The Buffer Values and the Bohr Effect of Human Fetal and Adult Whole Blood *in Vitro* in an Acid Range

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Extract

The buffering properties and the Bohr effect were studied *in vitro* on 10 human fetal and 9 adult whole blood samples. At the beginning of the experiments, the fetal and adult blood samples were similarly acidotic ($BE = -9$ mEq/liter).

In order to obtain the respiratory and the metabolic buffer values, the blood was titrated either by varying the P_{CO_2} at constant buffer base or by adding known amounts of base at constant P_{CO_2} . The mean fetal and adult *in vitro* apparent respiratory buffer values (β') were 28.30 mEq HCO_3^- /liter·pH unit $\pm$ 2.23 SEM and 23.66 mEq HCO_3^- /liter·pH unit $\pm$ 0.80 SEM, respectively. These values were not statistically different. The *in vitro* apparent metabolic buffer value (λ) rose significantly when P_{CO_2} increased. At P_{CO_2} of 30, 40, and 60 mm Hg, the mean values were, respectively, for fetal blood: 55 $\pm$ 1.75 SEM, 61.25 $\pm$ 1.41 SEM, and 71.25 $\pm$ 3.21 SEM mEq H^+ /liter·pH unit; for adult blood: 61.94 $\pm$ 2.76 SEM, 65.83 $\pm$ 2.47 SEM, and 71.94 $\pm$ 2.97 SEM mEq H^+ /liter·pH unit. Only at a P_{CO_2} of 30 mm Hg was the difference between adult and fetal blood statistically significant. In order to determine the magnitude of the Bohr effect, the iso- P_{CO_2} pH change (ΔpH_{ox}) associated with the oxygenation of a desaturated blood sample was measured and the O_2 contents were determined by Natelson's micromanometric method. Thus, by the following equation: $\Delta H^+ = \lambda \cdot \Delta pH$, the ratio $-\Delta H^+/\Delta O_2$ was calculated. The mean values obtained for P_{CO_2} of 30, 40, and 60 mm Hg were, respectively, 0.330 $\pm$ 0.027 SEM, 0.365 $\pm$ 0.028 SEM, and 0.401 $\pm$ 0.032 SEM mEq H^+ /mm O_2 for fetal blood; and 0.391 $\pm$ 0.029 SEM, 0.414 $\pm$ 0.029 SEM and 0.445 $\pm$ 0.032 SEM mEq H^+ /mm O_2 for adult blood. No significant differences were found at each P_{CO_2} between the adult and the fetal blood.

Speculation

These results show that the *in vitro* buffering properties and the Bohr effect were similar in the adult and the fetal whole blood. The observed difference between the adult and fetal blood for the metabolic buffer value at P_{CO_2} of 30 mm Hg could be explained by differences in hemoglobin (Hb) concentration or different carbamino reaction with fetal hemoglobin (HbF). However, the heterogeneity and the complexity of whole blood hinder simple interpretation. The practical implication of this study is that the use of the Siggaard-Andersen's nomogram for fetal blood is justified, at least *in vitro*.

Introduction

It is often assumed that fetal blood has the same fundamental acid-base properties as adult blood; for instance, the acid-base status of fetal or neonate blood is usually determined by using Siggaard-Andersen's nomogram, although this nomogram was constructed from values of adult blood [17, 19]. However, comparing fetal and adult blood, physicochemical differences, such as the higher hemoglobin concentration, the presence of fetal hemoglobin, or lower fetal protein concentration could result in different buffering properties for fetal blood. Furthermore, when partly desaturated blood is analyzed by the Astrup method, a correction depending on the Bohr effect must be introduced [18, 19]. The magnitude of this correction could also be modified by the possible fetal buffering peculiarities or the HbF properties. Thus, this study was undertaken in order to evaluate the properties of the fetal blood buffers and the magnitude of the fetal Bohr effect in comparison with adult blood.

The *in vitro* buffering properties of blood were studied by measuring the respiratory and metabolic buffer values. According to the data of Van Slyke [22], the buffer value, dB/dpH, represents the amount of added base, dB, causing a pH change, dpH, in 1 liter of solution. This concept can be used in the titration of whole blood, although there are more complicated relationships than in simple buffer solutions. As a consequence of this complexity, the buffer values measured in whole blood are empiric and can be called apparent buffer values. Thus, the *in vitro* titration of blood with addition or release of carbonic acid by changing the P_{CO_2} at constant buffer base permits the calculation of the apparent respiratory buffer value (β') = $-\Delta HCO_3^-/\Delta pH$, whereas the *in vitro* titration at constant P_{CO_2} by varying the buffer base gives the apparent metabolic buffer value (λ) = $-\Delta H^+/\Delta pH$. As described by Wyman (24), the linkage of O_2 and H^+ by the Hb molecule can be expressed either as the ratio $-\Delta H^+/\Delta O_2$ for a given pH, or as the ratio $\Delta \log P_{O_2}/\Delta pH$ for a given O_2 saturation level. Many authors have studied and compared this last expression of the Bohr effect for fetal and adult blood, but the results are inconsistent. Some found lower values for adult (0.45) than for fetal blood (0.60), others obtained higher values for adult (0.50) than for fetal blood (0.41) [8, 9]. Besides, Kirschbaum found, for fetal blood, values from 0.46 to 0.57, depending on the O_2 saturation [12]. These experiments were usually made by varying the P_{CO_2} in order to change the pH. However, it has re-

cently been proved that both pH and P_{CO_2} influence independently the magnitude of the Bohr effect [1, 2, 20]. In this study, the $-\Delta H^+/\Delta O_2$ ratio was chosen as an expression of the Bohr effect and it was measured for fetal and adult whole blood at constant P_{CO_2} and well defined acid-base conditions.

Our results show that the *in vitro* respiratory buffer value and the Bohr effect were similar for adult and fetal blood at least in the range of P_{CO_2} and pH studied. The *in vitro* metabolic buffer value was also similar for adult and fetal bloods at P_{CO_2} of 40 and 60 mm Hg, but dependent on the P_{CO_2} . However, at P_{CO_2} of 30 mm Hg it was significantly lower for the fetal than for the adult blood.

Methods

The method was thoroughly described in a previous paper (1) and only a few specific points will be mentioned.

Principle

The apparent metabolic buffer value (λ) was found by *in vitro* titration of saturated whole blood with known amounts of base and by determining the corresponding iso- P_{CO_2} pH shifts. Titration curves could be drawn at any constant P_{CO_2} and their slopes, $\lambda = -\Delta H^+/\Delta pH$ were calculated.

The apparent respiratory buffer value (β') was obtained by measuring the pH of fully oxygenated blood after *in vitro* equilibration with known P_{CO_2} (~30 and 60 mm Hg); using the Davenport diagram [5], it was possible to determine graphically the slope $\beta' = -\Delta HCO_3^-/\Delta pH$.

The Bohr effect, $-\Delta H^+/\Delta O_2$, was determined by measuring the pH shift, ΔpH_{ox} , of a given desaturated blood sample while undergoing oxygenation at constant P_{CO_2} . Since $\Delta pH_{ox} = \Delta H^+/\lambda$, the ratio, $-\Delta H^+/\Delta O_2$, can be calculated when the amount of bound O_2 is determined.

Material

Blood from full term fetuses was obtained at delivery by puncture of a cord vessel before the separation of the placenta. We ascertained the absence of maternal blood by measuring the immunoglobulins in each sample. No IgA was detected, thus excluding contamination by maternal blood. The mean pH ($\pm$ SEM) determined at P_{CO_2} 40 mm Hg after saturation was 7.260 ± 0.009 . Adult blood was taken by venous punc-

ture from healthy adults. In order to obtain the same pH at P_{CO_2} 40 as for the fetal blood, 0.5 ml 0.3 N isotonic lactic acid was added to 40 ml adult blood. The mean pH at P_{CO_2} 40 after saturation was then 7.244 ± 0.01 .

Techniques

The following modifications of the previously described method [1] were utilized. [1] Each aliquot of blood was 3 ml. [2] The titration solutions contained,

Table I. Fetal blood¹

	P_{CO_2} , mm Hg		
	30	40	60
ΔpH_{ox}	0.057 ± 0.003	0.055 ± 0.003	0.052 ± 0.004
β' , mEq HCO_3^- /liter·pH unit	28.30 ± 2.23	28.30 ± 2.23	28.30 ± 2.23
λ , mEq H^+ /liter·pH unit	55.00 ± 1.75	61.25 ± 1.41	71.25 ± 3.21
$-\Delta H^+/\Delta O_2$, mEq H^+ / mm O_2	0.330 ± 0.027	0.365 ± 0.028	0.401 ± 0.032
[BE], mEq/liter	-8.80 ± 0.55	-8.80 ± 0.55	-8.80 ± 0.55
[Hb], g/100 ml	17.52 ± 0.71	17.52 ± 0.71	17.52 ± 0.71
O_2 bound, mm/liter	9.25 ± 0.30	9.25 ± 0.30	9.25 ± 0.30
pH _{ox}	7.335 ± 0.010	7.260 ± 0.009	7.156 ± 0.007

¹ Values are expressed as means $\pm$ SEM. $N = 10$. See text for definition of symbols.

Table II. Adult blood¹

	P_{CO_2} , mm Hg		
	30	40	60
ΔpH_{ox}	0.053 ± 0.005	0.052 ± 0.004	0.051 ± 0.004
β' , mEq HCO_3^- /liter·pH unit	23.66 ± 0.80	23.66 ± 0.80	23.66 ± 0.80
λ , mEq H^+ /liter·pH unit	61.94 ± 2.76	65.83 ± 2.47	71.94 ± 2.97
$-\Delta H^+/\Delta O_2$, mEq H^+ / mm O_2	0.391 ± 0.029	0.414 ± 0.029	0.445 ± 0.032
[BE], mEq/liter	-9.37 ± 0.52	-9.37 ± 0.52	-9.37 ± 0.52
[Hb], g/100 ml	15.82 ± 0.27	15.82 ± 0.27	15.82 ± 0.27
O_2 bound, mm/liter	8.18 ± 0.23	8.18 ± 0.23	8.18 ± 0.23
pH _{ox}	7.322 ± 0.010	7.244 ± 0.010	7.135 ± 0.010

¹ Values are expressed as means $\pm$ SEM. $N = 9$. See text for definition of symbols.

respectively, 0, 80, 120, and 160 mEq/liter NaOH and the amount of NaCl required for isotonicity. The constant quantity of the solution added to blood was 109 μ l. [3] Inasmuch as only small amounts of blood were obtainable, the O_2 contents could not be measured by Van Slyke's method, and the Natelson microgazometer was chosen. Since the Natelson micromethod used as described either by Holaday and Verosky [10] or by the Instruction booklet no. 5 [14] gave very inconsistent results, the technique was modified (see *Appendix*). Then the macromethod and the modified micromethod were compared. Eleven comparisons of the macro- and micromethods made on whole blood samples with less than 1 mm O_2 /liter showed that the O_2 contents obtained by both methods were not statistically different (paired t test: $P > 0.5$). Eleven analyses of fully oxygenated samples (mean O_2 content 8 mm/liter) showed that the modified Natelson technique gave consistently lower results than Van Slyke's method (paired t test: $P < 0.001$). The mean difference ($\pm$ SEM) was 0.52 mm/liter $\pm$ 0.09. This quantity was added as a correction factor to each oxygen content obtained for fully saturated blood. This correction factor was not statistically different from that obtained by Holaday and Verosky on five samples in the same range of O_2 content ($P > 0.5$) [10].

Results

Ten experiments were performed with fetal blood and nine with adult blood. Tables I and II summarize the results for fetal and adult blood at P_{CO_2} of 30, 40, and 60 mm Hg.

In Vitro Apparent Respiratory Buffer Value = β'

The adult and fetal CO_2 titration curves are shown in Figure 1; their slopes, β' , were not statistically different (Tables I and II, Fig. 1).

In Vitro Apparent Metabolic Buffer Value = λ

Figure 2 demonstrates, for a P_{CO_2} of 40 mm Hg, the relation between the amount of base added and the mean ($\pm$ SEM) associated pH shift. As shown on Tables I and II, λ depends markedly on the P_{CO_2} , for either fetal or adult blood. No significant difference was found between fetal and adult λ at a P_{CO_2} of 40 and 60 mm Hg. But, at a P_{CO_2} of 30 mm Hg, λ was significantly lower for fetal than for adult blood ($P < 0.05$). Siggaard-Andersen's base excess scale could be tested by comparing the quantity of base added to the read base excess changes. Figure 3 proves the validity

Fig. 1. *In vitro* CO₂ titration curves. [—■—]: Mean pH values $\pm$ SEM for adult blood; [—●—]: mean pH values $\pm$ SEM for fetal blood.

of this nomogram for fetal blood, at least in the acid pH range studied.

$$\text{Bohr Effect} = -\Delta^+/\Delta O_2$$

Fetal $-\Delta H^+/\Delta O_2$ ratios did not differ significantly from the ratios for adults, although values obtained from fetal blood were, on the whole, slightly lower than those from adult blood (Tables I and II).

Discussion

The study of the apparent respiratory and metabolic buffer values makes it possible to evaluate the properties of the noncarbonic and carbonic buffers. Indeed, when whole blood is titrated by varying the P_{CO_2} , *i.e.*, by addition or release of carbonic acid, buffer value (β') depends on the noncarbonic buffer system, chiefly Hb. On the other hand, when whole blood is titrated by an acid or a base at fixed P_{CO_2} , buffer value (λ) depends on the noncarbonic and carbonic buffers.

No significant difference was found between the fetal and adult β' , although fetal β' was on the average higher than adult β' , probably because of the significantly more concentrated Hb in fetal blood compared with adult blood (Tables I and II). Thus the noncarbonic buffers (Hb, proteins, phosphates) had on the whole the same buffering effects in fetal as in adult

Fig. 2. Mean titration curves of adult and fetal blood at a P_{CO_2} of 40 mm Hg. Symbols are the same as in Figure 1.

Fig. 3. Relation between the amount of base added and the mean ($\pm$ SEM) read base excess changes for saturated fetal blood.

blood. The metabolic buffer value, λ , provides an index of the properties of the two buffer systems. The marked increase of λ with rising P_{CO_2} at constant buffer base, either in fetal or adult blood, has pre-

viously been explained [11]; however, it should be pointed out that when P_{CO_2} increases, the HCO_3^- concentration rises and the metabolic buffer value rises also, according to the special buffer properties of the carbonic system.

Fetal λ was significantly lower than adult λ at $P_{CO_2} = 30$ mm Hg. However, this discrepancy disappeared at higher P_{CO_2} . Many factors could be responsible for this peculiarity. One can speculate that the fetal whole blood had a lower HCO_3^- concentration at low P_{CO_2} than adult blood because of its higher hematocrit, and therefore had a lower carbonic buffer value. Beginning from an identical plasma bicarbonate level at a given P_{CO_2} , the same ΔP_{CO_2} would result in a greater ΔHCO_3^- in fetal than in adult blood, if fetal β' was slightly higher than that of adult blood. Thus, at higher P_{CO_2} , the difference of the carbonic buffer value is cancelled and the λ would be the same for both fetal and adult blood. Differences in noncarbonic buffers of fetal and adult blood could also be involved. Indeed, in the presence of CO_2 , Hb forms carbamate compounds [7], which increase the buffer power of Hb [15]. The different structure of HbF, in particular, the NH_2 -terminal glycine in γ chains instead of the NH_2 -terminal valine in α chains [16] or peculiarities of the fetal erythrocyte could inhibit this specific action of carbaminos. However, it must be pointed out that all interpretations remain very uncertain because of the complexity of the whole blood system, in which two phases, erythrocytes and plasma, are in equilibrium. This fact and its implications previously discussed [1] must also be considered for the evaluation of the Bohr effect.

Measurements of the Bohr effect on adult (HbA) and fetal hemoglobin solutions gave a $\Delta \log P_{O_2}/\Delta pH$ ratio of 0.48 in both cases [13]. Our values for whole blood are lower than those for Hb solutions, but were almost identical for fetal and adult blood. The discrepancy between the Hb solutions and the whole blood has already been discussed for adult blood [1]. *A priori*, the absence of significant differences between the adult and fetal $-\Delta H^+/\Delta O_2$ ratios seems surprising in regard to the possible influences of CO_2 and 2,3-diphosphoglycerate (2,3-DPG). Indeed, in the case of HbA, the $-\Delta H^+/\Delta O_2$ ratio is decreased by the carbamino reactions [5], but this effect is inhibited by 2,3-DPG [4, 20]. Furthermore, the interaction of 2,3-DPG and O_2 is less marked for HbF than for HbA [3, 6, 21] because of the structural differences of the HbF molecule. One can assume that the decreasing effect of car-

bamino on the Bohr effect of HbF is not inhibited by 2,3-DPG and, therefore, the $-\Delta H^+/\Delta O_2$ ratio would be smaller in fetal than in adult blood. It must also be noticed that, although no significant difference was found, fetal values seem to be slightly lower than adult values. Besides, as stated above, HbF could primarily bind less CO_2 than HbA, but no prediction can be made for whole blood because of its complexity. This complexity and the different conditions of measurements mentioned in the *Introduction* prevents the comparison of our results with those of other authors. According to the data of Wyman [25] and the known effect of CO_2 on the Bohr effect, the ratios $\Delta \log P_{O_2}/\Delta pH$ and $-\Delta H^+/\Delta O_2$ are identical only when the first ratio is defined for given O_2 saturation level and P_{CO_2} , the second one for given pH and P_{CO_2} . Furthermore, these equations are relevant in the simple situation of Hb solutions when the possible linkages are known and the medium conditions can be controlled.

It must be emphasized that only empiric values can be measured in whole blood. These values are valid for a given situation of measurement, *e.g.*, in our conditions, at P_{CO_2} levels of 30 to 60 mm Hg and a moderately acidotic state ($BE = -9$ mEq/liter). When desaturated fetal blood is analyzed by the Astrup method, it must be kept in mind that the correction for the Bohr effect differs from the values obtained on Hb solutions and depends on the acid-base condition of the blood.

Summary

In conclusion, the *in vitro* respiratory and metabolic apparent buffer values for the adult and fetal blood are in the same range. This fact is moreover enhanced by the observed validity of the base excess scale for fetal blood *in vitro* (see Fig. 3). Thus, the use of the Siggaard-Andersen's nomogram is allowable for the fetal blood, with the restriction that the *in vitro* conditions may not reflect the *in vivo* state [23].

The theoretical question of the "normality" of the fetus concerning his acid-base status must be considered. Has the fetal or neonate's acid-base status to be regarded as pathologic in comparison with the adult's "normality" or must it be considered as normal when the values are similar to the average observed in the majority of neonates? When it is known that the fundamental *in vitro* acid-base properties of fetal blood are the same as for adult blood, this question becomes a matter of definition of terms.

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Appendix

Modified Natelson's Technique

Reagents. Saponin-ferricyanid solution (0.4 g saponin + 1.6 g K-ferricyanid, H_2O to make 50 ml. 1 % lactic acid to make 100 ml); caprylic alcohol; NaOH, 3 N; Na-hydrosulfite solution (1 g $Na_2S_2O_4$ + 5 ml KOH, 1 N).

All reagents were degassed and kept under vacuum.

Method. Anaerobic introduction of: 0.03 ml blood, 0.01 ml Hg, 0.01 ml caprylic alcohol, 0.1 ml saponin-ferricyanid, 0.02 ml caprylic alcohol, 0.1 ml saponin-ferricyanid, 0.01 ml caprylic alcohol, Hg to the 0.12 ml mark. This was followed by: vacuum + shaking for 5 min, read P_1 ; anaerobic introduction of 0.03 ml NaOH, 3 N, Hg to the 0.12 ml mark; vacuum + shaking for 3 min, read P_2 ; anaerobic introduction of 0.03 ml Na-hydrosulfite, Hg to the 0.12-ml mark, vacuum + shaking for 3 min, read P_3 .

The measuring chamber was carefully washed after each measure and the stopcocks regreased.

Blank analyses were made after each two or three measures; blood was replaced with saponin-ferricyanid solution.