THE CO_2 TITRATION CURVE OF FETAL AND ADULT BLOOD *IN VITRO*

by

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A Thesis

Presented to
The University of Oregon Health Sciences Center
School of Nursing
in partial fulfillment
of the requirements for the degree of
Master of Nursing

June 12, 1981

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This study was supported in part by the Department of Health, Education, and Welfare Professional Nurse Traineeship, Grant Number 5 A11 NU00250-02, Grant Number 5 A11 NU00250-03, and Grant Number 5 A11 NU00250-04, and the Widmer Research Fund.

ACKNOWLEDGEMENTS

It is with deep gratitude that I express my thanks and appreciation to Jack Keyes, my advisor, teacher, and most respected critic. I also wish to thank him and Wilma Peterson for the use of the laboratory facilities. I would also like to express my appreciation to the following: to committee members Wilma Peterson and Mary McFarland for their careful reading and helpful advise; to Kent Thornburg and Sam Connell for the fetal and adult sheep blood; and to Fred Arfmann for his technical assistance. Finally to my family, my husband and my son, and my parents near and far, a simple thanks will never be sufficient. Their love, encouragement, and support have carried me through this effort.

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Chapter I

INTRODUCTION

Numerous acid-base studies have been done on normal full-term and pre-term newborn human infants. The authors of these studies universally conclude that the newborn is acidotic compared to the normal adult. Kildeberg (1964) concludes that the newborn acidosis has two possible causes. One may be an accumulation of combustible organic acids during the asphyxiating birth process. The other is hypoxia which causes cell membranes to depolarize, allowing hydrogen ions to leak into the plasma.

The pH of the blood of the newborn infant reaches and stabilizes within adult normal limits within the first 24 hours of life (Albert & Winters, 1966). Valcana (1972) suggests that the infant's low plasma bicarbonate concentration may be due to a low bicarbonate reabsorption in the proximal renal tubule. Albert and Winters (1966) speculate that as a result of the proportionately larger protein intake, the infant must excrete a greater amount of hydrogen ion at the expense of conjugate base forms.

In contemplating the acid-base equilibrium of the newborn infant, one must remember that there are four differences in body composition between the adult and the newborn

infant. These differences are as follows. 1) The percent of extracellular fluid volume of the newborn infant is approximately twice that of the adult (Nelson & Riegel, 1969; Strauss, 1966; Winters, 1973), and the percent of interstitial fluid volume is approximately three times greater than that of the adult (Friis-Hansen, 1961). The effect of this greater extracellular fluid volume on the acid-base status is dilution of available buffers, making the buffer system of the newborn infant less effective against changes in pH (Davenport, 1974; Dell, Lee, & Winters, 1971; Michel, 1968; Roos & Thomas, 1967; Winters, 1967). 2) The intracellular fluid volume of the newborn infant is 35% of the total body water compared to 40% for the adult (Friis-Hansen, 1961). Intracellular fluid contributes to buffering carbonic acid in the body, though it is not known to what extent this occurs in the infant. 3) The hematocrit of the newborn infant is approximately one and one-fourth times that of the normal adult female (Dittmer, 1961; Sisson & Whalen, 1960). The higher hematocrit, or specifically, hemoglobin concentration, will increase the ability of blood <u>in vitro</u> to buffer changes in pH (Davenport, 1974, p. 54). However, the effect the higher hematocrit has on the whole body response to changes in pH produced by changes in pCO_2 is determined by the ratio of the total volume of red cells in the body to

the volume of extracellular fluid. 4. The blood of the infant has a mixture of fetal and adult hemoglobin. This is of significance because fetal hemoglobin has a greater affinity for oxygen than adult hemoglobin (Bard, 1979; Versmold, Seifert, & Riegel, 1973). These differences in and of themselves could potentially create differences in the normal acid-base status between the infant and the adult. Since there are these four differences, it is not necessarily appropriate to use adult normal values as a comparison, let alone as guidelines for the clinical management of the newborn infant.

Winters (1967) addresses the effect of the greater blood volume, hemoglobin concentration, and extracellular fluid volume on the *in vivo* CO_2 titration curve. Taking these differences of the infant into account, Winters mathematically constructed a hypothetical *in vivo* CO_2 titration curve for the newborn infant comparing base excess (ordinate) and pCO_2 (abscissa). The slope of this CO_2 titration curve was greater than and displaced downward from the *in vivo* CO_2 titration curve of the adult. However, base excess was obtained from a nomogram constructed using adult normal values and therefore assumes a normal point of pH 7.40, pCO_2 40mmHg, and $[HCO_3^-]$ 24mEq/l. This is not the normal point for the blood gas composition of the infant (Nelson & Reigel, 1969).

The implications of a different CO_2 titration curve for the newborn are far reaching. This may preclude the use of the Siggaard-Andersen nomogram in determining buffer base and base excess as these were established using adult normal values (Nelson & Reigel, 1969). The classification, and therefore the treatment of an acid-base disturbance in an infant may be different because of the different slope of the CO_2 titration curve. It is therefore of importance to closely examine the possible effects of these differences between the adult and the infant. To begin this examination, I compared the slope of the *in vitro* CO_2 titration curve of full-term fetal sheep with that of adult sheep to determine whether the observed difference in the acid-base status of the newborn is in part due to the difference in the type and the concentration of hemoglobin.

Buffer Value and Buffer Capacity

In order to compare the slopes of the *in vitro* CO_2 titration curves, the following definitions must be clarified.

In 1922, Van Slyke defined the slope of the CO_2 titration curve at a given pH as the buffer value and expressed this as

$$= \frac{B}{pH}.$$

The figure B is the increment in gram equivalents per liter of strong base or acid added to a buffer solution. The pH is the resulting change in pH. When is divided by C (the concentration of buffer in moles/liter), the resulting quotient is the molecular buffer value (Van Slyke, 1922). However, when the buffer being measured is a protein such as hemoglobin, the unit of measurement is gm%, not moles/liter. It is incorrect in such a case to refer to the quotient $\frac{}{C}$ as molecular buffer value. To avoid this error, this quotient is called buffer value in this investigation, which is in keeping with the usage by Henderson (1928, p. 66). Buffer value as defined by Van Slyke, then, will be called buffer capacity (Bates, 1966; Siggaard-Andersen, 1966).

Implications for Nursing

Presently, parameters for acid-base status established for adults are used in the clinical management of the newborn infant. As is indicated, it may be an invalid assumption to do so. If it becomes established that the normal range of blood gas composition and the CO_2 titration curve of the infant differ significantly from those of the adult, the clinical assessment and care of the infant will change.

The nurse is a vital member of the health care team which identifies and treats the acid-base disturbances of the infant. Nurses make a unique contribution to the health

team in many ways. Collectively, they are the only members who are with a patient 24 hours a day. The nurse is therefore responsible for the continual assessment and care of the infant. This study contributes to the nursing knowledge which is used in the nursing process of assessing the infant, making a nursing diagnosis and plan, and implementing and evaluating the care.

For example, an infant who is now considered mildly acidotic may actually be within the normal range of acid-base parameters. This particular example would have the following effects on nursing and the nursing care given. The care of the infant would be more cost effective as intensive nursing care would not be necessary. The number of blood samples the nurse must draw for blood gas analysis would be reduced or eliminated. This would affect the well-being of the infant because there would be less discomfort and less chance the infant would become anemic as a result of the frequent sampling of blood necessary when monitoring acid-base status (Meites & Levitt, 1979). Also since the blood sample is usually drawn via a skin puncture of the heel, there would be less potential impairment of skin integrity and less chance of osteomyelitis occurring as a result of an accidental puncture of the heel bone (Meites & Levitt, 1979). The decreased number of blood samples the nurse must draw would also decrease the cost of care because less time would be spent in drawing the blood and less equip-

ment would be used. The nursing care could then center around promoting health and well-being rather than including the treatment of a non-existent disorder. Because an acid-base disorder does not exist, the child may not need to be separated from the mother, eliminating an iatrogenic barrier to attachment. The energies of the nurse can be more effectively directed to promoting parenting skills and the well-being of the mother-infant pair.

A nurse working with a foundation of current knowledge potentially may be more aware and effective in caring for the infant with an acid-base disturbance. This research supplements what is known about newborn acid-base regulation. In so doing, it contributes to the professional practice of nursing. In their argument for a discipline of nursing, Donaldson and Crowley (1978) state that "appropriately prepared nurses may elect to conduct research within other disciplines because of the critical importance of this non-nursing research to professional practice or the growth of the discipline (p. 119)."

Review of Literature

Graham and Wilson (1954) studied blood-gas and pH values of 64 full-term newborn human infants for the purpose of determining the probable respiratory stimuli of the newborn infant. The blood gas composition (pO_2,

pCO_2, pH, and $[HCO_3^-]$) of capillary blood during the first 24 hours of life was determined. The average pH, measured at 38°C, was 7.33 within one hour of birth and 7.43 at 24 hours. The average pCO_2 was 35mmHg at birth and 29mmHg at 24 hours. The plasma CO_2 content* remained essentially the same from birth to 24 hours at an average 19.8mEq/l. Oxygen saturation was 85% at birth, corresponding to a pO_2 of 55-60mmHg on the oxygen dissociation curve for adult type hemoglobin. The oxygen saturation was 91% at 24 hours which corresponds to a pO_2 of 65mmHg.

Using the normal adult as the basis for their decisions, Graham and Wilson drew two conclusions. First, the acidosis at birth can not be due to an accumulation of carbon dioxide because contrary to what is now known, their results showed a pCO_2 at birth which is below the adult normal range. By comparing the oxygen saturation of the newborn infant's blood with the oxygen saturation at which an oxygen deficit becomes a respiratory stimulus for the adult, they further concluded that the primary stimulus for respiration in the newborn infant is hypoxia.

Weisbrot, James, Prince, Holladay, and Apgar (1958)

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* The authors did not define plasma CO_2 content. Because of the year in which this study was published, it was most likely determined from the Van Slyke analysis for CO_2 content.

compared the blood gas composition of arterial blood from 21 newborn human infants and 12 mothers. The newborns weighed more than 2500 grams and had Apgar scores of six or greater. The blood samples at birth were drawn from the umbilical artery. Then at 1, 3, and 24 hours of life, samples were obtained from the left atrium. The maternal blood samples were drawn from multiparous women having repeat cesarean sections. The samples were drawn during the surgical procedure while the women were under regional anesthesia, and not in labor. All blood samples were collected anaerobically in greased syringes. The pH was measured at 37°C. The pCO_2 was calculated from the Henderson-Hasselbalch equation. The buffer base was determined from the Singer and Hastings nomogram which assumes certain values for serum protein concentration, carbonic acid pK' and a CO_2 solubility factor which are not correct for the newborn infant (Oliver, Demis, & Bates, 1961).

Weisbrot et al. reported a mean pH of 7.23 (S.D.±0.062) at birth and 7.41 (S.D.±0.039) at 24 hours. Contrary to Graham and Wilson's results, Weisbrot et al. measured a mean pCO_2 of 58.4mmHg (S.D.±10.5) at birth. At 24 hours the mean pCO_2 was 33.6mmHg (S.D.±3.4). The plasma CO_2 content* was 25mEq/l (S.D.±3.89) at birth and 21.4mEq/l

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* The authors did not define plasma CO_2 content. Because of the year in which the paper was published, it was most likely determined from the Van Slyke analysis for total CO_2 content.

(S.D.±1.63) at 24 hours. The mean values obtained from the infants at 24 hours were not statistically different from the mean values for pH, pCO_2, and CO_2 content of blood obtained from the mothers. Weisbrot et al. do not agree with Graham and Wilson (1954) that the respiration of the newborn infant at birth is driven by hypoxia. Instead, they postulate that the respiratory center of the infant is conditioned by the low pCO_2 of the in utero environment. As a result, the respiratory center drives respiration to return the blood pCO_2 back to that low level after birth. However, it is now known that the pCO_2 of fetal arterial blood is not low as is the mother's, but is approximately 45mmHg (Dawes, 1968, p. 106).

Oliver, Demis, and Bates (1961) performed blood gas analyses on serial arterial blood samples from 40 full-term infants during the first hour of life. The blood samples were taken from the umbilical artery and from the left atrium. The results of the analyses showed a mean pH at birth of 7.10 (S.D.±0.09) and 7.34 (S.D.±0.06) at one hour; a mean pCO_2 of 76.4mmHg (S.D.±11.6) at birth and 38.0mmHg (S.D.±6.9) at one hour; a mean CO_2 content as measured on a Kopp-Natelson microgasometer of 24.5mM/l (S.D.±2.8) at birth and 21.6mM/l (S.D.±2.3) at one hour; and a mean pO_2 of 19.5mmHg (S.D.±12.3) at birth and 61.7 mmHg (S.D.±13.8) at one hour of life. The low oxygen tension was attributed by Oliver et al. to the following:

1) a right to left shunt through the ductus arteriosus because the pO_2 values of the left atrial blood samples were higher than the pO_2 values of the umbilical artery samples, and 2) a low ventilation-perfusion ratio. They also concluded that the acid-base disturbance present at birth is best explained by asphyxiation during the birth process.

Prod'hom, Levison, Cherry, Drorbaugh, Hubbell, and Smith (1964) measured the intrapulmonary gas exchange and the blood gas composition of 20 newborn infants born by cesarean section to diabetic mothers. The blood samples were drawn from an umbilical arterial catheter at 20 minutes, 1, 4, and 24 hours of life. The following are the results of the blood gas analyses: a mean pH of 7.26 (S.D.±0.09) at 20 minutes and 7.43 (S.D.±0.04) at 24 hours; a mean pCO_2 of 47mmHg (S.D.±8) at 20 minutes and 26mmHg (S.D.±3) at 24 hours; a mean CO_2 content* of 21.9mM/l (S.D.±3.2) at 20 minutes and 23.8mM/l (S.D.±1.7) at 24 hours; a mean pO_2 of 59mmHg (S.D.±17) at 20 minutes and 71mmHg (S.D.±10) at 24 hours. The comparison of the blood pO_2 composition with the results of the intrapulmonary gas exchange analyses indicated a right to left shunt of 22% and an uneven ventilation/perfusion ratio.

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* CO_2 content was determined on plasma using the Kopp-Natelson microgasometer.

By the fourth hour, alveolar ventilation and pulmonary perfusion had adjusted so that the alveolar-arterial pO_2 difference remaining was caused by the right to left shunt. This shunting continued for the 24 hour duration of the study. Prod'hom et al. noted no statistically significant arterial-alveolar pCO_2 difference. They also noted that even though the 24 hour mean value for pCO_2 was low, the ventilation equivalent was normal (according to adult normal parameters), indicating that the infants were not hyperventilating.

Prod'hom et al. point out that even though many studies have been done on the acid-base status of the newborn infant, the exact measurement of the acid-base balance is not satisfactory. They state "the biology of fetal and neonatal blood is still not well known (p. 688)." They also state that one of the things not known is the slope of the CO_2 titration curve.

Malan, Evans, and Heese (1965) measured the blood gas composition of arterialized capillary blood from 20 pre-term and 16 full-term infants at 2, 4, 6, 12, 24, 48, and 72 hours of life. The acid-base values were determined using the Astrup method. The mean values for the full-term infants at two hours were pH 7.34 (S.D.±0/06), pCO_2 41.4mmHg (S.D.±8.8), and a bicarbonate concentration (determined from the Siggaard-Andersen nomogram) of

21.3mEq/l (S.D.±4.3). At 24 hours of life the mean values for the full-term infants were pH 7.41 (S.D.±0.04), pCO_2 34.9mmHg (S.D.±4.0), and bicarbonate concentration 21.7mEq/l (S.D.±1.9). At 72 hours of life the mean values for pH, pCO_2, and bicarbonate concentration for the full-term infants were 7.42 (S.D.±0.04), 35.5mmHg (S.D.±5.4), and 22.2mEq/l (S.D.±3.1). Malan et al. point out that it may not be acceptable to use the Siggaard-Andersen nomogram for adults in determining acid-base values of the newborn infant. Furthermore, they state that the normal acid-base parameters of newborn infant blood are not well delineated.

Kildeberg (1964) measured the blood lactic acid concentration of 30 acidotic infants to determine if anaerobic metabolism contributes to neonatal acidosis. Blood lactic acid concentrations were plotted against base excess and against pCO_2. There was no correlation between these values in cases of mild and moderate degrees of acidosis. In severe acidosis, the blood lactic acid levels accounted for only one-third of the excess acid present.

Research using an animal model was conducted by Dell, Lee, and Winters (1971). They measured the effect of acute changes in body composition on the slope (log pCO_2/ pH) of the <u>in vitro</u> CO_2 titration curve of nephrectomized

and spleenectomized hypercapnic dogs. The effect of doubling the extracellular fluid volume was a significant decrease in the slope of the *in vivo* CO_2 titration curve. Increasing the blood volume of the dogs significantly increased the slope while changes in the hemoglobin concentration did not significantly change the slope of the *in vivo* CO_2 titration curve.

The changes in body compostion Dell et al. produced in the dogs mimic those normally found in the newborn infant. Because the dogs were artificially ventilated to a hypercapnic state, the results are of significance in assessing the infant with respiratory distress. Dell et al. conclude that the seeming metabolic acidosis associated with respiratory distress in infants is mostly the result of the dilution of bicarbonate in the larger extracellular fluid compartment.

Conclusion

The newborn infant is acidotic at birth, recovering to a steady-state different from the normal adult within 24 hours. The acidosis following birth appears to be the result of many possible factors: the asphyxiating birth process, shunting of blood through the ductus arteriosus, a low ventilation-perfusion ratio, and possibly some anaerobic metabolism.

After this initial 24 hours, the blood gas composi-

tion usually stabilizes to the approximate values of pH 7.38, pCO_2 35mmHg, and $[HCO_3^-]$ 20mM/l (Nelson and Riegel, 1969). This low bicarbonate concentration may be due to the dilution of bicarbonate in the larger extracellular fluid volume of the infant. In the past, the infant has been considered acidotic at this steady-state, an interpretation which is now open to question (Dell, Lee, & Winters, 1971).

It is apparent that the normal acid-base parameters of the newborn infant are not well delineated, let alone well understood. There are several differences between the adult and infant which, if taken as a whole, could account for the differences observed in the normal acid-base status of the two groups. Studies need to be performed which establish the effect of each of these differences.

Problem Statement

In this study I compared the slope of the *in vitro* CO_2 titration curve of full-term fetuses with that of the adult model. This comparison was made to determine whether the different acid-base status of the newborn infant is in part due to the difference in the type and concentration of hemoglobin.

Hypothesis I: The slope of the *in vitro* CO_2 titration curve, buffer capacity, of the full-term fetal animal will be significantly greater than that for the adult animal.

Hypothesis II: The buffer value of the full-term fetal blood will be significantly greater than the buffer value of the adult blood.

Chapter II

METHODS

Thirteen blood samples, ranging from 12 to 21 ml in quantity, were obtained from seven pregnant ewes and six full-term fetal lambs. Each of the thirteen blood samples contained heparin and was divided into three sub-samples. Each sub-sample was equilibrated in a Dynex tonometer for 20 minutes at 37°C with one of three gases of known composition (±0.5%). These gases were as follows: 3% CO_2 in oxygen, 5% CO_2 in oxygen, and 10% CO_2 in oxygen. Two determinations of hematocrit were then performed on each sub-sample just prior to blood gas analysis. The blood for gas analysis was withdrawn from the tonometer anaerobically using greased glass syringes. Blood gas analysis was then performed immediately on a BMS 3 Mk 2 Radiometer blood gas analyzer. The blood gas analyzer was calibrated before and checked after each sample was analyzed according to the calibration technique described in the instrument manual. The bicarbonate concentration was determined using the Henderson-Hasselbalch equation:

$$pH = pKa' + \log \frac{[HCO_3^-]}{S \cdot pCO_2}$$

where pKa' is equal to 6.1 at 37°C and S is 0.0301 $\frac{mM\ CO_2}{mmHg \cdot pCO_2 \cdot L}$.

The results from each of the three sub-samples were plotted on a [HCO_3^-](ordinate)/pH(abscissa) graph. A line was drawn through the three points to yield the CO_2 titration curve for that sample. Since the curve was linear, the slope of the line was then calculated using linear regression analysis.

To eliminate the effect of hemoglobin concentration on the slope of the CO_2 titration line, the value for the slope of the line was divided by the concentration of hemoglobin in the sample. The hemoglobin concentration was determined from the hematocrit.

Time was of the essence when the blood samples were equilibrated in the tonometer and then analyzed. Consequently, the determination of hemoglobin concentration on each sample was not done as it was not possible to include this in the schedule. Therefore the following method was used at a later time to establish the relationship between hematocrit and hemoglobin concentration for adult and fetal sheep blood.

The cyanmethemoglobin method was used to convert hemoglobin to cyanmethemoglobin which is stable and can be measured by spectrophotometry. The reactions which take place in this conversion are as follows. Hemoglobin is oxidized to methemoglobin by ferricyanide. The methemoglobin is then converted into cyanmethemoglobin in the presence of potassium cyanide (Tietz, 1976, p. 411).

In order to delineate the relationship between hemoglobin concentration and absorbance values of cyanmethemoglobin, certified cyanmethemoglobin standard, for which the hemoglobin concentration is known, was used to construct a standard curve. The hemoglobin concentration of the sheep blood can then be determined by plotting absorbance values on this curve.

The working standards were prepared as shown in the following table.

Table 1

Working Standards Used in Determining the Absorbance/Hemoglobin Concentration Relationship

Tube no.	Cyanmethemoglobin certified standard in ml (80 mg/dl)	Drabkin's solution in ml	Hgb (mg/dl)
S_1	0	4	0
S_2	1	3	20
S_3	2	2	40
S_4	4	0	80

The Drabkin's solution consisted of 100 parts sodium bicarbonate, 20 parts potassium ferricyanide, 5 parts potassium cyanide, and 0.5 ml of 30% Brij-35 solution (Sigma Chemical Company) dissolved in distilled water and diluted to 1000 ml (Tietz, 1976, p. 412).

The hemoglobin concentrations of adult and fetal sheep blood were determined as follows. Five different hemoglobin concentrations were established for each sample. The first hemoglobin concentration was that of the initial blood sample. The other four were obtained in the following manner. Two aliquots of the blood sample were centrifuged for two minutes at 3000 rpms in a Beckman model TJ-6 centrifuge. Then approximately one-half of the plasma from one aliquot was transferred into a third tube of the blood sample and three-fourths of the plasma of the second aliquot was transferred into a fourth tube of the blood sample. Duplicate hematocrit determinations were performed on each of the resulting five blood samples. Triplicate dilutions (1:225) were then made by pipetting 20 microliters of each blood sample into five ml of Drabkin's solution. The absorbance was then determined for these dilutions and the working standards using a Beckman model 25 spectrophotometer set at a wavelength of 540 nm (Appendix A, table 1A). The standard curve was constructed (Appendix A, figure 1A) and the hemoglobin concentrations for the adult and fetal sheep blood were determined. The hemoglobin concentrations were then plotted against the respective hematocrit values and a line was drawn through the points showing the relationship (Appendix A, figures 2A and 3A). Linear regression analysis was used to determine the slopes of the

lines. The hemoglobin concentrations of the tonometered blood samples were then calculated from the hematocrit values.

Chapter III

RESULTS

The results from the blood gas analyses, the hematocrit determinations, and the calculated hemoglobin concentrations of the tonometered blood samples are shown in tables 2 and 3. The data of five samples of each of the seven ewe and the six term fetal blood samples were used. The initial adult sheep sample was eliminated from the data because a hematocrit determination was inadvertently not done. Also the data of one adult and one fetal sheep sample were eliminated because the points of the CO_2 buffer line were not linear and it was impossible to determine which point was in error or why.

The <u>in vitro</u> CO_2 buffer lines obtained from the samples are shown in figures 1 and 2. The correlation coefficient "r" ranged from -0.98 to -1.00. The vertical displacement of the CO_2 buffer lines reflects the bicarbonate concentration of the blood when obtained from the sheep. The sheep blood was obtained from other laboratories within the institution where the sheep were subjected to different surgical and respiratory manipulations. Consequently the initial bicarbonate concentrations varied considerably.

The slope of the CO_2 buffer line is expressed by the

change of bicarbonate concentration per unit change of pH. Figure 3 depicts the slopes (buffer capacity) of the CO_2 buffer lines of the samples.

In order to eliminate the effect of the variable hemoglobin concentrations the value of each slope was divided by its respective hemoglobin concentration. The resulting quotient is the buffer value of the blood. Figure 4 depicts the buffer value of each sample. Figure 5 shows the mean buffer value of each group (adult vs. fetal blood). A Student's t test for significant difference was performed on the mean buffer values of the two groups. No significant difference exists. The probabliity that the two mean buffer values are the same is greater than 0.9.

Table 2

Mean Values of Adult Sheep Blood Equilibrated with Gases

Containing Three Different CO_2 Concentrations

Sample		3% CO_2*			5% CO_2*			10% CO_2*			Hct	Hgb (g/dl)
		pH	pCO_2 (mmHg)	HCO_3^- (mEq/l)	pH	pCO_2 (mmHg)	HCO_3^- (mEq/l)	pH	pCO_2 (mmHg)	HCO_3^- (mEq/l)		
1	$\bar{X}$	7.658	20.7	22.5	7.481	34.5	25.0	7.232	71.2	29.0	30	9.3
	S.D.	0.002	0.1		0.002	0.1		0.001	0.4			
	N	7	7		7	7		6	6			
2	$\bar{X}$	7.658	22.8	24.8	7.504	35.3	27.0	7.236	73.8	30.4	25	7.8
	S.D.	0.0005	0.05		0.0005	0.05		0.001	0.4			
	N	5	6		7	7		7	7			
3	$\bar{X}$	7.667	21.4	23.7	7.498	35.0	26.4	7.226	75.5	30.3	27	8.4
	S.D.	0.002	0.1		0.001	0.1		0.001	0.3			
	N	3	5		7	6		6	4			
4	$\bar{X}$	7.645	22.0	23.2	7.472	35.4	25.1	7.227	70.6	28.4	27	8.4
	S.D.	0.001	0.1		0.001	0.1		0.001	0.1			
	N	6	4		7	8		5	4			
5	$\bar{X}$	7.686	22.7	26.4	7.516	36.4	28.5	7.259	73.2	31.8	24	7.5
	S.D.	0.002	0.4		0.002	0.1		0.001	0.05			
	N	4	9		3	7		6	4			

* percentages of CO_2 in the equilibrating gas mixtures are approximate and may vary ±0.5%. N = number of samples. S.D. = ± one standard deviation.

Table 3

Mean Values of Fetal Sheep Blood Equilibrated with Gases

Containing Three Different CO_2 Concentrations

Sample		3% CO_2*			5% CO_2*			10% CO_2*			Hct	Hgb (g/dl)
		pH	pCO_2 (mmHg)	HCO_3^- (mEq/l)	pH	pCO_2 (mmHg)	HCO_3^- (mEq/l)	pH	pCO_2 (mmHg)	HCO_3^- (mEq/l)		
1	$\overline{X}$	7.600	22.3	21.2	7.445	36.7	24.4	7.200	76.6	29.0	39	11.2
	S.D.	0.001	0.1		0.001	0.1		0.001	0.1			
	N	5	7		8	8		4	4			
2	$\overline{X}$	7.610	21.9	21.3	7.451	36.0	24.3	7.207	72.9	28.1	36	10.1
	S.D.	0.002	0.1		0.001	0.1		0.002	0.4			
	N	5	4		6	8		6	7			
3	$\overline{X}$	7.308	22.0	10.7	7.136	38.5	12.6	6.953	73.2	15.7	34	9.6
	S.D.	0.0005	0.2		0.0005	0.2		0.002	0.3			
	N	4	4		4	4		5	4			
4	$\overline{X}$	7.530	22.4	18.2	7.402	35.6	21.5	7.144	74.5	24.8	34	9.6
	S.D.	0.003	0.05		0.0004	0.1		0.002	0.2			
	N	6	4		6	8		4	4			
5	$\overline{X}$	7.168	21.6	7.6	7.080	34.4	9.9	6.924	72.7	14.6	48	13.5
	S.D.	0.001	0.1		0.001	0.1		0.001	0.2			
	N	8	8		7	8		5	5			

* percentages of CO_2 in the equilibrating gas mixtures are approximate and may vary ±0.5%.
N = number of samples. S.D. = ± one standard deviation.

Figure 1. The *in vitro* CO_2 titration curves of five samples of adult sheep blood. The slope of each curve is the buffer capacity of that blood sample.

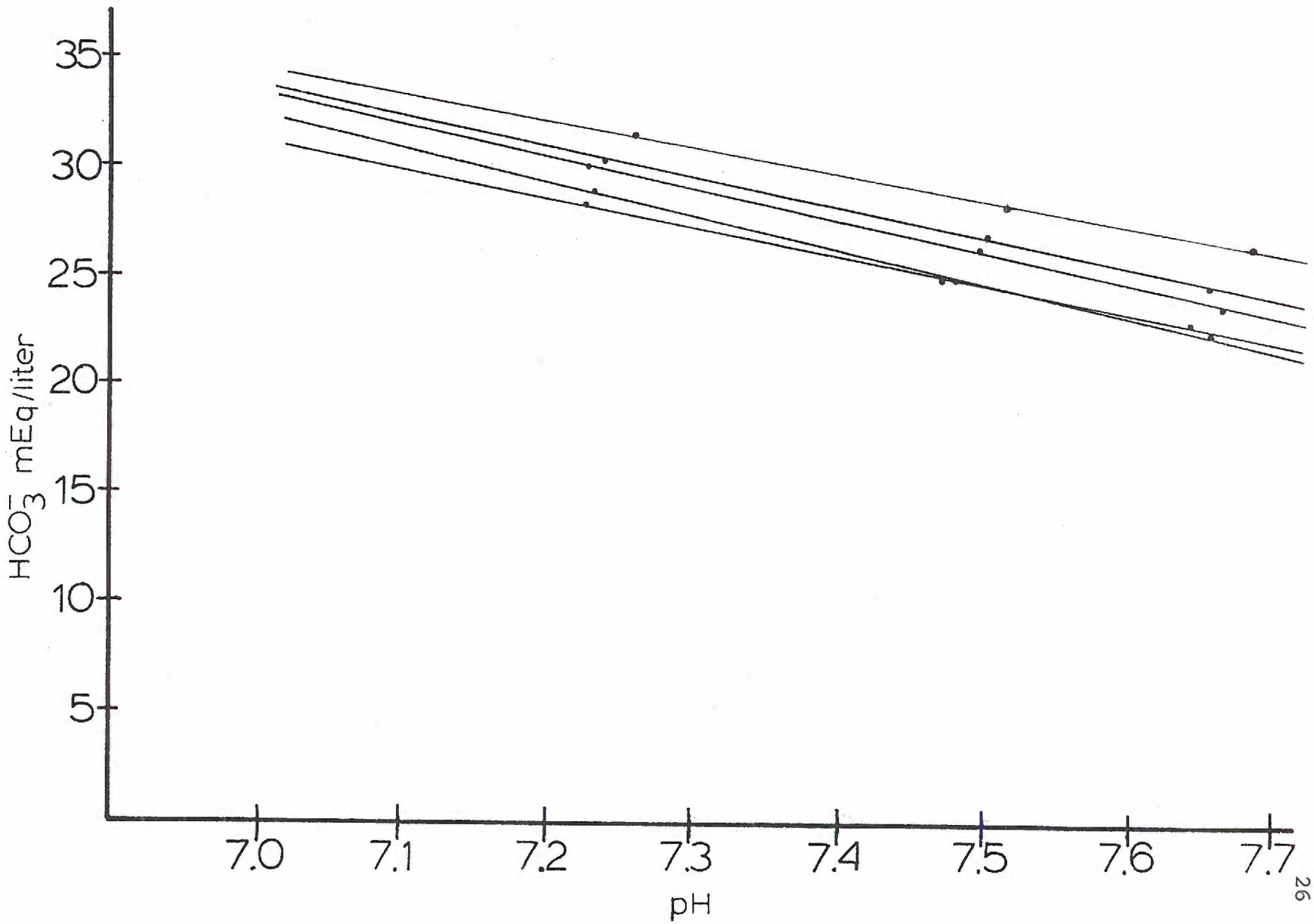

35
30
25
20
15
10
5
HCO_3^- mEq/liter
7.0
7.1
7.2
7.3
7.4
7.5
7.6
7.7
pH

Figure 2. The *in vitro* CO_2 titration curves of five samples of full-term fetal sheep blood. The uppermost curve includes the results of two separate blood samples. The slope of each curve is the buffer capacity of that blood sample.

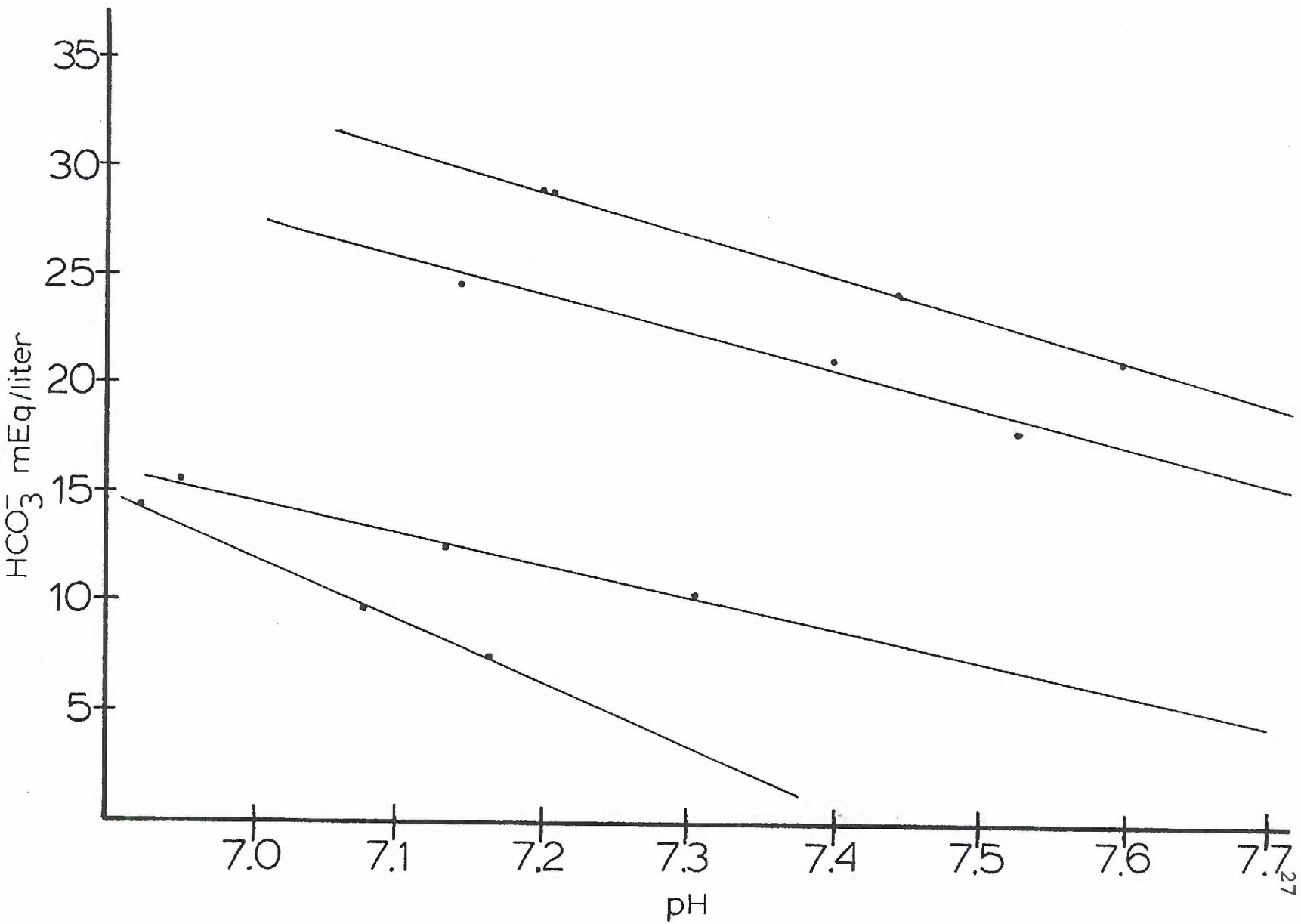

35
30
25
20
15
10
5
HCO_3^- mEq/liter
7.0
7.1
7.2
7.3
7.4
7.5
7.6
7.7
pH

Figure 3. A comparison of the buffer capacities of all blood samples. Each line represents the slope of the CO_2 titration curve of the sample.

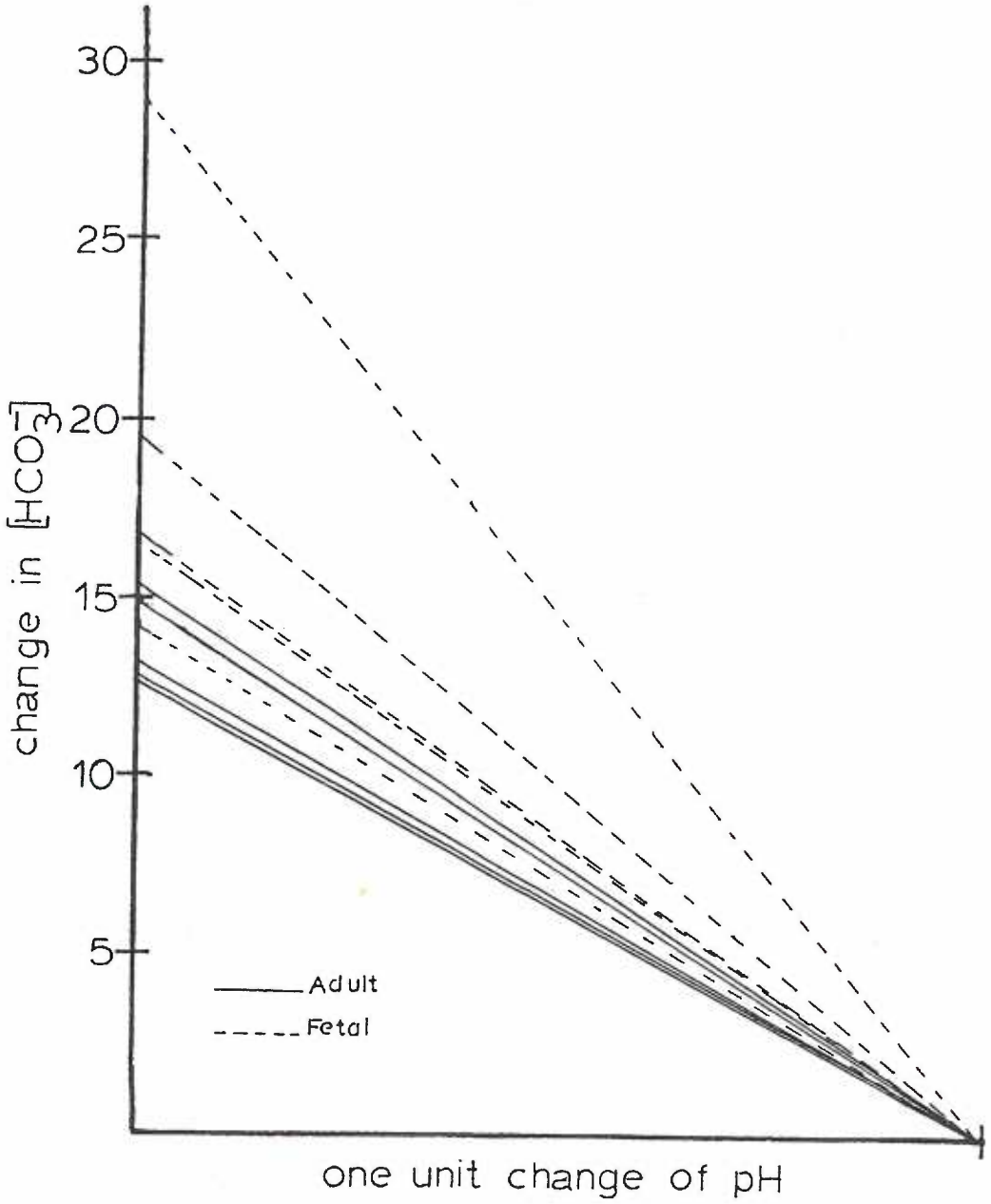

30
25
20
15
10
5
change in [HCO3-]
Adult
Fetal
one unit change of pH

Figure 4. A comparison of the buffer values of all blood samples. The buffer value of a sample is the buffer capacity divided by the hemoglobin concentration of the sample.

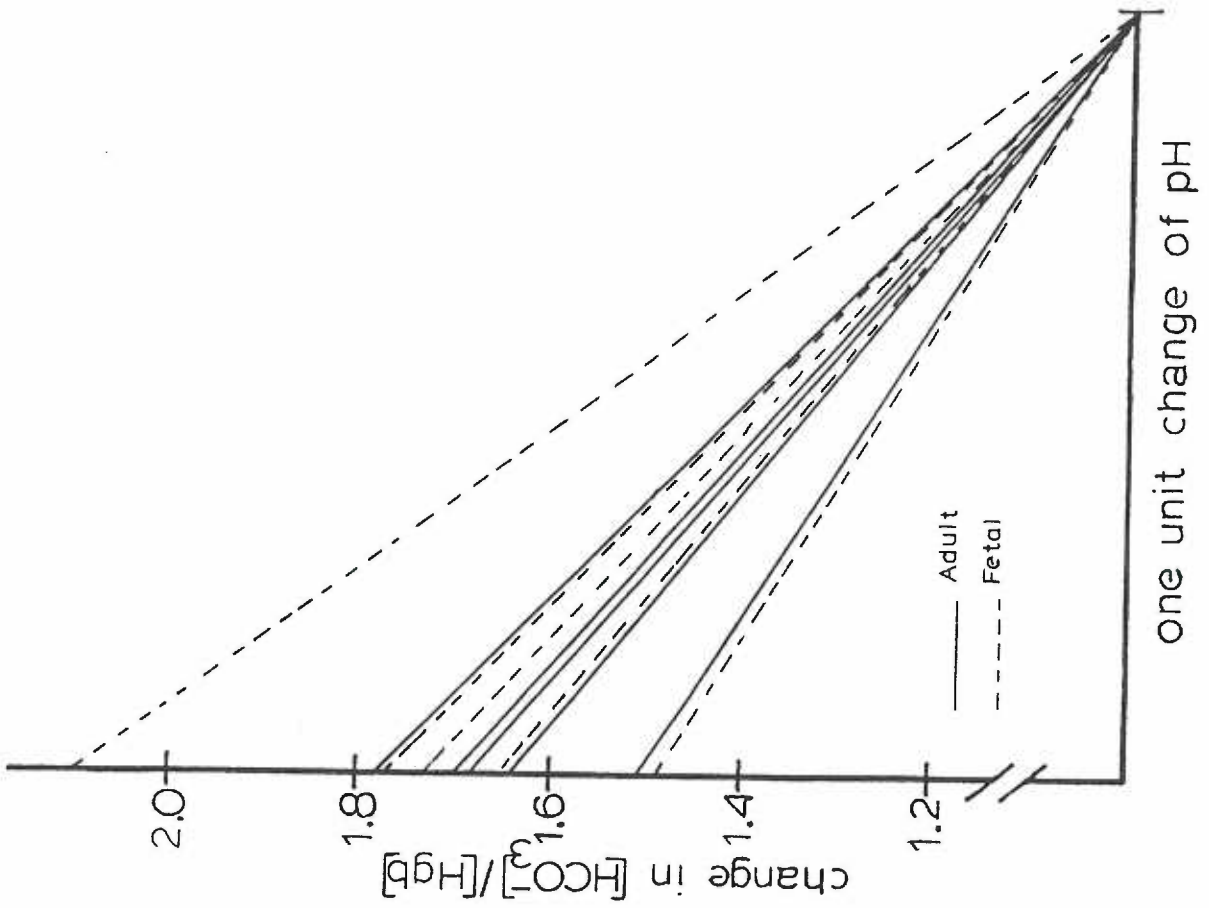
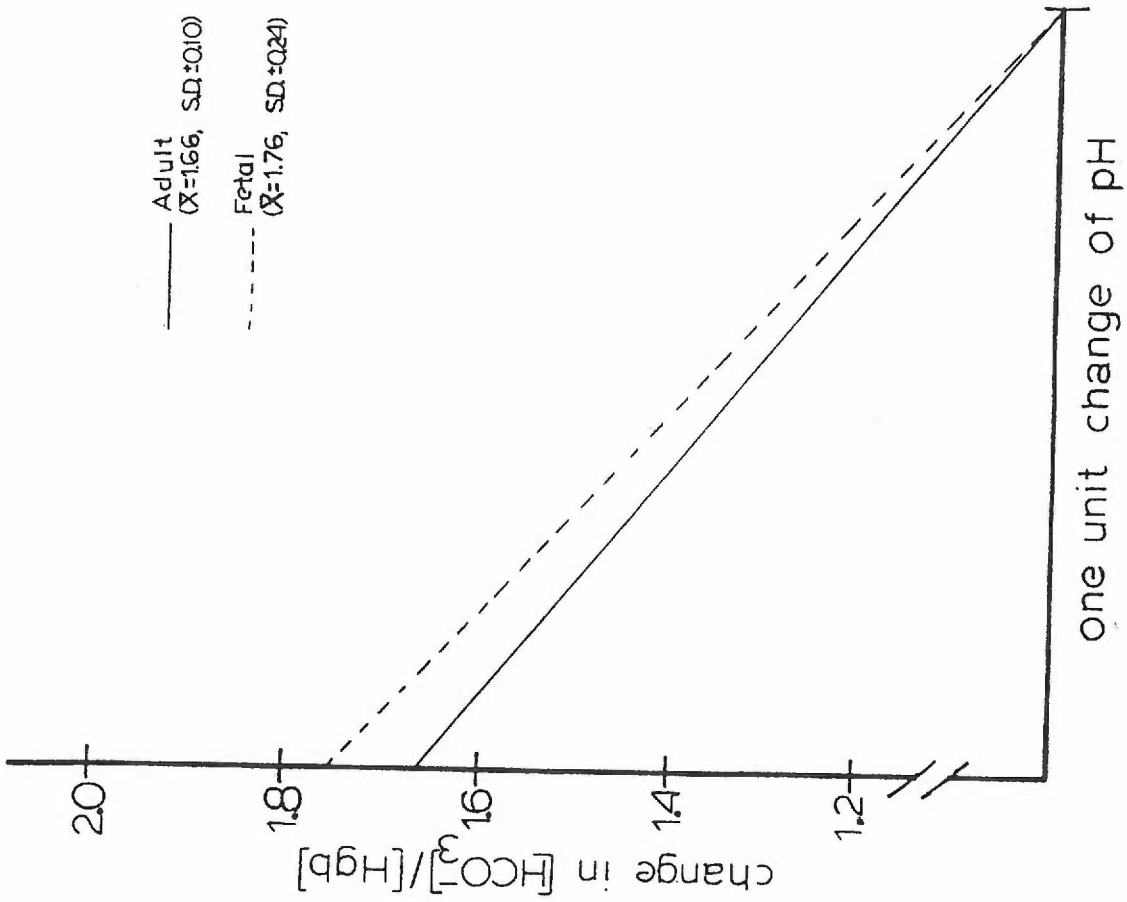

change in $[HCO_3^-]/[Hgb]$
2.0
1.8
1.6
1.4
1.2
Adult
Fetal
One unit change of pH

Figure 5. The mean buffer values of the adult and full-term fetal sheep blood are shown. The mean buffer values are not significantly different.


Adult
(X=1.66, SD.±0.10)
Fetal
(X=1.76, SD.±0.24)
change in [HCO3-]/[Hgb]
2.0
1.8
1.6
1.4
1.2
one unit change of pH

Chapter IV

DISCUSSION

The hemoglobin concentration of the blood in the newborn infant is greater than that of the adult. Furthermore, the infant has a mixture of fetal and adult hemoglobin. In order to determine the effects these differences have on acid-base status, one must compare the blood of the adult and the newborn infant *in vitro*. To do so eliminates the effect of the proportionally larger extracellular and proportionally smaller intracellular fluid volumes of the infant.

The slope of the *in vitro* CO_2 titration curve is the buffer capacity of that sample of blood. Differences in the buffer capacity between the two groups could be explained on the basis of 1) the hemoglobin concentration and 2) the types of hemoglobin. The buffer value of the blood is the buffer capacity divided by the hemoglobin concentration. The difference in the blood buffer value between the adult and the newborn infant, or in this case the full-term fetus, reflects the effect of the different types of hemoglobin.

The results of this study show that buffer capacity varies directly with hemoglobin concentration in both

adult and fetal blood. No comparison of buffer capacity can be made between the two groups in this investigation because of the wide variation in hemoglobin concentrations of the samples.

The mean buffer value of the two groups did not differ significantly (figure 5). It can therefore be concluded that at 100% oxygen saturation of hemoglobin, fetal hemoglobin does not affect the slope of the CO_2 titration curve. However, because of the higher oxygen affinity of fetal hemoglobin and therefore possibly lower affinity for CO_2, it would be of importance to compare the buffer value of adult and newborn infant blood at low oxygen concentrations before concluding that the presence of fetal hemoglobin has no effect on the slope of the CO_2 titration curve for values of pO_2 in the physiological range.

It is clear from the results that the initial $[HCO_3^-]$ does not affect either the buffer capacity or the buffer value. Though the positions of the CO_2 titration curves on the $[HCO_3^-]$/pH graph varied widely, the slopes varied according to hemoglobin concentration only.

The Bohr effect on the slope of the CO_2 titration curve was eliminated by the high concentration of oxygen in the equilibrating gases. The lowest mean pO_2 obtained in the samples was 517mmHg. Hemoglobin is 100% saturated at a pO_2 of 140mmHg or greater (Slonin & Hamilton, 1976, p. 84).

In order to compare the *in vivo* CO_2 titration curve of the adult with that of the newborn infant, one must take into account the following factors: the types of hemoglobin, the hemoglobin concentrations, and the proportion of extracellular and intracellular fluid volumes to total body weight. Because the hemoglobin in the blood of a newborn infant is usually well saturated with oxygen after 24 hours (Graham & Wilson, 1954), the mixture of fetal and adult hemoglobin probably has no effect on the *in vivo* CO_2 titration curve. The hemoglobin concentration of the newborn infant is 25% greater than that of the adult female. However, the extracellular fluid volume is proportionally 100% greater in the infant than in the adult. Although the higher hemoglobin concentration of the infant will increase the slope of the CO_2 titration curve, the effect of the proportionally greater extracellular fluid volume will dominate. Theoretically then, the over-all effect is to decrease the slope of the *in vivo* CO_2 titration curve of the infant compared to that of the adult. It is not known what effect the 5% proportionally smaller intracellular fluid volume of the newborn infant has on the buffer capacity of the infant. However, if the intracellular buffer value of the infant is the same as that of the adult, one may conclude that the effect of the proportionally smaller

intracellular fluid volume is to also decrease the slope of the CO_2 titration curve.

Chapter V

SUMMARY AND CONCLUSIONS

It has already been established that the normal point for the blood gas composition of the infant varies from that of the adult. From this standpoint alone, one must question the validity of using adult normal values in assessing and managing the acid-base status of the infant. The values for buffer base and base excess determined from nomograms are not correct for the infant because the point of reference is adult normal values. In addition, since the slope of the *in vivo* CO_2 titration curve of the two groups is different, the error incurred by the clinical use of adult normal values and nomograms may be even greater. Further research is needed to delineate the normal range of blood gas composition and the slope of the *in vivo* CO_2 titration curve in the newborn infant. With these data, the classification and therefore the treatment of acid-base disturbances may change.

There are four differences in body composition between the newborn infant and the adult which could explain the difference in the points of normal acid-base equilibrium for the two groups. In order to examine the effect hemoglobin concentration and fetal hemoglobin have on this

difference, the *in vitro* CO_2 titration curves of the blood of five full-term fetal sheep and five pregnant ewes were compared.

The slopes of the *in vitro* CO_2 titration curves, buffer capacities, varied directly with hemoglobin concentration. The mean buffer value of the blood of the two groups was the same, indicating that fetal hemoglobin has no effect on the buffer capacity of blood when the hemoglobin is 100% saturated with oxygen.

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APPENDIX A

Table and Figures of

Hgb/Hct Relationship Determination

Table 1A

Absorbance and Hgb Concentration of Samples

Tube No.	Absorbance a	b	c	Average Absorbance	Hct	Hgb (mg/dl)
Standards						
S_1	0.000			0.000		0
S_2	0.135			0.135		20
S_3	0.267			0.267		40
S_4	0.539			0.539		80
Unknowns						
Adult						
A_1	0.225	0.228	0.228	0.227	25.0	33.9
A_2	0.344	0.342	0.340	0.342	37.0	51.0
A_3	0.179	0.179	0.180	0.179	18.5	26.8
A_4	0.415	0.418	0.419	0.417	45.5	62.3
A_5	0.157	0.157	0.157	0.157	16.5	23.4
Fetal						
F_1	0.290	0.289	0.291	0.290	35.0	43.3
F_2	0.403	0.400	0.402	0.402	48.0	59.9
F_3	0.221	0.222	0.222	0.222	26.0	33.1
F_4	0.481	0.478	0.478	0.479	58.0	71.5
F_5	0.204	0.203	0.203	0.203	24.0	30.3

Figure 1A. The standard curve for hemoglobin concentration. This curve was determined from dilutions of standard cyanmethemoglobin read in a spectrophotometer at 540nm. The hemoglobin concentrations of the sheep blood samples were determined from this curve.

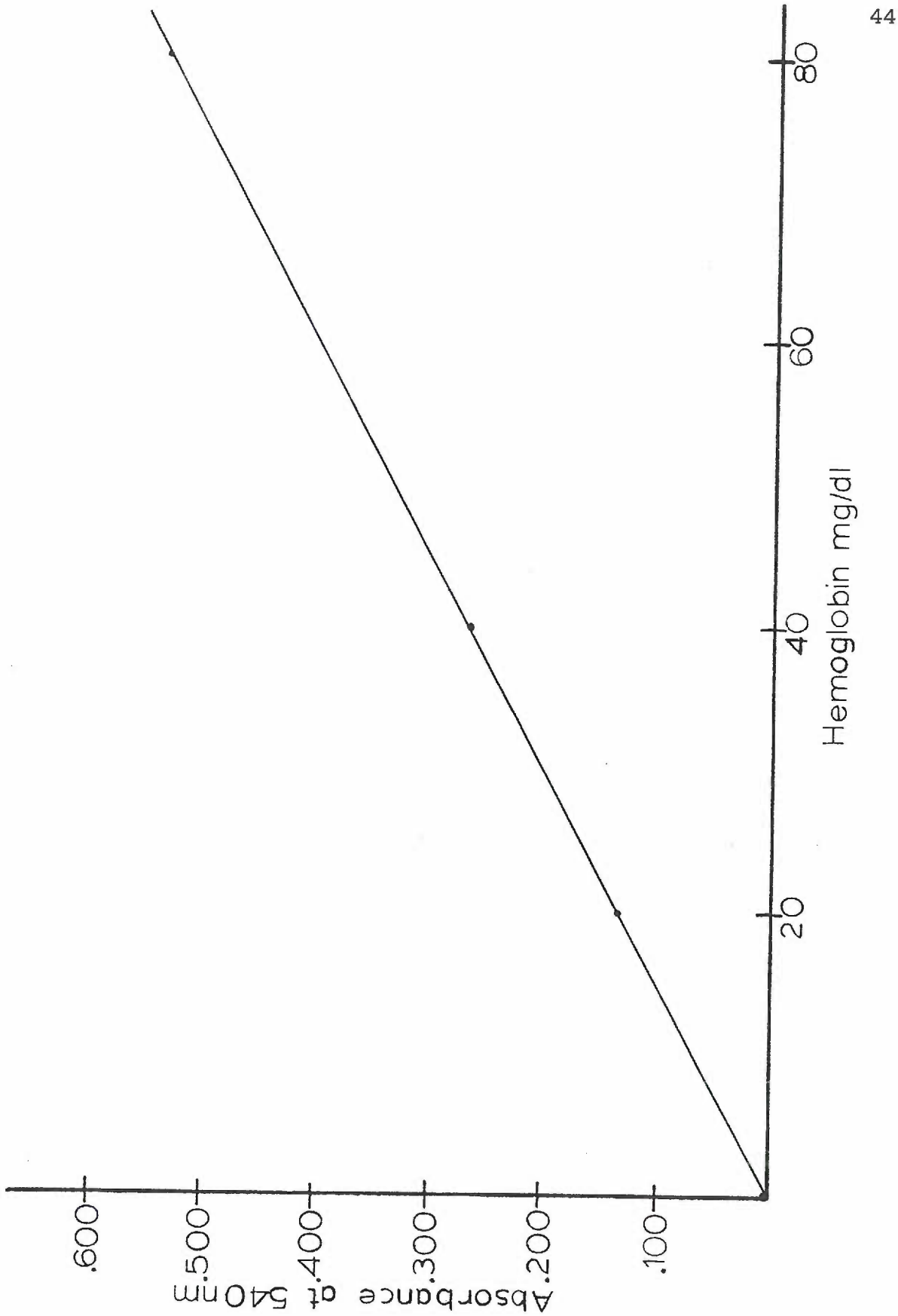

Absorbance at 540 nm
.100
.200
.300
.400
.500
.600
20
40
60
80
Hemoglobin mg/dl

Figure 2A. The line depicts the linear relationship between the hemoglobin concentration and the hematocrit for adult sheep blood.

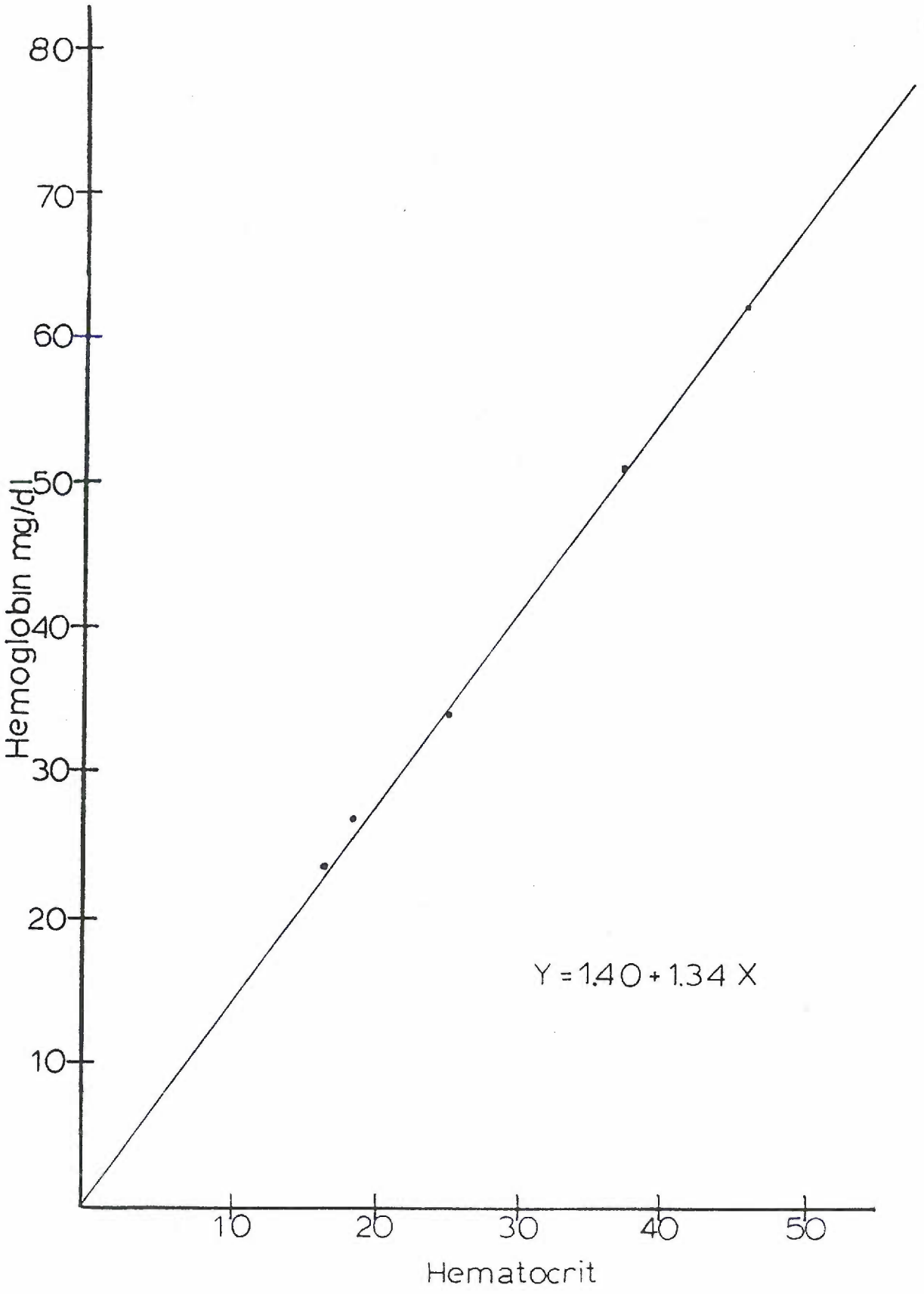
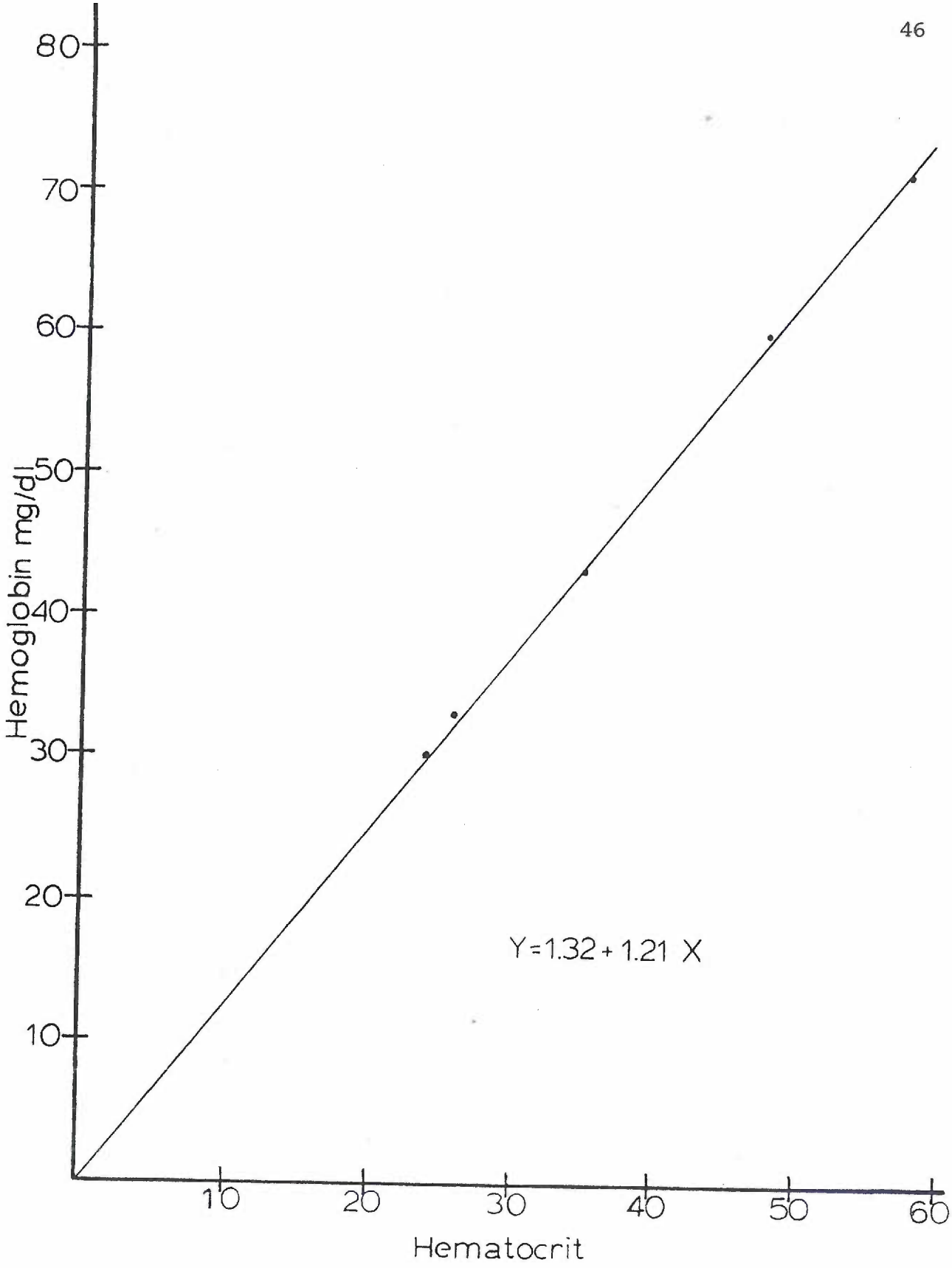

80
70
60
50
40
30
20
10
Hemoglobin mg/dl
10
20
30
40
50
Hematocrit
Y = 1.40 + 1.34 X

Figure 3A. The line depicts the linear relationship between the hemoglobin concentration and the hematocrit of full-term fetal sheep blood.


80
70
60
50
40
30
20
10
Hemoglobin mg/dl
10
20
30
40
50
60
Hematocrit
Y=1.32+1.21 X

AN ABSTRACT OF THE THESIS OF

PAMELA J. ISRAEL

For the MASTER OF NURSING

Date of Receiving this Degree: June 12, 1981

Title: THE CO_2 TITRATION CURVE OF FETAL AND ADULT BLOOD IN VITRO

Approved: [redacted]
Jack L. Keyes, Ph.D., Thesis Advisor

The blood samples of five full-term fetal lambs and five pregnant ewes were equilibrated with three gases of known composition: 3% CO_2 in oxygen, 5% CO_2 in oxygen, and 10% CO_2 in oxygen. Blood gas analysis was performed on the samples and bicarbonate concentrations were calculated using the Henderson-Hasselbalch equation. The resulting $[HCO_3^-]$ and pH values were plotted on a graph to give the in vitro CO_2 titration curve for each sample.

Analysis of the results indicated that the slope of the in vitro CO_2 titration curve varied directly with hemoglobin concentration. Buffer capacity per unit hemoglobin concentration was identified for the blood obtained from adult and fetal sheep. It was concluded that fetal hemoglobin has no effect on the in vitro CO_2 titration curve when the hemoglobin is saturated with oxygen.