added to 2 ml control plasma; then 2 ml 10% TCA were added and the supernatant was counted in the same fashion as the other samples. The standards prepared in this manner provided information not only about the number of counts injected but also about the number of ^{36}Cl counts appearing in the tritium channel. (No tritium counts appeared in the ^{36}Cl channel.)

The volume of dilution of both tritium and ^{36}Cl was calculated by the standard dilution equation after the observed counting rates had been corrected for quenching, coincidence, and ^{36}Cl in the tritium channel [14]. The number of counts injected was decremented by the amount of isotope lost during blood sampling. All of the isotope calculations were carried out on the IBM 7090/94 at Columbia University Computer Center with a program written in FORTRAN IV. The data were automatically recorded on punch cards by attaching the output of the counter to a solenoid deck placed on the keyboard of an IBM 026 key punch machine. All samples were counted in triplicate and the resulting range of counts of each run was tested to determine whether the counts followed Poisson counting statistics. Contrary to a previous report in the literature [15], the spread of the triplicate counts was found to exceed 95% confidence limits for a Poisson distribution only one-twentieth of the time on the average.

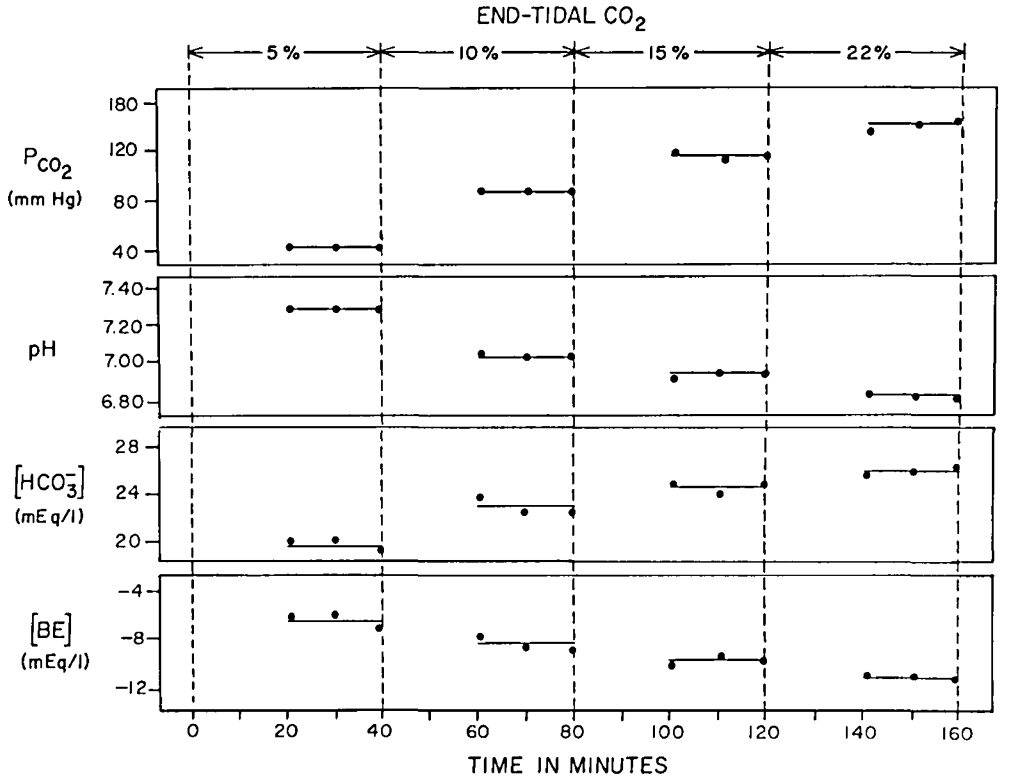

Fig. 2. Illustration in a single control dog of the basic experimental design used in the linearity studies showing the establishement of steady state end tidal CO_2 concentrations and the collection of acid-base data during four such steady states at 5, 10, 15, and 22% end tidal CO_2.

Results

Table I. Correlation coefficients for a linear least squares fit of various pairs of the acid-base variables

Variable pair	Correlation coefficients		
	Normal dogs	Dogs with ECF[1] expansion	Dogs with BV[1] expansion
pH-log P_{CO_2}	0.996	0.997	0.998
$[H^+]$-P_{CO_2}	0.994	0.997	0.992
pH-$[HCO_3^-]$	0.935	0.929	0.976
pH-P_{CO_2}	0.978	0.984	0.964
pH-log $[HCO_3^-]$	0.934	0.926	0.982
P_{CO_2}-$[HCO_3^-]$	0.932	0.902	0.970
P_{CO_2}-log $[HCO_3^-]$	0.923	0.890	0.946
log P_{CO_2}-log $[HCO_3^-]$	0.964	0.953	0.990
log P_{CO_2}-$[HCO_3^-]$	0.964	0.955	0.986
$[H^+]$-$[HCO_3^-]$	0.880	0.868	0.974
pH-[BE]	0.924	0.964	0.912
pH-log [BE]	0.925	0.963	0.914
P_{CO_2}-[BE]	0.870	0.938	0.812
P_{CO_2}-log [BE][2]	0.871	0.939	0.812
log P_{CO_2}-log [BE][2]	0.950	0.941	0.898
log P_{CO_2}-[BE]	0.951	0.940	0.898

[1] ECF: extracellular fluid. BV: blood volume.
[2] log [BE] was calculated as log ([BE] + 100).

Linearity Studies

Figure 2 presents data from a single control dog to illustrate the experimental design used in the linearity studies. During the initial period, end tidal CO_2 concentration was held at 5%, resulting in a mean steady state value for Pa_{CO_2} of 43.7 mm Hg. End tidal CO_2 concentration was then abruptly increased to 10%, and after a 20-min period for equilibration three acid-base determinations were carried out on blood collected at 10-min intervals. These showed that Pa_{CO_2} had risen to a mean value of 84.6 mm Hg and that this was associated with a fall in blood pH and whole blood base excess and a rise in plasma bicarbonate concentration. In the third experimental period, end tidal CO_2 concentration was readjusted to 15%, while in the fourth period it was again adjusted to 22%. With each increment in end tidal CO_2 concentration, plasma P_{CO_2} rose to a new steady state, being 114 mm Hg at 15% end tidal CO_2 concentration and 160 mm Hg at 22% end tidal concentration. With each of these segments of hypercapnia, pH showed a further fall

associated with further rises in plasma bicarbonate concentration and further falls in whole blood base excess.

In none of the experimental groups was a significant or consistent difference found in the mean plateau values for pH, plasma bicarbonate concentration, or whole blood base excess among the samples taken at 20, 30, and 40 min during the periods of hypercapnia, suggesting that a steady state with respect to blood acid-base status had indeed been achieved in each period under the conditions of the experimental design (see Fig. 2).

The adequacy of a straight line fit to 16 pairs of four relevant acid-base variables including appropriate logarithmic or antilogarithmic transformations was evaluated by calculating the correlation coefficient for every variable pair in each dog using all 12 data points. The correlation coefficients were then averaged for each experimental group using Fisher's Z transformation [10]. The results of this analysis are shown in Table I for the three experimental groups, a group with normal body composition, a group with expanded extracellular fluid, and a group with expanded blood volume. The correlation coefficients for the pH-log P_{CO_2} and $[H^+]$-P_{CO_2} relations were very high for the normal dogs, being 0.996 and 0.994, respectively, demonstrating that a straight line would fit either of these two relations extremely well. Furthermore, these two correlation coefficients were significantly higher in the group of six normal dogs than the correlation coefficient for any other variable pair tested.

The correlation coefficients were equally high for the pH-log P_{CO_2} and the $[H^+]$-P_{CO_2} pairs in each of the other two experimental groups studied, being 0.997 for both in the group with expanded ECF and 0.998 and 0.992, respectively, in the group with expanded blood volume. In the six dogs with expanded ECF the correlation coefficient of 0.997 was significantly higher than any other variable pair tested. In the three dogs with expanded blood volume the correlation coefficients for log $[HCO_3^-]$-pH, log $[HCO_3^-]$-log P_{CO_2}, and $[HCO_3^-]$-log P_{CO_2} were also high and did not differ significantly from the correlation coefficients for the pH-log P_{CO_2} or $[H^+]$-P_{CO_2} relations, possibly because this group included fewer dogs.

Body Composition Studies

Figure 3 presents the acid-base data obtained from a single dog given 2 liters mock ECF and illustrates the pattern of results obtained in the second series of experiments. Each dog served as his own control so that the slope of the *in vivo* CO_2 equilibration curve was determined by a two-point equilibration before and after body composition was altered (see *Materials and Methods*). Each point on the equilibration curve represents the mean of three arterial acid-base analyses. Before infusion mean Pa_{CO_2} rose, in this dog, from an average of 39 to 153 mm Hg while mean bicarbonate concentration rose from 18.7 to 25.5 mEq/liter, mean base excess fell from −7.0 to −14.0 mEq/liter and mean blood pH fell from 7.301 to 6.860. After infusion of the mock ECF a similar rise in Pa_{CO_2} produced a smaller rise in mean bicarbonate concentration, 18.6 to 21.3 mEq/liter, a nearly equivalent change in base excess, −6.6 to −13.7 mEq/liter, and a larger fall in pH 7.311 to 6.830.

The mean acid-base data for all six experimental groups and the mean body composition data for each group are shown in Tables II and III. In the control group there were no significant differences between the rise in bicarbonate or the fall in base excess before and after a time period equivalent to that required for the infusion in the other experimental groups. Although the bicarbonate concentration at both levels of Pa_{CO_2} was lower during the second titration this result was presumably due to endogenous acid production in the nephrectomized animal. The increase in total body water and ECF was largely accounted for by the ongoing infusion of isotonic saline (0.9% NaCl) which was required for administration of succinylcholine and to keep the venous catheter patent.

In the two groups infused with a mock ECF solution, total body water and ECF compartment increased considerably in volume (Table III) while intracellular fluid (ICF) volume remained virtually unchanged. Packed cell volume also fell as a result of the dilution of the ECF. Examination of the acid-base data (Table II) shows that after infusion of the mock ECF the plasma bicarbonate concentration at normal P_{CO_2} was higher, probably due to a higher bicarbonate concentration of the infusate than was present in the dogs prior to this infusion. The degree of rise of plasma bicarbonate due to the change in P_{CO_2}, however, was less after expansion of the ECF than before even though there was an equivalent rise in plasma P_{CO_2}. As a consequence pH fell more after expansion than before. Base excess changes were nearly equal before and after expansion in both groups.

The animals infused with heparinized whole blood (*group 4*) showed not only a rise in mean blood volume of 25 ml/kg but also an even larger increase in

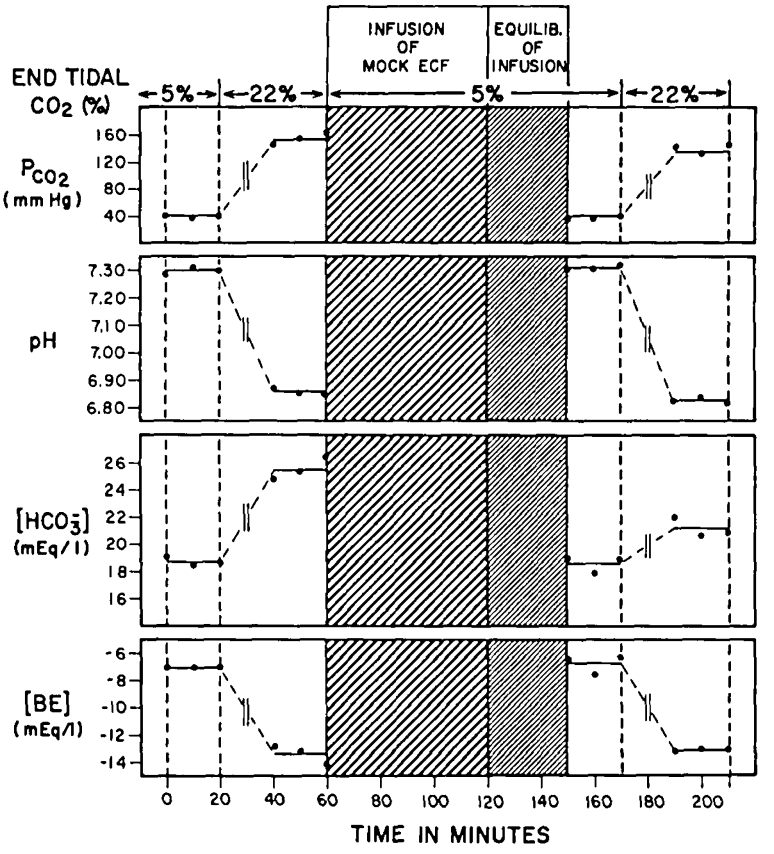

Fig. 3. Illustration in a single dog of the basic experimental design used in the body composition studies showing the collection of acid-base data at two steady state end tidal CO_2 tensions before and after the infusion of mock ECF.

mean interstitial fluid (ISF) volume amounting to nearly 40 ml/kg. Part of this rise in ISF volume presumably reflected plasma leaving the blood volume after infusion, causing packed cell volume (PCV) to increase even though the blood infused had a lower PCV than the blood of the recipient. The P_{CO_2}-induced rise in plasma bicarbonate (Δ plasma bicarbonate) was greater after infusion (8.2 mEq/liter plasma) than before infusion (7.1 mEq/liter plasma). The change in base excess was nearly equal in both groups.

In animals infused with 50 ml/kg plasma after removal of 20–25 ml/kg blood (*group 5*), the mean PCV fell from 52.6 to 39.6%. Blood volume also fell while ISF volume rose. The change in plasma bicarbonate after the PCV was decreased was 8.5 mEq/liter compared with the control value of 9.5 mEq/liter. In contrast, in animals given 25–30 ml/kg of packed cells (*group 6*), the change in plasma bicarbonate was 7.9 mEq/liter before infusion whereas after infusion it was 8.7 mEq/liter. In the latter group PCV rose from 42.4 to 55.8%, and both blood volume and ISF volume increased.

The slope of the *in vivo* CO_2 equilibration curve, defined as $\Delta\log P_{CO_2}/\Delta pH$, was calculated for each individual animal before and after body composition was altered (or similar time interval in the case of the control animals). The mean slopes for each experimental group before and after alteration of body composition are shown in Table IV along with the grand mean for all before titrations, which represents the mean control slope for all 38 animals.

In evaluating these data there are two complicating factors which immediately arise: the control acid-base status of the animal, *i.e.*, at normal P_{CO_2}, was changing because of the intervening infusion and of the passage of time and in no animal was a single variable of body composition altered but rather all three, blood volume, ISF volume, and PCV, were changed to some extent, although the predominant change occurred in the primary variable being studied. It is thus necessary to adjust mathematically these data before they can be properly evaluated.

The effect of changing acid-base status on the $\Delta\log P_{CO_2}/\Delta pH$ slope can be shown to depend on initial

Table II. Summary of acid-base data

Group	Start				Finish				
	pH	P_{CO_2}, mm Hg	BE[1]	HCO_3	pH	P_{CO_2}, mm Hg	BE	HCO_3^-	ΔHCO_3^-
			mEq/liter				mEq/liter		
First equilibration									
Control	7.346	37.6	−4.7	20.2	6.868	161.8	−10.6	27.5	7.3
	± 0.027[2]	± 3.4	± 1.7	± 1.7	± 0.039	± 15.9	± 1.6	± 1.6	
+1 liter ECF[3]	7.316	34.3	−7.7	17.3	6.858	147.3	−13.9	24.7	7.4
	± 0.063	± 1.9	± 3.4	± 2.4	± 0.051	± 8.5	± 3.1	± 3.0	
+2 liter ECF[3]	7.305	36.8	−7.5	17.8	6.853	159.0	−12.8	26.6	8.8
	± 0.024	± 4.2	± 1.7	± 2.0	± 0.020	± 18.9	± 1.8	± 2.7	
↑ BV[3]	7.309	35.4	−7.8	17.5	6.831	159.0	−12.5	24.6	7.1
	± 0.025	± 1.9	± 1.6	± 1.4	± 0.047	± 20.3	± 2.4	± 1.9	
↓ Hemog[3]	7.358	31.9	−6.4	17.6	6.794	190.6	−13.6	27.1	9.5
	± 0.034	± 2.1	± 1.9	± 1.4	± 0.037	± 35.9	± 1.9	± 2.9	
↑ Hemog[3]	7.350	36.5	−4.9	19.8	6.825	180.5	−10.3	27.7	7.9
	± 0.031	± 4.3	± 0.9	± 1.1	± 0.020	± 18.7	± 1.6	± 2.1	
Second equilibration									
Control	7.325	35.5	−6.8	18.2	6.849	162.2	−12.1	26.4	8.2
	± 0.047	± 3.0	± 2.5	± 1.9	± 0.022	± 13.6	± 2.7	± 2.8	
+1 liter ECF	7.336	36.8	−5.5	19.4	6.853	156.0	−12.3	25.7	6.3
	± 0.056	± 1.7	± 3.1	± 2.2	± 0.040	± 6.5	± 3.2	± 3.1	
+2 liter ECF	7.309	38.1	−6.6	18.6	6.821	152.4	−13.1	23.7	5.1
	± 0.061	± 3.3	± 3.2	± 2.4	± 0.047	± 19.4	± 4.0	± 3.7	
↑ BV	7.307	35.1	−8.0	17.4	6.855	155.4	−12.8	25.6	8.2
	± 0.057	± 3.2	± 3.6	± 2.7	± 0.027	± 25.6	± 3.6	± 4.1	
↓ Hemog	7.339	33.4	−6.8	17.7	6.791	184.0	−12.3	26.2	8.5
	± 0.032	± 2.3	± 1.9	± 1.5	± 0.027	± 17.7	± 1.8	± 2.2	
↑ Hemog	7.325	34.3	−7.4	17.5	6.819	173.4	−14.2	26.2	8.7
	± 0.021	± 1.9	± 1.6	± 1.4	± 0.020	± 17.6	± 1.5	± 1.9	

[1] Base excess.
[2] All values are mean ± standard deviation of the observations.
[3] ECF: extracellular fluid increased by 1 liter, by 2 liters. BV: blood volume; Hemog: hemoglobin, decreased and increased.

Table III. Body composition data

Group	First equilibration				Second equilibration			
	BV[1]	ISF[1]	ICF[1]	PCV,[1] %	BV	ISF	ICF	PCV, %
	ml/kg				ml/kg			
Control	64.0 ± 4.7[2]	222 ± 22	376 ± 24	48 ± 6	64.4 ± 7.5	236 ± 25	374 ± 26	50 ± 7
50% ECF[3]	60.0 ± 4.8	250 ± 40	372 ± 43	51 ± 7	63.3 ± 10.3	350 ± 52	372 ± 42	42 ± 6
100% ECF[3]	67.2 ± 4.1	210 ± 26	393 ± 78	49 ± 3	75.9 ± 11.2	388 ± 40	402 ± 79	42 ± 2
↑ BV[3]	60.8 ± 4.0	245 ± 21	380 ± 42	45 ± 7	86.1 ± 7.0	285 ± 22	376 ± 41	49 ± 5
↓ Hemog[3]	60.7 ± 6.1	214 ± 18	358 ± 46	52 ± 5	54.2 ± 9.5	232 ± 20	357 ± 40	40 ± 5
↑ Hemog[3]	63.3 ± 3.0	228 ± 22	361 ± 30	42 ± 6	78.4 ± 7.7	257 ± 28	359 ± 28	56 ± 7

[1] BV: blood volume. ISF: interstitial fluid. ICF: intracellular fluid. PCV: packed cell volume.
[2] All values are mean ± standard deviation of the observations.
[3] See Table II for definition of abbreviations.

bicarbonate concentration in a manner similar to the *in vitro* $\Delta \log P_{CO_2} - \Delta pH$ slope (see derivation in *Appendix*). This dependence on initial plasma bicarbonate $[HCO_3^-]$ can be expressed by the following equation:

$$\frac{\Delta \log P_{CO_2}}{\Delta pH} = -1 + \frac{0.434}{[HCO_3^-]} \frac{\Delta [HCO_3^-]}{\Delta pH}$$

Since $\Delta[HCO_3^-]/\Delta pH$ is a negative quantity during changes in P_{CO_2}, at a constant $\Delta[HCO_3^-]/\Delta pH$ (a constant nonbicarbonate buffer capacity), the slope of the *in vivo* CO_2 equilibration curve will become steeper (more negative) as $[HCO_3^-]$ falls. The correlation between the control slopes of all dogs and their initial plasma $[HCO_3^-]$ is high ($r = 0.59$) (Fig. 4). This rela-

Table IV. Mean *in vivo* CO_2 equilibration slopes ($\Delta\log P_{CO_2}/\Delta pH$) for each experimental group before and after body composition was altered

Group	First equilibration	Second equilibration
Control	−1.32546 ± 0.04077[1]	−1.38740 ± 0.03077[1]
+50% ECF[2]	−1.38680 ± 0.07076	−1.29710 ± 0.04515
+100% ECF[2]	−1.40410 ± 0.11112	−1.23490 ± 0.04540
↑ BV[2]	−1.35880 ± 0.02137	−1.43040 ± 0.19826
↓ Hemog[2]	−1.37380 ± 0.04149	−1.35030 ± 0.02836
↑ Hemog[2]	−1.32180 ± 0.02304	−1.39180 ± 0.03050
Grand mean	−1.3644 ± 0.06192	

[1] All values are means ± SEM.
[2] See Table II for definition of abbreviations.

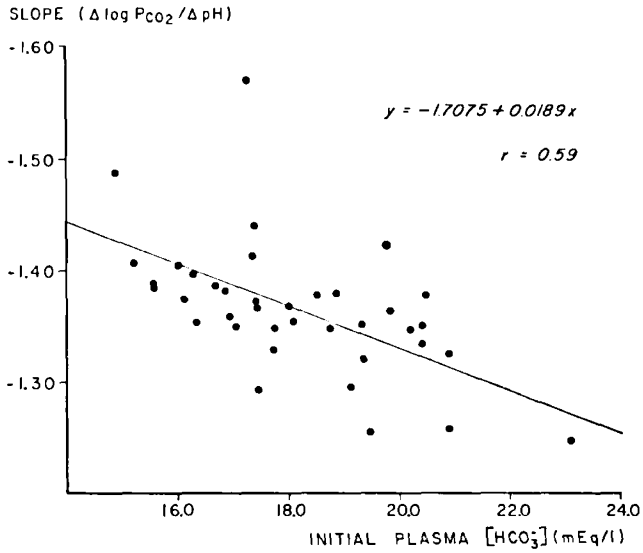

Fig. 4. Illustration of the dependence of slope ($\Delta\log P_{CO_2}/\Delta pH$) on initial plasma bicarbonate concentration.

tion implies that before any conclusions are drawn from changes in the slope of the *in vivo* CO_2 equilibration curve the slopes must be corrected for changes in initial bicarbonate concentration.

The second complicating factor, *i.e.*, all variables of body composition being altered when one variable is changed, can also be handled statistically. The problem is to find a linear combination of the body composition variables which discriminates best between the slopes of the *in vivo* CO_2 equilibration curves before and after body composition was altered and then to determine which of the variables did not add significantly to the discrimination. Those variables which did not add significantly to the discrimination are not relevant or significant. The statistical technique used for finding these discriminant functions was that of multivariate analysis. The present problem, however, can be simplified since our interest is in comparing the change in slope when body composition is changed. Therefore, one can find the equation which best expresses the delta slope as a function of the delta body composition variables by a simple multiple regression technique. It is also possible to include the delta initial bicarbonate concentration in the multiple regression equation, thereby correcting the slope for changing acid-base status as well. The final multiple regression equation then takes the following form:

$$\Delta\text{slope} = a + b_1\,\Delta[HCO_3^-]_i + b_2\Delta\text{ISF} + b_3\Delta\text{BV} + b_4\Delta\text{PCV}$$

where Δslope is $\Delta\log P_{CO_2}/\Delta pH$ before body composition is altered minus $\Delta\log P_{CO_2}/\Delta pH$ after body composition is altered, $\Delta[HCO_3^-]_i$ is the change in the initial bicarbonate concentration (the bicarbonate concentration at a normal P_{CO_2}), ΔISF, ΔBV, and ΔPCV represent the changes in ISF volume, blood volume, and PCV, b_1, b_2, b_3, and b_4 are the regression coefficients for the respective variables, and a is the intercept of the regression plane.

The problem at hand differs from the usual multiple regression problem in that the interest is not in determining the regression coefficients *per se* but in determining which of the variables did not add significantly to the change in slope. If a variable significantly influenced the slope then its regression coefficient would be significantly different from 0. The sign of the regression coefficient indicates direction of the effect, a positive sign indicating that the variable increases the slope while a negative sign indicates the opposite ef-

Table V. Results of multiple regression analysis

Variable	Regression coefficient	Standard error of regression coefficient	*t* value
ΔHCO_3^-	0.04396	0.00587	7.49[1]
ΔISF[2]	0.00087	0.00024	3.67[1]
ΔBV[2]	−0.00388	0.00118	3.28[1]
ΔPCV[2]	0.00308	0.00185	1.66

[1] $P < 0.01$.
[2] See Table III for definition of abbreviations.

Table VI. Summary of electrolyte data[1]

Group	Na	K	Cl	*R*	Lac	Na	K	Cl	*R*	Lac	Δ*R*
First equilibration											
Control	143	3.5	112	11.7	1.46	144	4.0	108	6.4	1.37	5.3
	± 2	± 0.3	± 6	± 4.7	± 0.35	± 3	± 0.7	± 3	± 6.0	± 0.06	
+1 liter ECF[2]	146	4.3	115	11.3		149	4.9	122	−2.1		13.4
	± 3	± 0.3	± 1	± 2.5		± 6	± 0.9	± 10	± 8.2		
+2 liter ECF[2]	151	4.3	123	9.8		156	4.6	126	2.2		7.6
	± 5	± 0.7	± 7	± 4.5		± 5	± 0.6	± 6	± 5.8		
↑ BV[2]	147	4.5	115	14.3	1.85	147	4.7	113	9.1	1.10	5.2
	± 3	± 0.5	± 4	± 2.3	± 0.87	± 2	± 0.4	± 3	± 3.3	± 0.31	
↓ Hemog[2]	148	4.0	118	13.1	1.64	149	4.1	116	5.9	1.29	7.2
	± 2	± 0.5	± 5	± 3.4	± 0.59	± 2	± 0.4	± 6	± 7.2	± 0.37	
↑ Hemog[2]	145	3.8	114	12.9	1.15	147	4.0	113	5.2	1.11	7.7
	± 1	± 0.4	± 3	± 3.1	± 0.59	± 3	± 0.5	± 4	± 3.5	± 0.46	
Second equilibration											
Control	145	4.2	110	17.7	1.89	146	4.6	111	7.8	1.55	9.9
	± 3	± 0.4	± 3	± 5.4	± 0.82	± 3	± 0.3	± 8	± 9.2	± 1.30	
+1 liter ECF	147	4.8	122	2.2		148	5.5	124	−3.8		6.0
	± 2	± 0.4	± 4	± 3.9		± 3	± 0.6	± 6	± 4.7		
+2 liter ECF	153	4.9	129	5.9		156	5.3	129	3.4		2.5
	± 4	± 1.2	± 3	± 4.3		± 4	± 0.9	± 2	± 5.1		
↑ BV	148	5.9	115	15.9	1.35	150	6.3	112	11.5	1.06	4.4
	± 4	± 1.7	± 7	± 7.8	± 0.61	± 4	± 1.3	± 4	± 6.4	± 0.99	
↓ Hemog	148	5.0	112	19.8	2.58	151	4.5	114	12.1	2.16	7.7
	± 2	± 1.2	± 3	± 2.3	± 0.50	± 4	± 0.5	± 4	± 3.6	± 0.61	
↑ Hemog	145	4.2	114	12.4	1.64	147	4.6	114	7.3	1.10	5.1
	± 1	± 1.2	± 2	± 3.4	± 0.16	± 1	± 0.9	± 1	± 3.0	± 0.30	

[1] All values are in milliequivalents per liter and represent mean ± standard deviation of the observations.
[2] See Table II for definition of abbreviations.

fect. The results of the analyses are shown in Table V and demonstrate: (*1*) that as initial bicarbonate falls the slope significantly increases; (*2*) that as ISF volume rises the slope significantly falls; (*3*) that as blood volume increases the slope significantly increases; and (*4*) that as PCV increases, the slope does not change significantly. (All significance tests were done at the overall error rate of 0.01; *i.e.*, a penalty was adopted for multiple comparisons so that the overall error rate applied to the whole experimental series.)

The mean plasma electrolyte data and mean arterial whole blood lactate concentration are shown for each plateau for each experimental group (Table VI). There were no striking changes in the plasma Na^+ and Cl^- concentration; plasma K^+ tended to rise slightly but not significantly. *R*, a measure of the undetermined anions and defined as $[Na^+] - [Cl^-] - [HCO_3^-]$, fell approximately 7 mEq/liter as P_{CO_2} rose because of a rise in bicarbonate since $[Cl^-]$ and $[Na^+]$ did not change. In every case mean lactate concentration fell as P_{CO_2} rose, as has previously been reported [11].

Discussion

Linearity Studies

The results of the linearity study demonstrate that in all three groups of dogs the data representing the *in vivo* CO_2 equilibration curve can be represented linearly with a high degree of confidence on either the pH-log P_{CO_2} coordinate system or on the $[H^+]$-P_{CO_2} coordinate system. Furthermore, in no group was there a significant difference in the correlation coefficients noted between these two methods of representation of the data so that a choice as to which to use has to be made on grounds other than purely statistical ones. We prefer the pH-log P_{CO_2} relation for two general reasons: (*1*) the pH-log P_{CO_2} coordinate system is well known and widely used [22] for portraying the *in vitro* behavior in blood, hence facilitating the comparison of the *in vivo* with the *in vitro* curve; (*2*) pH is a directly measured variable, its antilogarithmic transformation being difficult to interpret in a thermodynamic sense [1]. Furthermore, as pointed out by Davis [8], since pH is directly related to chemical potential, it may be

Table VII. Mean slopes and correlation coefficients of *in vivo* CO_2 equilibration curves on the log plasma P_{CO_2}-blood pH coordinate system in normal dogs

Study	No. of dogs	Mean slope, $\Delta\log P_{CO_2}/\Delta pH$	Corrected mean slope,[1] $\Delta\log P_{CO_2}/\Delta pH$	Correlation coefficient
Brown and Miller [6]	5	−1.215	−1.263	
Brown [4]	7	−1.199	−1.225	
Siggaard-Anderson [21]	3	−1.299	(−1.299)	
Cohen, Brackett, and Schwartz [7]	14	−1.309	−1.353	0.995
Brown and Clancy [5]	24	−1.207	−1.270	1.000
Refsum and Kim [16]	5	−1.309	(−1.309)	0.999
Present study	41	−1.365	(−1.365)	0.996[2]
Weighted mean			−1.3204	

[1] Corrected for variations in pK_1' with pH.
[2] $n = 6$.

the more useful representation of the effects of acidity upon physiologic processes. For these reasons we have adopted the pH-log P_{CO_2} coordinate system for portraying the *in vivo* behavior of blood, although this implies no inherent superiority over the $[H^+]$-P_{CO_2} coordinate system used by others.

The observed linearity of the *in vivo* CO_2 equilibration curve when plotted on the pH-log P_{CO_2} coordinate system is supported by data previously reported by others. Table VII shows the correlation coefficients calculated for three previously reported studies [5, 7, 16] in which the data are sufficient for their evaluation and in which a steady state of acute hypercapnia was documented. In each of these three previously reported studies the calculated correlation coefficients are in excess of 0.99, indicating that a straight line fits these data extremely well.

To the authors' knowledge, there is no fundamental explanation for the remarkably good fit of a straight line to the physiologic data describing the *in vivo* CO_2 equilibration curve and the pH-log P_{CO_2} coordinate system. There is no *a priori* reason to expect that the summation of such separate effects as blood buffering, diffusion of bicarbonate from blood to ISF, and transfer of bicarbonate from cells into ISF should be linearly represented on the pH-log P_{CO_2} coordinate system. Indeed, even in the simpler *in vitro* system, where such uniquely physiologic factors as bicarbonate diffusion from blood into ISF and cellular-extracellular fluid transfer of bicarbonate are not present, there exists no adequate theoretical explanation for the linearity of the CO_2 equilibration curve on this coordinate system. The lack of any theoretical explanation for the observed linearity of the *in vivo* curve need not, however, detract from the practical usefulness of the observation. Just as in the case of blood *in vitro*, where it is possible to characterize the slope of the curve with the determination of only two points on the curve, it is likewise possible to characterize the *in vivo* curve by the determination of only two points, thus simplifying experiments designed to evaluate factors which may influence the slope of the curve.

Table VII summarizes the mean slopes of all 41 normal "titrations" in our series of experiments (six control dogs from the linearity studies plus the control observations from 35 dogs in the body composition studies) along with the mean slopes for all of the data in the literature [4–7, 16, 21] recalculated for the pH-log P_{CO_2} coordinate system. It is apparent (Table VII) that the values for the slopes show considerable variation, ranging from −1.365 in our studies to −1.199 in the study of Brown [4]. Several reasons can be advanced to explain this variation. First, there are methodologic differences between the various studies. In five studies [4–7, 16] blood pH and total CO_2 content of plasma were measured and plasma P_{CO_2} was calculated by means of the Henderson-Hasselbalch equation. Only Refsum and Kim [16], however, appear to have taken into account the known variations in pK'_1 with blood pH [19]. The failure of other investigators to take account of this effect leads to an underestimation of the true value for the slope. Thus, according to Severinghaus [19] the value for pK'_1 is 6.095 at pH 7.40 and 38°. If a pK'_1 of 6.10 is used instead of 6.095, the calculated P_{CO_2} for normal blood with a P_{CO_2} of 40 mm Hg will be about 1% too high. At pH 6.80 the value for pK'_1 is 6.113 [19], and if the true value for P_{CO_2} were 200 mm Hg, the P_{CO_2} calculated from the Henderson-Hasselbalch equation at this pH using a pK'_1 of 6.10 would be too low by about 2.5%. These effects of calculating a falsely high value for P_{CO_2} at a normal value for blood pH and a falsely low value for P_{CO_2} at a low value for blood pH, although separately small, summate to cause the apparent slope of the *in vivo* CO_2 equilibration curve in the pH-log P_{CO_2} coordinate system to be too low to a significant extent. The results of recalculation of the slopes for those studies in which this effect was not originally taken into account appear in Table VII. These recalculated slopes are higher, and accordingly the agreement among the various studies is considerably better than at first appears.

In one study [21], the equilibration method was used, but no attempt was made to correct for nonlinearity of the *in vitro* curve at high values for plasma P_{CO_2}. Such nonlinearity would tend to make the *in vivo* slope reported by this investigator somewhat low,

although the error in the slope is likely to be no more than 2–3% since the high P_{CO_2} values used in this study were relatively low (83–123 mm Hg) compared with most other studies.

A second possible explanation for the variation of previously reported data concerns possible variations in body composition of the animals being studied. It was apparent from our results that expansion of ECF volume or expansion of blood volume can affect the value of the slope obtained. Thus, it is conceivable that variations in hemoglobin concentration, blood volume, and the status of hydration in randomly selected mongrel dogs might be considerable and might account for some of the variation from study to study noted in the table. An additional factor to be considered concerns the fact that in our experiments the spleen and kidneys were ligated so that the circulating blood volume was 15–20% lower and was constant throughout the experiment. In acute experiments of 2–3-hr duration it is doubtful that renal compensation could significantly account for any variation in slopes.

A third possible explanation for variability involves a variation in the initial bicarbonate concentration of the animals which were titrated. Fig. 5 which presents *mean* data in the literature shows that the slope of the *in vivo* CO_2 equilibration curve bears a definite relation ($r = 0.88$) to the initial plasma bicarbonate concentration in that the slope increases as initial (control) plasma bicarbonate concentration decreases. The failure of others to recognize and adjust for this effect may likewise be a source of discrepancy between the various reported studies.

Despite the variation in reported *in vivo* slopes, all studies including our own are in agreement that the *in vivo* slope is definitely less than the *in vitro* slope. Thus, a weighted mean for all studies is −1.3204 (Table VII), while the mean *in vitro* slope is -1.754 ± 0.128 ($P < 0.001$). (It may be noted that the mean *in vitro* slope for the dogs reported here is steeper than the value of −1.57 reported for normal man by Siggaard-Andersen [22]. In normal man, however, the base excess is 0 mEq/liter whereas in our control anesthetized dogs the initial base excess was −7.3 mEq/liter. It is known that the *in vitro* slope becomes steeper as base excess falls. The observed *in vitro* slope can be corrected for the initially low base excess by adding the arithmetic value of the base excess to the observed base excess and to the observed buffer base, thereby correcting the base excess to 0 mEq/liter to correspond with that of normal man. The slope is now recomputed using two points: a base excess of 0 mEq/liter and the buffer base as computed above. The mean *in vitro* slope so corrected is -1.596 ± 0.110, a value which is not significantly different from that obtained in normal man.) This difference in slope is mainly due to bicarbonate redistribution between blood and ISF whereby the apparent buffer capacity of blood *in vivo* is decreased. Thus, *in vivo* blood pH falls farther at any given elevated value for plasma P_{CO_2} than it does *in vitro,* a fact first noted by Shaw and Messer [20].

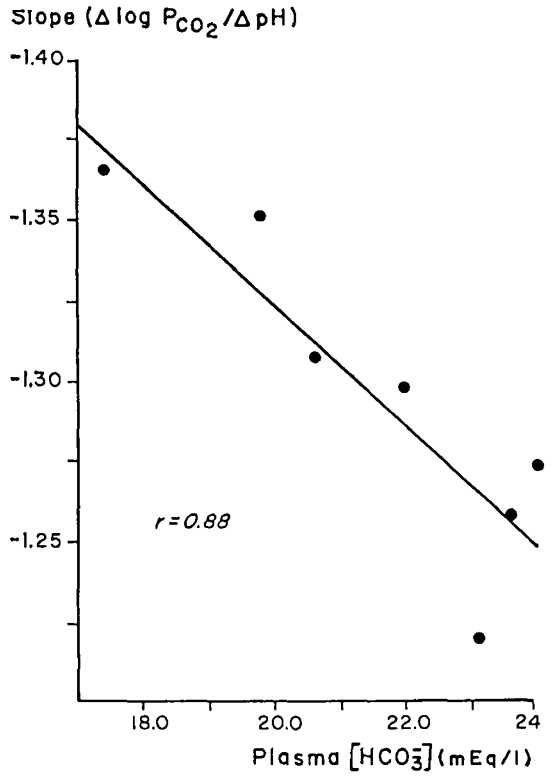

Fig. 5. Relationship between the mean initial plasma bicarbonate concentration and the slope of the *in vivo* CO_2 equilibration curve for all previously reported studies as well as for the present study. The slopes are the recalculated slopes of Table VII. The regression equation is: slope = $1.700 + 0.0188HCO_{3p}$.

Body Composition Studies

The results of the second series of experiments tended to confirm the qualitative predictions of the model discussed earlier. As ISF volume increases a greater "sink" is created for bicarbonate diffusion. Assuming that the cellular contribution of bicarbonate did not increase markedly, more bicarbonate would leave the blood compartment under these conditions. Thus for the same ΔP_{CO_2}, pH would fall further and the slope ($\Delta \log P_{CO_2}/\Delta pH$) of the *in vivo* CO_2 equili-

bration curve would decrease, an effect which was actually observed (Table IV).

As blood volume increases, the amount but not the concentration of the nonbicarbonate buffers in the blood compartment increases so that at any elevated P_{CO_2} value more bicarbonate is produced and is available for diffusion into the interstitial fluid. Hence, there is a greater rise in plasma bicarbonate concentration with increasing P_{CO_2} when compared with a normal blood volume so pH will not fall as much with an increased blood volume and the slope of the *in vivo* CO_2 equilibration curve would increase, as was observed (Table IV).

As PCV increases the concentration of the nonbicarbonate buffers increases so that at any elevated P_{CO_2} value more bicarbonate is generated by the buffer reactions but at a higher concentration gradient so that more must diffuse into the ISF compartment before an equilibrium is attained. However, at equilibrium there should be a rise in plasma bicarbonate concentration with less of a fall in blood pH and an increase in the slope of the *in vivo* CO_2 equilibration curve. Similar reasoning in case of a decreased PCV leads to the conclusion that slope should fall. Statistical analysis of the data showed that although the slope changed in the expected direction this change was not statistically significant. This was somewhat surprising since Refsum and Kim [16] found a significant increase in the $\Delta HCO_3^-/\Delta pH$ slope with increasing hemoglobin concentration. Several explanations may be advanced for this apparent discrepancy. First, the experimental design was different in the two studies in that Refsum and Kim either bled or infused the dogs 2 or 3 days prior to the study whereas in our study the bleeding or infusion was completed 0.5 hr before the study. Second, Refsum and Kim did not exclude the spleen before equilibration with high P_{CO_2}. As the plasma P_{CO_2} is increased, contraction of the spleen might increase the amount of nonbicarbonate buffer in the circulating blood volume, thus causing the slope of the *in vivo* CO_2 equilibration curve to change continuously during the experiment (and incidentally to cause a straight line to fit the HCO_3^--pH relation better than previously reported). While the initial plasma bicarbonate concentration was reported for all experiments in the study of Refsum and Kim, no data on body composition were reported so that it is not possible to correct the slopes for changes in blood volume and interstitial fluid volume which may have been introduced by a physiologic response to the bleeding or infusion.

It was not possible with the data at hand to evaluate quantitatively the fourth factor identified by our model (*i.e.*, the nonextracellular contribution of HCO_3^-). All acid-base determinations of the present study as well as all previously reported data (including [12]) represent changes in arterial blood. A more precise evaluation of the cellular contribution, however, must involve determination of the plasma bicarbonate concentration of the mixed venous blood since this more closely approximates the bicarbonate contribution of the ISF. An accurate estimate of the bicarbonate concentration of the ISF is necessary since it contains approximately two-thirds of the extracellular pool of bicarbonate. As pointed out recently by Roos and Thomas [17] there is no constant relation between arterial and mixed venous plasma bicarbonate in acute hypercapnia, since cardiac output continuously increases as P_{CO_2} rises. Under these conditions metabolic CO_2 is then carried in a larger volume of blood at a lower bicarbonate concentration so that the arterial-venous difference for bicarbonate will fall as cardiac output rises. Thus the relation between arterial and mixed venous bicarbonate (and hence ISF bicarbonate) is continuously changing and what may appear to be a movement of bicarbonate from the cells to the ISF may be in fact a movement of bicarbonate from the ISF to the mixed venous blood as a consequence of the smaller arterial-venous difference for bicarbonate. Other published studies purporting to examine the cellular contribution of bicarbonate in acute respiratory acidosis have utilized arterial blood for acid-base analysis and are therefore subject to the same error.

Bicarbonate which is generated from buffer reactions in the erythrocyte exchanges with Cl^- in the plasma so that, *in vitro*, a fall in plasma Cl^- is observed. Similarly, HCO_3^- in plasma would be expected to exchange in ISF Cl^-, causing plasma Cl^- to rise. The fact that plasma Cl^- did not change significantly suggests that these two effects offset each *in vivo*. The increase in plasma HCO_3^- was accompanied by a fall in the *R* fraction (defined as $Na^+ - Cl^- - HCO_3^-$ and is an indication of the undetermined anions; see *R* in Table VI). The increase was caused in large part by the decrease in charge on the plasma proteins (the fall in Pr^- can be calculated from $Pr^- = 0.104(Pr)\Delta pH = 0.104 \times 70 \times 0.6 = 4.4$ mEq/liter [24]. On the assumption that protein concentration is 70 g/liter and the pH falls from 7.4 to 6.8 ($\Delta pH = 0.6$), solving the equation yields: $\Delta Pr^- = 0.104 \times 70 \times 0.6 = 4.4$ mEq/liter) so that electroneutrality was maintained by a transfer of change from the conjugate bases of the plasma protein to HCO_3^- with the Cl^-

shifts into erythrocytes and from the ISF offsetting each other. In all cases there was a small but significant fall in whole blood lactate concentration.

The importance of alterations in body composition in the quantitative interpretation of acid-base displacement in patients with acute respiratory acidosis is obvious. If a newborn infant or an edematous patient (both of whom have an increased ISF volume compared with normal adult subjects) develops acute respiratory acidosis, the rise in plasma bicarbonate will be less than that expected for the normal healthy adult male studied by Brackett, Cohen, and Schwartz [2]. This smaller rise in plasma bicarbonate need not necessarily represent the development of a concomitant metabolic acidosis, but may only represent the effect of an increased amount of bicarbonate redistribution due to the increased ISF volume. Thus the quantitative relation between Pa_{CO_2} and plasma bicarbonate observed in healthy adult males [2] should not be used as a standard for identifying a metabolic acidosis complicating the acute hypercapnia of a premature infant with RDS or of an older patient who has an abnormal body composition. In the past a considerable amount of effort has been expended in trying to find the cause of the apparent metabolic acidosis in infants with RDS without much success. Although an elevation of lactic acid concentration of approximately 2–3 mEq/liter may occur, no other significant accumulations of anions of nonvolatile acids has been found. The work presented in this paper shows that most of this metabolic acidosis is "apparent" and is most likely attributable to a redistribution of HCO_3^- in the larger ISF volume of the infant born prematurely.

While it may be dangerous to apply quantitatively to man data collected on the dog, the order of magnitude of the change in bicarbonate at a given increased P_{CO_2} value may be calculated from our data. For example, if the ISF were doubled and P_{CO_2} rises to 100 mm Hg then the rise in plasma bicarbonate would be approximately 2 mEq/liter less than if ISF were normal, a 30% effect.

Summary

The *in vivo* CO_2 equilibration curve is linear in normal dogs and in dogs with induced abnormality of body composition when plotted on the pH-log P_{CO_2} coordinate system. The slope of such curves rises as plasma HCO_3^- falls, decreases as interstitial fluid volume increases, increases as blood volume increases, and does not change significantly with acute changes in hemoglobin. These results indicate that the slope of the *in vivo* CO_2 equilibration curve is dependent upon these variables of body composition and imply that the interpretation of blood acid-base data in acute hypercapnia must include a consideration of the patient's body composition.

Appendix

The Henderson-Hasselbalch equation may be written as follows:

$$\log P_{CO_2} = pK - pH + \log HCO_3^- - \log S$$

Differentiating with respect to pH yields:

$$\frac{d \log P_{CO_2}}{d\,pH} = -1 + \frac{d \log HCO_3^-}{d\,pH}$$

but

$$d \log HCO_3^- = \frac{0.434}{HCO_3^-}\, d\, HCO_3^-$$

Substituting this expression into the previous one yields:

$$\frac{d \log P_{CO_2}}{d\,pH} = -1 + \frac{0.434}{HCO_3^-}\,\frac{d\, HCO_3^-}{d\,pH}$$

Therefore, at a constant nonbicarbonate buffer capacity (*i.e.*, constant $d\ HCO_3^-/d$ pH) the slope of the pH-log P_{CO_2} relationship ($d \log P_{CO_2}/d$ pH) becomes steeper as HCO_3^- falls.

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26. Radiochromate, Abbott Laboratories, North Chicago, Ill.
27. The authors wish to express their thanks to RONNIE B. GERARD and AGNETE THOMSEN for their excellent technical assistance.
28. Supported by Public Health Service Research Grants nos. HE-10257 and 5 RO1 HD-03993 from the National Institutes of Health.
29. DR. RALPH B. DELL is a recipient of Research Career Development Award no. 5 KO3 GM-19779, National Institutes of Health, and CHRISTINE E. LEE is supported by National Institutes of Health Training Grant no. 5 TO1 GM-00257.
30. DR. ROBERT W. WINTERS is a Career Scientist, Health Research Council, New York City Grant no. I-309.
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Printed in U.S.A.