A COMPARISON OF CARBON DIOXIDE TRANSPORT BY HUMAN ADULT AND FETAL BLOOD

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
JUDITH LORRAINE HOFMAN
1969


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ABSTRACT

A COMPARISON OF CARBON DIOXIDE TRANSPORT BY HUMAN ADULT AND FETAL BLOOD

by Judith Lorraine Hofman

Equations have been derived which make inferences possible concerning the relative ionizations of fetal and adult hemoglobins in intact cells. Using measurements of the effect of oxygen saturation and changes in PCO_2 on the total CO_2 content of cells, it has been shown that little or no difference exists in the intracellular dissociation constants of adult and fetal hemoglobin.

A COMPARISON OF CARBON DIOXIDE TRANSPORT BY HUMAN ADULT AND FETAL BLOOD

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Pathology

1969

656 201
6-25-69

ACKNOWLEDGMENTS

To Dr. A. E. Lewis, my major professor, I wish to express my gratitude for his suggestions and encouragement in my research project and for the many hours he has given me of his time. His abilities cover a wide area.

I wish to express my thanks to Dr. R. Heisey for serving on my committee and for the suggestions he has given in my research project.

I thank Dr. C. Sander for his encouragement and for serving on my committee.

My sincere thanks to Dr. George Eyster for allowing me to use the Cardiopulmonary Laboratory and Radiometer equipment for my research, to Janet Eyster for her helpful suggestions and to Dr. Robert Whipple for his help with the anesthetic machine.

I am grateful to the students and staff at the Veterinary Clinic who donated blood samples and especially to John Dickason who gave me support and encouragement through many long hours.

To the laboratory staff at Olin Health Center, I wish to express my appreciation for the cooperation they gave me in obtaining blood samples.

To Mrs. Granger and the nurses in the Department of Obstetrics, Edward W. Sparrow Hospital, who have been very helpful with their assistance in obtaining blood samples, I am very grateful.

I am grateful for the financial assistance of the U. S. Public Health Service Allied Health Traineeship and express gratitude to Dr. C. C. Morrill, Head of the Department of Pathology, and the others who made it available.

I wish to express my thanks to Mary Schwab for her assistance in my research project, to the secretaries of the department for giving me assistance in numerous and invaluable ways and to all who have helped directly and indirectly in this project.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Studies of the oxygen dissociation curves of maternal and fetal whole blood (Eastman, Geiling, DeLauder, 1933; Leibson, Likhnitsky and Sax, 1936; Darling et al., 1942; McCarthy, 1943; Barcroft, 1947) and hemoglobin solutions (Allen, Wyman and Smith, 1953; Rooth, Sommerkamps, and Bartells, 1962) indicate that the differences in the oxygen dissociation curves are due to the intracellular environment rather than the function of the fetal hemoglobin molecule.

A study of the Bohr effect (Mann and Romney, 1968) showed that at pH 7.2 - 7.8 the Bohr effect of fetal hemoglobin in solution is the same as that of adult hemoglobin in solution, but that at a pH lower than 7.2 the Bohr effect of fetal hemoglobin was greater.

The purpose of this study was to compare carbon dioxide transport of fetal and adult blood to determine if fetal hemoglobin had a direct or indirect effect on the amount of carbon dioxide transported.

REVIEW OF LITERATURE

At birth, the hemoglobin of the full-term infant is up to 80% fetal hemoglobin, hemoglobin F; (Brinkman and Jonxis, 1935) the remaining hemoglobin is adult hemoglobin, hemoglobin A (White and Beaven, 1959). The concentration of hemoglobin F is correlated with the gestational age of the infant (Beaven, Ellis and White, 1960) and after birth, decreases until, at the age of 1 - 2 years, the level is 1 - 5% of the total hemoglobin (Betke, 1960).

Oxygen Transport.

A comparison of the oxygen dissociation curves of fetal and maternal blood reveals a greater oxygen saturation of fetal than maternal blood at the same O_2 tension and hydrogen ion concentration (Eastman et al., 1933; Leibson et al., 1936; Darling et al., 1942; McCarthy, 1943; Barcroft, 1947). Nelson et al., established that the fetal or neo-natal oxygen dissociation curves at pH 7.4 are identical to those for adult blood at pH 7.6. These observations used intact erythrocytes. However, studies from lysed cells manifest little difference in the oxygen dissociation curves of fetal and maternal hemoglobin (McCarthy, 1943; Allen et al., 1953). Rooth et al. (1962) were able to shift the position of the curve by varying the acid-base concentration in the hemoglobin solutions.

These results suggest that the differences observed in the oxygen dissociation curves are not due to the fetal hemoglobin molecule but to the intracellular environment.

Bohr Effect.

Changes in the acidity of the blood are reflected in a shift of the oxygen dissociation curve. As the pH decreases, the oxygen dissociation curve shifts to the right so that a higher oxygen tension is needed to give the same oxygen saturation. As the pH increases, the opposite phenomenon is observed. This effect of pH on the oxygen dissociation curve is known as the Bohr effect. On oxygenation there is a shift in the dissociation constant of hemoglobin; and oxyhemoglobin is more acidic than deoxyhemoglobin. (Christiansen, Douglas and Haldane, 1914; German and Wyman, 1937). The shift in the isoelectric point of the hemoglobin molecule when oxygenated is due to the large reversible change in the conformation (the arrangement of the globin chains) between oxyhemoglobin and deoxyhemoglobin. (Benesch, 1962; Benesch and Benesch, 1962; Benesch and Benesch, 1963). This change of conformation is probably responsible for the Bohr effect. A comparison of the Bohr effect of fetal and adult hemoglobin in solution by Mann and Romney (1968) showed that between pH 7.2 and pH 7.8, the Bohr effect was the same for fetal and adult hemoglobin. Below a pH of 7.2, the Bohr effect of fetal hemoglobin was greater.

Carbon Dioxide Transport.

Carbon dioxide as well as oxygen is transported by the blood. Small amounts of carbon dioxide are carried directly in the plasma as dissolved carbon dioxide and carbamino compounds. Carbon dioxide carried by whole blood is distributed as follows: 5% as dissolved carbon dioxide, 15-20% as carbamino compounds and 75-80% as bicarbonate ions. The reaction of water and carbon dioxide in the presence of carbonic anhydrase within the erythrocyte forms carbonic acid which then dissociates to form hydrogen ions and bicarbonate ions. The bicarbonate ions diffuse into the plasma to maintain an equilibrium with a corresponding diffusion of chloride ions into the erythrocyte to establish ionic equilibrium (Donnan equilibrium). The carbamino compounds are formed by the reversible reaction of carbon dioxide and the free amino groups of the proteins.

Hemoglobin, as a buffer, functions indirectly in the transport of bicarbonate ions. As stated before, oxyhemoglobin is more acidic than deoxyhemoglobin; that is, deoxyhemoglobin electrochemically balances less cation than oxyhemoglobin. Therefore, as hemoglobin is deoxygenated, an amount of carbon dioxide diffuses into the cell sufficient to match the cations no longer balanced by hemoglobin. Donnan equilibrium conditions are attained by subsequent exchanges of bicarbonate and chloride ions.

The purpose of these studies was to determine the possible role of differences in the dissociation constants of adult and fetal hemoglobins and differences in carbon dioxide carrying capacities of adult and fetal red cells.

MATERIALS AND METHODS

Instrumentation

1. Coleman Junior Spectrophotometer - used for measuring optical density (O.D.) of cyanmethemoglobin solutions.
2. Microhematocrit centrifuge - used to measure packed cell volume.
3. American Optical Microoximeter - used for measuring oxygen saturations.
4. Radiometer pH microelectrode type E5021 and PCO_2 electrode type E5036.
5. Anesthetic machine, Ohio Company - used for preparing gas mixtures.
6. Constant temperature water bath 38°C.
7. Kopp Natelson microgasometer - for measuring carbon dioxide content of plasma and whole blood.

Preparation and Analysis of Blood Samples

Eleven samples of human adult blood and eleven samples of human cord blood were used in these studies. All of the samples were collected in 10 milliliter (ml.) heparin tubes.* Immediately after collection the blood samples were refrigerated (2 to 10° C.) and analyzed within twelve hours.

The adult blood samples were drawn from the antecubital vein of healthy male and female adults.

The cord blood samples were obtained through the courtesy of the Department of Obstetrics, Edward W. Sparrow Hospital. The blood samples were taken from the cord after it had been tied and cut or from the placental vein after delivery of the placenta.

Hemoglobin concentration was measured on each sample by the cyanmethemoglobin method (Wintrobe). The packed cell volume was determined on each sample by filling a microhematocrit tube 2/3 to 3/4 full, plugging one end with clay and centrifuging in a micro-hematocrit centrifuge for 5 minutes at a R.C.F. = 13,307 x gravity. (R.C.F. = relative centrifugal force).

The percentage of hemoglobin F was measured on each fetal blood sample by the one minute alkali denaturation procedure of Singer, Chernoff and Singer (1951). The procedure was modified for use with 1 to 2 ml. samples of blood. (See Appendix I for details of method.) On all samples the pH and PCO_2 were measured with Radiometer electrodes which insures anaerobic conditions.

* B-D Vacutainers 3200 KA

One 3 to 4 ml. portion of each sample was equilibrated with a gas mixture having a low PCO_2 (10.0 - 21.0 mm. Hg) and a second 3 to 4 ml. portion was equilibrated with a gas mixture having a high PCO_2 (66.0 - 110.0 mm. Hg).

The gas mixtures were prepared with an anesthetic machine which had flow meters for CO_2, O_2 and N_2O. The gas mixture was saturated with water vapor at room temperature by passing the gas mixture over glass beads which had been moistened with water. The gas mixture proportions were checked for accuracy by comparing the PCO_2 values calculated from flow meter data with the direct PCO_2 measurements at 38°C. using the Radiometer electrode. Although the gas was saturated with water vapor at room temperature and equilibrated with the blood sample at 38° C., the drop in water saturation was negligible.

For an equilibration chamber, a 250 ml. separatory funnel was used (Peters and Van Slyke, 1932), which was placed in a 38°C. water bath. Each refrigerated blood sample was warmed in the funnel for 3 minutes. The funnel was then rotated to distribute the blood in a thin film over the interior of the funnel. The gas mixture was allowed to flow through the funnel for five minutes during which time the funnel was rotated 3 to 4 times. In specimens with relatively high packed cell volumes, it took 1 to 2 minutes longer for the blood to equilibrate completely with the gas as indicated by obvious color changes. After 5 minutes the funnel

was closed and the blood allowed to collect in the bottom of the funnel.

Each blood sample was thoroughly mixed, drawn into a syringe and the following measurements taken: oxygen saturation, pH and PCO_2, and total CO_2 content of whole blood. The remainder was then transferred anaerobically, to prevent loss of CO_2, to a small closed tube for centrifugation. After centrifugation, the total CO_2 content of plasma was measured. (See Appendix I for procedures for measuring CO_2 contents of plasma and whole blood.) All of the blood samples were treated in this manner except for three of the adult samples and seven of the fetal blood samples. These were covered with a layer of mineral oil and stored in the refrigerator prior to centrifugation. Plasma total CO_2 contents obtained in the samples stored under mineral oil were variable because of stability factors and are not included in the analysis of the data.

Equations for Calculations

The partial pressure of carbon dioxide was calculated from flow meter data according to the following equation:

$$PCO_2 = \frac{(B.P. - V.P.H_2O)\ vol.\%\ CO_2}{100} \qquad (1)$$

PCO_2 = partial pressure of CO_2

B.P. = barometric pressure

$V.P.H_2O$ = vapor pressure of water at the temperature of gas (PCO_2) measurement

The total CO_2 content of packed cells was calculated on the basis of the plasma and whole blood total CO_2 contents and the packed cell volume by the use of the following equation:

$$TCO_2\ mM/L.\ pc = \frac{TCO_2\ mM/L.\ wb - TCO_2\ mM/L.\ p\ (1 - PCV)}{PCV} \qquad (2)$$

pc = packed cells

wb = whole blood

p = plasma

mM/L. = millimoles per liter

PCV = packed cell volume

The means of the total CO_2 content of the adult and fetal blood samples were statistically compared by using the t - test for unpaired data with unequal samples as discussed by Lewis (1966).

The total cation within erythrocytes is electrochemically balanced by bicarbonate, chloride, mono- and di-hydrogen phosphates, small amounts of organic acid ions, and the anions formed by the

dissociation of oxyhemoglobin and deoxyhemoglobin. Usually that portion of the total cation balanced by buffering anions is referred to as "buffer base" (Peters and Van Slyke, 1932). For present purposes our interest is necessarily confined to the amount of cation balanced only by HCO_3^-, $HgbO_2^-$ and Hgb^- (for definitions see below) hereinafter referred to as "available cation." Assuming that the changes in amount of cation balanced by phosphates are small in the range studied and that exchanges of bicarbonate and chloride ions between cells and plasma maintain a constant proportion, we may summarize the approximate quantitative relationships with the following equation:

$$B^+ = \left[HCO_3^-\right] + \left[HgbO_2^-\right] + \left[Hgb^-\right] \qquad (3)$$

B^+ = available intracellular cation (mEq/L-milliequilivants per liter)

$\left[HCO_3^-\right]$ = concentration of bicarbonate ions within the erythrocyte (mEq./L.)

$\left[HgbO_2^-\right]$ = concentration of dissociated oxyhemoglobin (mEq./L.)

$\left[Hgb^-\right]$ = concentration of dissociated deoxyhemoglobin (mEq./L.)

Using this assumption, the following equation has been derived (see Appendix III for details of derivation) to clarify the relationships between hydrogen ion concentration within the cell, PCO_2, oxygen saturation, hemoglobin concentration and dissociation constants of oxyhemoglobin and deoxyhemoglobin:

$$PCO_2 = \left[B+ - \frac{K_2 G\emptyset}{K_2 + h} - \frac{K_3 G(1 - \emptyset)}{K_3 + h} \right] \frac{h}{K_1 \beta} \qquad (4)$$

PCO_2 = partial pressure of carbon dioxide

$\emptyset$ = oxygen saturation fraction

G = hemoglobin concentration (gms per 100 ml.)

K_1 = dissociation constant of carbonic acid

K_2 = dissociation constant of oxyhemoglobin

K_3 = dissociation constant of deoxyhemoglobin

β = solubility constant for intracellular CO_2 (0.0254) as determined by Van Slyke, et al., (1928)

h = hydrogen ion concentration mEq./L within the erythrocyte.

Similar equations using similar assumptions and definitions have been derived by Siggaard-Andersen (1964) and Singer and Hastings (1948) but these relate only to fully oxygenated hemoglobin. These equations were intended for clinical investigations of total buffer base content in various disturbances in water and electrolyte metabolism. Equation (4) considers both oxyhemoglobin and deoxyhemoglobin. Dividing equation (4) by total hemoglobin concentration (G) yields the following equation:

$$\frac{PCO_2}{G} = \left[\frac{B+}{G} - \frac{K_2\emptyset}{K_2 + h} - \frac{K_3(1-\emptyset)}{K_3 + h} \right] \frac{h}{K_1 \beta} \qquad (5)$$

Letting $y = \frac{PCO_2}{G}$, equation (5) can be differentiated with respect to $\emptyset$ to give:

$$\frac{\Delta y}{\Delta \emptyset} = \left[- \frac{K_2}{K_2 + h} + \frac{K_3}{K_3 + h} \right] \frac{h}{K_1 \beta} \qquad (6)$$

RESULTS

Estimates of the carbon dioxide carried by whole blood, plasma and packed cells, along with measurements of packed cell volume are summarized in Tables 1 and 2 for adult and fetal blood samples, respectively. The total carbon dioxide content in millimoles per liter (mM/L.) of packed cells was examined to determine if hemoglobin F had any direct effect on the amount of carbon dioxide carried. Results of statistical analysis of the data in Tables 1 and 2 are presented in Table 3. No significant difference is shown in the amount of carbon dioxide transported by fetal blood when compared to adult blood.

Equation (6) was used to compare $\Delta y/\Delta\beta$ in fetal and adult blood. From this comparison inferences can be made about the dissociation constants of fetal oxy- and deoxyhemoglobin and adult oxy- and deoxyhemoglobin. Tables 4 and 5 show the details of this evaluation. From this evaluation it is determined that:

$$-0.34 = \left[- \frac{K_2}{K_2 + h} + \frac{K_3}{K_3 + h} \right] \frac{h}{K_1 \beta} \qquad \text{for adult samples}$$

and $$-0.35 = \left[- \frac{K_2'}{K_2' + h} + \frac{K_3'}{K_3' + h} \right] \frac{h}{K_1 \beta} \qquad \text{for fetal samples,}$$

where K_2, K_3 are dissociation constants for adult oxy- and deoxyhemoglobin respectively and K_2', K_3' are dissociation constants for

fetal oxy- and deoxyhemoglobin respectively. Using known values for adult K_2 and K_3 ($pK_2 = 6.95$, $pK_3 = 8.25$) from Benesch and Benesch (1963) and substituting the extreme values of hydrogen ion concentration observed in plasma, the changes in the total values of $\frac{K_2}{K_2 + h}$ and $\frac{K_3}{K_3 + h}$ were less than 10%. Presumably the range of hydrogen ion concentration within the cell would be less because of the greater buffer capacity of hemoglobin as compared to plasma protein. Thus, these changes would be less than 10% and would be negligibly small when compared to the tenfold changes of $\Delta y/\Delta \emptyset$.

K_2, $K_3 \cong K_2'$, K_3', that is, any difference in dissociation constants of oxy- and deoxyhemoglobin F and hemoglobin A are too small to account for differences in oxygen saturation or carbon dioxide transport.

Table 1--Summary of total CO_2 contents in mM/Liter of plasma, whole blood and packed cells of adult blood samples

Sample	PCV	TCO_2 mM/L. plasma	TCO_2 mM/L. whole blood	TCO_2 mM/L. packed cells
A 1 a	49	16.1	11.3	6.3
b		29.7	21.3	12.7
A 2 a	42	18.8	12.9	4.8
b		31.3	24.5	15.2
A 3 a	43	19.0	12.4	3.7
b		32.9	24.5	13.3
A 4 a	43	22.3	13.7	2.3
b		33.5	23.0	9.1
A 5 a	48	20.0	11.5	3.0
b		34.7	23.2	10.8
A 6 a	41	14.0	12.1	9.3
b		33.3	24.3	11.5
A 7 a	54	21.7	11.8	3.3
b		35.8	25.4	16.5
A 8 a	49	23.8	14.2	4.3
b		39.1	25.4	11.2

PCO_2 values are given in Table 3. In the above table the sample labeled a is always the lower value; b is the higher of the two samples measured.

Table 2.--Summary of total CO_2 contents in mM/Liter of plasma, whole blood and packed cells of fetal blood samples

Sample	PCV	TCO_2 mM/L. plasma	TCO_2 mM/L. whole blood	TCO_2 mM/L. packed cells
F 1 a	43	18.0	12.5	5.2
b		33.0	24.7	13.7
F 2 a	48	21.8	12.8	3.0
b		35.4	23.4	10.4
F 3 a	45	13.2	9.1	4.1
b		31.9	21.1	7.9
F 4 a	50.5	20.5	11.7	3.1
b		32.5	22.2	12.1

PCO_2 values are given in Table 3. In the above table the sample labeled a is always the lower value; b is the higher of the two samples measured.

Table 3.--Comparison of total CO_2 contents in mM/Liter packed cells of adult and fetal blood samples

	ADULT	FETAL
PCO_2 of whole blood mm Hg (range)	15.3 (12.0-23.0)	16.4 (14.9-18.5)
mean TCO_2 mM/L. packed cells	4.4	3.9
std. dev.	2.3	1.0
n	8	4
Comparison of means using t-test		
t = 0.46 no significant difference		
PCO_2 of whole blood mm Hg (range)	92.7 (71.0-110.0)	102.6 (89.0-112.0)
mean TCO_2 mM/L. packed cells	12.5	11.0
std. dev.	2.4	2.5
n	8	4
Comparison of means using t-test		
t = 1.00 no significant difference		

Table 4.--Summary of results and calculations for adult bloods

Sample	ϕ_1	ϕ_2	$\Delta\phi$	Hgb gms%	$(PCO_2)_1$	y_1	$(PCO_2)_2$	y_2	Δy	$\Delta y/\Delta\phi$
A 1	64.0	59.5	4.5	15.2	13.9	0.91	98.0	6.45	-5.54	-1.23
A 2	65.0	28.0	37.0	13.5	15.2	1.13	100.0	7.41	-6.28	-0.17
A 3	81.5	42.0	39.5	14.3	12.0	0.84	71.0	4.97	-4.13	-0.10
A 4	75.5	58.0	17.5	14.0	14.2	1.01	83.0	5.93	-4.92	-0.28
A 5	57.5	51.5	6.0	15.4	16.7	1.08	94.5	6.14	-5.06	-0.84
A 6	81.5	49.0	32.5	13.2	14.1	1.07	99.0	7.50	-6.43	-0.20
A 7	67.5	43.0	24.5	17.1	13.1	0.77	86.0	5.03	-4.26	-0.17
A 8	58.0	33.0	25.0	15.7	23.0	1.46	110.0	7.01	-5.55	-0.22
A 9	58.0	39.5	18.5	16.0	14.9	1.93	99.0	6.19	-4.26	-0.23
A 10	83.5	44.0	39.5	13.6	13.8	1.01	90.0	6.62	-5.61	-0.14
A 11	55.0	34.0	21.0	16.8	14.3	0.85	79.0	4.70	-3.85	-0.18

mean $\Delta y/\Delta\phi = -0.34 \pm 0.35$

ϕ_1, ϕ_2 = % oxygen saturations of samples equilibrated with low CO_2 and high CO_2 gas mixtures, respectively. $\Delta\phi = \phi_1 - \phi_2$. $(PCO_2)_1$, $(PCO_2)_2$ = partial pressure of carbon dioxide in mm Hg of samples equilibrated with low and high CO_2 gas mixtures, respectively. $y_1 = (PCO_2)_1/G$, $y_2 = (PCO_2)_2/G$ and $\Delta y = y_1 - y_2$.

Table 5.--Summary of results and calculations for fetal bloods

Sample	ϕ_1	ϕ_2	$\Delta\phi$	Hgb gms%	$(PCO_2)_1$	y_1	$(PCO_2)_2$	y_2	Δy	$\Delta y/\Delta\phi$
F 1	55.5	39.0	16.5	13.1	14.9	1.14	99.5	7.60	-6.46	-0.39
F 2	32.0	26.0	6.0	15.1	18.5	1.23	112.0	7.42	-6.19	-1.03
F 3	88.0	34.0	54.0	15.4	16.2	1.05	110.0	7.14	-6.09	-0.11
F 4	86.0	77.0	9.0	17.2	15.9	0.92	89.0	5.17	-4.25	-0.47
F 5	47.0	34.0	13.0	15.3	16.5	1.08	93.0	6.08	-5.00	-0.38
F 6	85.0	62.0	23.0	17.0	12.7	0.75	95.0	5.59	-4.84	-0.21
F 7	76.5	62.0	14.5	16.1	15.2	0.94	82.0	5.09	-4.15	-0.29
F 8	40.0	28.0	12.0	16.6	13.1	0.79	69.0	4.16	-3.37	-0.28
F 9	68.5	33.0	35.5	19.1	12.7	0.66	86.0	4.50	-3.84	-0.11
F 10	60.0	34.0	26.0	17.0	12.9	0.76	101.0	5.94	-5.18	-0.20
F 11	50.0	39.5	10.5	19.8	15.7	0.79	105.0	5.30	-4.51	-0.43

mean $\Delta y/\Delta\phi = -0.35 \pm 0.26$

ϕ_1, ϕ_2 = % oxygen saturations of samples equilibrated with low CO_2 and high CO_2 gas mixtures, respectively. $\Delta\phi = \phi_1 - \phi_2$. $(PCO_2)_1$, $(PCO_2)_2$ = partial pressure of carbon dioxide in mm Hg of samples equilibrated with low and high CO_2 gas mixtures, respectively. $y_1 = (PCO_2)_1/G$, $y_2 = (PCO_2)_2/G$ and $\Delta y = y_1 - y_2$.

DISCUSSION

At one time, it appeared that fetal hemoglobin represented a biological adaptation providing increased efficiency for the transport of oxygen from placental circulation to the fetal tissues (Eastman et al., 1933; Leibson et al., 1936; Darling et al., 1942). Further studies show that this increased efficiency of oxygen transport is not a function of the hemoglobin F molecule itself but is a consequence of the intracellular environment (McCarthy, 1943; Allen et al., 1953; Rooth et al., 1962). This is confirmed by studies on metabolic acidosis indicating acidosis is responsible for the greater oxygen saturation of fetal than adult blood at the same oxygen tension (Rooth and Caligara, 1963).

Apparently an increase in the efficiency of carbon dioxide transport also does not exist, although the level of carbon dioxide is increased in the fetus (Kaiser, 1959; Haraguchi, 1951; Prystowsky, Hellegers, Bruns, 1961; Newman, Braid and Wood, 1967). The results of the study reported in this thesis show no significant difference in the amount of carbon dioxide carried by adult and fetal blood at a given PCO_2.

Huisman (1963) reviewed the chemical and structural properties of hemoglobin. The essential conclusions are that hemoglobins A and

F consist of an identical heme portion and four globin chains: two α and two β chains in hemoglobin A and two α and two γ chains in hemoglobin F. The β and γ chains differ slightly in the content and arrangement of amino acids. The four chains are arranged in a helical fashion. The interactions between the α and β chains of adult hemoglobin (Benesch and Benesch, 1963) and the α and γ chains of fetal hemoglobin upon oxygenation are responsible for the change in configuration of oxy- and deoxyhemoglobin which is related to the dissociation constants of oxy- and deoxyhemoglobin.

In this study an equation was derived to clarify the relationships between hydrogen ion concentration, PCO_2, oxygen saturation, hemoglobin concentration and the dissociation constants of oxy- and deoxyhemoglobin. This equation makes it possible to compare the dissociation constants of fetal and adult hemoglobin, and assumes that the change in intracellular hydrogen ion concentration is negligible over the range studied. The nearly equal values for the dissociation constants of adult and fetal hemoglobin imply that upon oxygenation the effect of the rearrangement of the globin chains of fetal hemoglobin is similar to the rearrangement effects in adult hemoglobin.

SUMMARY AND CONCLUSIONS

The purpose of these studies was to determine the possible role of differences in the dissociation constants of adult and fetal hemoglobins and differences in carbon dioxide-carrying capacities of adult and fetal red cells.

Samples of adult and fetal blood were equilibrated with low CO_2 and high CO_2 gas mixtures. pH, PCO_2, oxygen saturation, total CO_2 content of whole blood and plasma were measured. Prior to equilibration the hemoglobin concentration and PCV (packed cell volume) were measured in all samples. The percentage of hemoglobin F was measured in the fetal samples.

This study concludes that adult and fetal blood transport the same amount of carbon dioxide when equilibrated <u>in vitro</u> at similar PCO_2s. The dissociation constants of fetal oxy- and deoxyhemoglobin are nearly equal to the dissociation constants of adult oxy- and deoxyhemoglobin.

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APPENDIX I

Chemical Procedures

Alkali Denaturation

Reagents:

1. Alkali solution, N/12 KOH. Dissolve 4.67 gms. KOH in distilled water and make up to 1 liter with water. Titrate with standard N/2 HCl and correct normality with additional KOH or HCl.

2. Precipitating solution, 50% saturated $(NH_4)_2SO_4$. Add 500 ml. 100% saturated $(NH_4)_2SO_4$ to 500 ml. distilled water plus 2.5 ml. concentrated HCl.

3. Toluene, C.P.

Procedures:

1. Wash 1 to 2 ml. of blood once with 0.9% NaCl and centrifuge.

2. To sediment, add 1.5 volumes of distilled water and 0.4 volumes of toluene, C. P.

3. Mix on vortex mixer for 30 to 60 seconds.

4. Centrifuge for 10 minutes at 2500 rpm.

5. Discard upper two layers and remove layer with clear red solution.

6. Adjust concentration of hemoglobin to about 10 gms./ 100 ml. with distilled water, solution A.

7. Add 0.02 ml. of solution A to 4 ml. of distilled water, solution B. This is a 1:200 dilution.

8. Add 0.1 ml. of solution A to a 1.6 ml. of alkaline reagent (N/12 KOH).

9. To mix, rinse pipette 5 to 6 times while shaking tube.

10. At exactly 1 minute, add 3.4 ml. precipitating solution. Invert tube 3 to 4 times and filter immediately. The filtrate, solution C, is a 1:50 dilution of solution A.

11. Determine the optical density (O.D.) of solutions B and C against a distilled water blank in a 10 x 75 mm. cuvette.

$$\frac{1/4 \text{ O.D. soln. C}}{\text{O.D. soln. B}} \times 100 = \% \text{ alkali resistant hemoglobin (Hgb F)}$$

Note: Solution B (1:200) is 4 times more dilute than solution C, therefore, 1/4 is used as a correction factor.

Total CO_2 and O_2 Content of Whole Blood

Instrument:

Kopp Natelson microgasometer. Note: Check standard and reagent blank daily with each series.

Reagents:

1. 1.2% potassium ferricyanide. Dissolve 1.2 gms. potassium ferricyanide in distilled water and make up to 100 ml. with water. Store in refrigerator. Do not allow mercury to come in contact with this stock solution.

2. 1% saponin. Dissolve 1 gm. of saponin in distilled water and make up to 100 ml. with normal saline (0.9% NaCl). Dispense in 5 ml. quantities into 20 ml. vials and freeze.

3. Saponin ferricyanide mix. On the day of the test, mix 0.75 ml. of 1.2% potassium ferricyanide with 5 ml. of 1% saponin, and cover with caprylic alcohol (2 cm. height). Deaerate under vacuum in a vacuum dessicator until reagent blank is less than 1 vol. % O_2. Time 1 to 2 hours.

4. 3N sodium hydroxide. Dissolve 12 gms. of NaOH in distilled water and make up to 100 ml. with water. Allow it to cool to room temperature and place in a polyethylene bottle. Transfer a portion to a 20 ml. vial and cover with mineral oil. Add mercury to a height of 2 cm. in the vial. Deaerate.

5. 1N potassium hydroxide. Dissolve 5.6 gms. of KOH in distilled water and make up to 100 ml. with water. Store in a sealed polyethylene bottle.

6. Sodium hydrosulfite solution. Place 1 gm. of sodium hydrosulfite in a 20 ml. vial or test tube. Add 5 ml. of 1N KOH and dissolve. Add 2 ml. of mercury and cover with mineral oil. Deaerate.

7. 1N lactic acid. Dilute 90 ml. of 85% lactic acid to 1 liter with distilled water. Transfer a portion to a large vial.

8. Vial containing mercury.

9. Vial containing distilled water.

Procedure:

Record temperature at beginning of each procedure.

Rinse instrument with approximately 1 ml. 1N lactic acid and expel.

1. Draw in 0.01 ml. caprylic alcohol, 0.1 ml. saponin ferricyanide solution, 0.01 ml. caprylic alcohol.

2. Draw in 0.03 ml. specimen.

3. Repeat Step 1.

4. Draw in Hg to 0.12 ml. mark.

5. Close reaction chamber stopcock and draw sample into reaction chamber bowl and mix for 3 to 5 minutes.

6. Raise caprylic alcohol meniscus to 0.12 ml. mark and record pressure (P_1).

7. Advance Hg to the top of manometer and open reaction chamber stopcock.

8. Draw in 0.03 ml. NaOH and Hg to 0.12 ml. mark.

9. Close reaction chamber stopcock and draw sample into reaction chamber bowl and mix for 3 minutes.

10. Raise caprylic alcohol meniscus to 0.12 ml. mark and record pressure (P_2). (P_1 - P_2) x factor for mM/L. or vol.% = CO_2 mM/L. or vol.%.

11. Repeat Step 7.

12. Draw in 0.03 ml. hydrosulfite reagent and Hg to 0.12 ml. mark.

13. Close reaction chamber stopcock and draw sample into reaction chamber bowl and mix for 3 minutes.

14. Raise caprylic alcohol meniscus to 0.12 ml. mark and record pressure (P_3). (P_2 - P_3) x factor for mM/L. or vol.% = O_2 mM/L. or vol.%.

Standardization:

Sample 0.03 ml. of air in the place of blood sample. Use an air sample which is completely saturated with water vapor. To saturate, fill a flask halfway with distilled water. Allow to stand for 15 minutes and draw up an air sample just above the surface of the water. Calculations for standard:

$$\text{Initial vol.} \times \frac{273\ ^{\circ}\text{C.}}{\text{R.T.}+273} \times \frac{\text{B.P.} - \text{V.P.}}{760} = \text{Volume taken if measured dry at } 0^{\circ}\text{ C. and 760 mm. Hg} \quad (1)$$

(R.T. - room temperature, B.P. - barometric pressure,

V.P. - vapor pressure H_2O)

$P_2 - P_3$ x factor for vol.% = O_2 content vol.% in air sample (2)

$$\frac{300}{\text{Corr. vol.}} \times 10{,}000 \times O_2 \text{ content vol.\%} = O_2 \text{ content vol.\% in air sample} \quad (3)$$

Note: O_2 vol.% of air sample = 20.9 vol.% ± 1 vol. %

Total CO_2 Content of Plasma

Instrument:

Kopp Natelson microgasometer. Note: Check standard daily with each series.

Reagents:

1. Standard: Dissolve 1.191 gms. anhydrous sodium carbonate, dried at 100°C., in distilled water, dilute to 500 ml. with water and keep under mineral oil. Add 2 ml. to a small vial with plastic top, and add 2 ml. clean dry mercury and cover with mineral oil for working standard.

2. Acid-antifoam: Dilute 5.3 ml. 85% lactic acid and 10 ml. anti-foam (820) to 250 ml. with distilled water. Transfer a portion to a large vial and add mercury to a height of 2 cm. in the vial.

3. 1N lactic acid. Dilute 90 ml. of 85% lactic acid to 1 liter with distilled water. Transfer portion to a large vial.

4. Large vial containing distilled water.

5. Vial containing mercury.

Procedure:

Record temperature at beginning of each procedure.

Advance mercury until a small drop is held on the tip of the pipette.

Draw in from each vial as follows:

1. Add 0.03 ml. specimen and 0.01 ml. mercury.

2. Add 0.1 ml. acid-antifoam reagent followed by mercury to the 0.12 ml. mark.

3. Close reaction chamber stopcock and retreat with piston until a small bubble of mercury remains in the reaction chamber.

4. Shake for 1 minute.

5. Advance piston until the top aqueous meniscus is at the 0.12 ml. mark.

6. Record manometer reading P_1.

7. Advance piston until mercury is at top of manometer.

8. Open stopcock and expel mercury to just past the stopcock.

9. Close stopcock and retreat with piston until the meniscus is at the 0.12 ml. mark.

10. Record manometer reading P_2. $(P_1 - P_2)$ x factor for mM/L. or vol.% = CO_2 content mM/L. or vol.%.

11. Advance piston until mercury is at top of manometer.

12. Eject the mercury in the pipette and all aqueous matter and rinse with distilled water.

Standardization:

Sample working standard in place of plasma.

$(P_1 - P_2)$ x factor for vol.% = 22.5 vol.% CO_2 ± 1%

APPENDIX II

Raw Data

Table 6.--Hematological data for adult blood samples

Sample	Hemoglobin gms/100 ml.	% Packed Cell Volume
A 1	15.2	49.0
A 2	13.5	42.0
A 3	14.3	43.0
A 4	14.0	43.0
A 5	15.4	48.0
A 6	13.2	41.0
A 7	17.1	54.0
A 8	15.7	49.0
A 9	16.0	48.5
A 10	13.6	42.0
A 11	16.8	49.0

Table 7.--Oxygenation data for adult blood samples

Sample	% O_2 sat.	PO_2 mm Hg	O_2 content mM/L.**
A 1 a*	55.5	33.5	-
b	64.0	28.0	8.0
c	59.5	43.5	7.0
A 2 a	57.5	34.0	-
b	65.0	33.0	7.2
c	28.0	26.0	4.8
A 3 a	39.0	30.0	-
b	81.5	40.0	5.2
c	42.5	32.5	2.2
A 4 a	55.0	36.5	-
b	75.5	36.5	4.1
c	58.0	44.3	3.4
A 5 a	62.0	36.0	-
b	57.5	27.0	6.4
c	51.5	40.5	6.1
A 6 a	47.5	31.0	-
b	81.5	44.0	5.7
c	49.0	43.0	3.2
A 7 a	33.0	19.5	-
b	67.5	24.0	5.0
c	43.0	30.0	2.4
A 8 a	41.0	30.0	-
b	58.0	22.0	3.8
c	33.0	25.5	2.4
A 9 a	42.0	30.0	-
b	58.0	28.0	5.6
c	39.5	33.5	3.2
A 10 a	52.5	32.5	-
b	83.5	45.5	5.8
c	44.0	35.5	3.5
A 11 a	44.0	27.0	-
b	55.0	25.0	5.1
c	34.0	23.0	4.4

* The PCO_2 values are given in the following table. In the above table the sample labeled a is always the initial value, b is the lower value and c is the higher value, of the samples measured.

** O_2 contents were not measured on the initial sample.

Table 8.--pH and carbon dioxide data for adult blood samples

Samples	pH	PCO_2	TCO_2 mM/L. ** whole blood	TCO_2 mM/L. plasma
A 1 a*	7.33	57.0	-	23.0
b	7.62	13.9	11.3	16.1
c	7.19	98.0	21.3	29.7
A 2 a	7.29	67.0	-	26.0
b	7.59	15.2	12.9	18.8
c	7.15	100.0	24.5	31.3
A 3 a	7.33	52.5	-	26.4
b	7.67	12.0	12.4	19.0
c	7.26	71.0	24.5	32.9
A 4 a	7.35	44.5	-	24.1
b	7.64	14.2	13.7	22.3
c	7.24	83.0	23.0	33.5
A 5 a	7.34	53.0	-	25.5
b	7.62	16.7	11.5	20.0
c	7.16	94.5	23.2	34.7
A 6 a	7.34	46.0	-	21.0
b	7.61	14.1	12.1	14.0
c	7.12	99.0	24.3	33.3
A 7 a	7.34	48.0	-	26.3
b	7.67	13.1	11.8	21.7
c	7.14	86.0	25.4	35.8
A 8 a	7.28	60.0	-	25.6
b	7.54	23.0	14.2	23.8
c	7.11	110.0	25.4	39.1
A 9 a	7.31	64.0	-	28.9
b	7.61	14.7	10.2	25.2
c	7.17	99.0	24.8	44.5
A 10 a	7.31	53.0	-	25.9
b	7.57	13.8	12.8	22.1
c	7.13	90.0	24.5	38.7
A 11 a	7.30	67.0	-	30.3
b	7.68	14.3	12.4	24.0
c	7.21	79.0	24.0	42.6

* a is the initial blood sample. b was equilibrated with low CO_2 gas mixture. (PCO_2 = 10-21 mm Hg). c was equilibrated with high CO_2 gas mixture. (PCO_2 = 66-110 mm. Hg).

** TCO_2 contents of whole blood were not measured on the initial sample.

Table 9.--Hematological data for fetal blood samples

Sample	Hemoglobin gms/100 ml.	% Packed Cell Volume
F 1	13.1	43.0
F 2	15.1	48.0
F 3	15.4	45.0
F 4	17.2	50.5
F 5	15.3	47.5
F 6	17.0	51.5
F 7	16.1	47.0
F 8	16.6	47.0
F 9	19.1	54.0
F 10	17.0	49.0
F 11	19.8	58.0

Table 10.--Oxygenation data for fetal blood samples

Sample	% O_2 sat.	PO_2 mm Hg	O_2 content mM/L.**
F 1 a*	92.5	77.5	-
b	55.5	16.5	3.2
c	39.0	20.5	1.6
F 2 a	98.0	117.0	-
b	32.0	16.0	3.5
c	26.0	21.0	1.8
F 3 a	75.5	43.5	-
b	88.0	56.5	9.1
c	34.0	32.5	3.6
F 4 a	94.5	66.0	-
b	86.0	45.5	8.9
c	77.0	59.0	8.2
F 5 a	75.0	56.5	-
b	44.0	24.5	3.4
c	34.0	29.5	2.1
F 6 a	48.5	60.0	-
b	85.0	48.0	8.0
c	62.0	46.0	5.4
F 7 a	95.0	89.5	-
b	76.5	33.0	6.4
c	62.0	42.0	4.0
F 8 a	96.0	151.5	-
b	40.0	20.5	4.1
c	28.0	25.0	3.6
F 9 a	98.5	133.0	-
b	68.5	28.0	8.1
c	33.0	25.5	3.8
F 10 a	90.5	69.0	-
b	60.0	29.0	6.9
c	34.0	28.5	4.0
F 11 a	97.0	141.0	-
b	50.0	21.5	6.0
c	36.5	32.5	4.2

* The PCO_2 values are given in the following table. In the above table the sample labeled a is always the initial value, b is the lower value and c is the higher value of the samples measured.

** O_2 contents were not measured on the initial sample.

Table 11.--pH and carbon dioxide data for fetal blood samples

Samples	pH	PCO_2	TCO_2 mM/L** whole blood	TCO_2 mM/L plasma
F 1 a*	7.27	48.0	-	22.7
b	7.57	14.9	12.5	18.0
c	7.13	99.5	24.7	33.0
F 2 a	7.37	32.5	-	18.1
b	7.52	18.5	12.8	21.8
c	7.08	112.0	23.4	35.4
F 3 a	7.19	59.0	-	19.2
b	7.43	16.2	9.1	13.2
c	7.02	110.0	21.1	31.9
F 4 a	7.38	33.5	-	20.8
b	7.53	15.9	11.7	20.5
c	7.15	89.0	22.2	32.5
F 5 a	7.01	65.0	-	19.0
b	7.30	16.5	9.3	14.3
c	6.95	93.0	18.4	26.3
F 6 a	7.19	35.0	-	17.0
b	7.33	12.7	11.5	15.7
c	7.02	95.0	19.0	30.0
F 7 a	7.22	45.0	-	20.7
b	7.48	15.2	10.7	18.6
c	7.06	82.0	19.6	31.9
F 8 a	7.17	40.0	-	18.5
b	7.45	13.1	9.4	18.5
c	7.07	69.0	19.2	32.6
F 9 a	7.22	45.0	-	24.9
b	7.55	12.7	10.6	19.9
c	7.11	86.0	24.8	38.8
F 10 a	7.03	48.5	-	20.7
b	7.30	12.9	7.7	15.6
c	6.97	101.0	18.8	29.2
F 11 a	7.24	53.5	-	22.8
b	7.52	15.7	11.3	†
c	7.03	105.0	19.5	†

* a is theinitial blood sample. b was equilibrated with low CO_2 gas mixture (PCO_2 = 10-21 mm. Hg). c was equilibrated with high CO_2 gas mixture (PCO_2 = 66.0 - 110 mm. Hg).

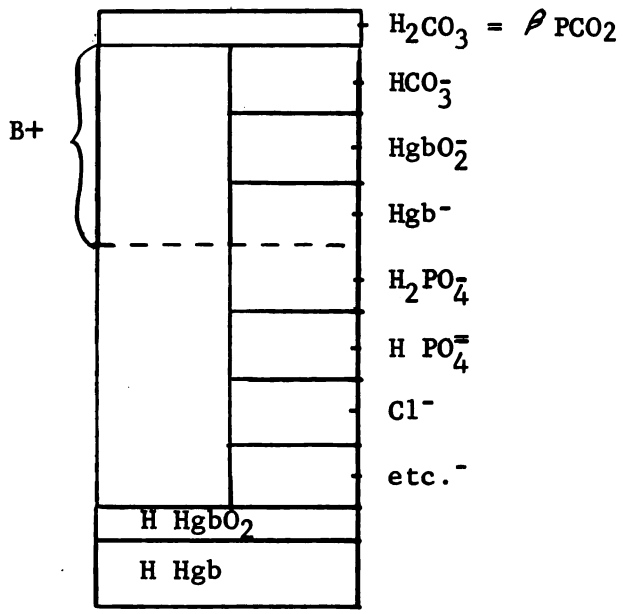
** TCO_2 contents of whole blood were not measured on the initial sample.

† Sample too hemolyzed to measure plasma TCO_2

APPENDIX III

Derivation of Formula Used in Text for $\Delta y / \Delta \phi$

Derivation of Formula Used in Text for $\Delta y/\Delta \phi$



(Proportions of anions shown are schematic and not exact.)

Notation:

B+ = available intracellular cation mEq./L. (milliequivalents per liter) as implied in illustration above

A = $[H\ HgbO_2]$ mEq./L. - concentration of undissociated oxyhemoglobin

a = $[HgbO_2^-]$ mEq./L. - concentration of dissociated oxyhemoglobin

B = $[H\ Hgb]$ mEq./L. - concentration of undissociated deoxyhemoglobin

b = $[Hgb^-]$ mEq./L. - concentration of dissociated deoxyhemoglobin

G = Total hemoglobin concentration grams/100 ml.

$\emptyset$ = Oxygen saturation fraction

h = hydrogen ion concentration of cell mEq./L.

K_1 = Dissociation constant of carbonic acid (H_2CO_3)

K_2 = Dissociation constant of adult oxyhemoglobin

K_3 = Dissociation constant of adult deoxyhemoglobin

β = Solubility constant for intracellular CO_2

TCO_2 = Total carbon dioxide content of cells

All of the above constants refer only to the dissociation constant of the first hydrogen ion as shown in the following generalized formula:

$$H_nA \rightarrow H+ + (H_{n-1}A)^-$$

The steps in the derivation are summarized below:

Dissociation constants for oxy- and deoxyhemoglobin

(1) $G = A+a + B+b$

(2) $\emptyset G = A+a \qquad G(1-\emptyset) = B+b$

$A = \emptyset G-a \qquad B = G(1-\emptyset)-b$

(3) $h = \frac{K_2 A}{a} \qquad h = \frac{K_3 B}{b}$

(4) $h = \frac{K_2(\emptyset G-a)}{a} = K_3 \frac{[(1-\emptyset)G-b]}{b}$

(5) $ah = K_2(\emptyset G-a) \qquad bh = K_3[(1-\emptyset)G-b]$

$a = \frac{K_2 G\emptyset}{K_2+h} \qquad b = \frac{K_3(1-\emptyset)\,G}{K_3+h}$

Dissociation constants for carbonic acid

$$(6)\quad h = \frac{K_1 [H_2CO_3]}{[HCO_3^-]} = \frac{K_1 \beta PCO_2}{TCO_2 - \beta PCO_2}$$

$$H_2CO_3 = \beta PCO_2$$

$$HCO_3^- = TCO_2 - \beta PCO_2$$

$$(7)\quad TCO_2 - \beta PCO_2 = K_1 \frac{\beta PCO_2}{h}$$

Relationships to B+

$$(8)\quad B+ \cong [HCO_3^-] + a + b$$

$$(9)\quad B+ \cong (TCO_2 - \beta PCO_2) + a + b$$

$$(10)\quad B+ = K_1 \frac{\beta PCO_2}{h} + \frac{K_2 G\phi}{K_2+h} + \frac{K_3 G(1-\phi)}{K_3+h}$$

$$(11)\quad \frac{K_1 \beta PCO_2}{h} = B+ - \frac{K_2 \phi G}{K_2+h} - \frac{K_3 G(1-\phi)}{K_3+h}$$

$$(12)\quad PCO_2 = \frac{h}{K_1 \beta}\left[B+ - \frac{K_2 G\phi}{K_2+h} - \frac{K_3 G(1-\phi)}{K_3+h} \right]$$

$$(13)\quad \frac{PCO_2}{G} = \frac{h}{K_1 \beta}\left[\frac{B+}{G} - \frac{K_2 \phi}{K_2+h} - \frac{K_3(1-\phi)}{K_3+h} \right]$$

$$\text{let } y = \frac{PCO_2}{G}$$

$$(14)\quad dy/d\phi \cong \Delta y/\Delta\phi \cong \left[- \frac{K_2}{K_2+h} + \frac{K_3}{K_3+h} \right] \frac{h}{K_1 \beta}$$

VITA

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