A Further Study of the Metabolic Buffer Value and the Bohr Effect in Human Fetal Whole Blood

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Extract

The metabolic buffer value ($\lambda = \Delta\text{H}^+/\text{liter} \cdot \text{pH unit}$) and the Bohr effect ($-\Delta\text{H}^+/\Delta\text{O}_2$) were studied *in vitro* in fetal blood at a base excess concentration [BE] of 0 mEq/liter and +10 mEq/liter by the same method as in a previous study [3]. At a P_{CO_2} of 30, 40, and 60 mm Hg, the mean fetal λ ($\pm\text{SEM}$) was, respectively, for [BE] = 0 mEq/liter: 74.00 ± 2.01 , 79.00 ± 1.35 , and 87.75 ± 2.42 mEq $\text{H}^+/\text{liter} \cdot \text{pH unit}$; for [BE] = +10 mEq/liter: 96.75 ± 3.24 , 97.50 ± 3.85 , and 103.75 ± 8.17 mEq $\text{H}^+/\text{liter} \cdot \text{pH unit}$. At P_{CO_2} of 30, 40, and 60 mm Hg, the mean fetal Bohr effect ($\pm\text{SEM}$) was respectively, for [BE] = 0 mEq/liter: 0.447 ± 0.021 , 0.469 ± 0.023 , and 0.505 ± 0.027 mEq $\text{H}^+/\text{mm O}_2$; for [BE] = +10 mEq/liter: 0.361 ± 0.033 , 0.395 ± 0.032 , and 0.467 ± 0.051 mEq $\text{H}^+/\text{mm O}_2$. These values together with the previous results obtained at [BE] = -9 mEq/liter were compared with the corresponding values obtained previously for adult blood with the same conditions and method. This comparison between adult and fetal blood demonstrates that the pH-dependent variation of λ is more marked, at low P_{CO_2} , in fetal blood than in adult blood, and the relation between the plasma pH and the Bohr effect is different in fetal blood compared with adult blood.

Speculation

The present study makes apparent some differences between fetal and adult blood in regard to the buffering properties and the Bohr effect. These results are difficult to interpret in term of physicochemical properties of fetal hemoglobin; however, the following practical implications result from the present data. (1) The [BE] scale of the Siggaard-Andersen nomogram is not strictly adequate for fetal blood *in vitro* in alkaline pH range. (2) Because the $-\Delta\text{H}^+/\Delta\text{O}_2$ ratio is greater in fetal blood than in adult blood, the results obtained by the Astrup method for desaturated fetal blood must be corrected in accordance with the desaturation level.

Introduction

The differences in quality and in quantity of hemoglobin in fetal blood in regard to adult blood prompted us to determine and compare the buffer values and the Bohr effect of human fetal and adult blood. A study of these variables made in adult and fetal blood at the

original acid state of fetal blood, *i.e.*, at a [BE] of -9 mEq/liter, showed that the metabolic buffer value ($\lambda = \text{mEq H}^+/\text{liter} \cdot \text{pH unit}$) and the Bohr effect ($-\Delta\text{H}^+/\Delta\text{O}_2 = \text{mEq H}^+/\text{mm O}_2$) were similar in fetal and adult blood [3]. However, it has been demonstrated in adult whole blood that the λ and the

$-\Delta\text{H}^+/\Delta\text{O}_2$ ratio depend at any constant P_{CO_2} on the pH, *i.e.*, on the [BE]; a rise of pH at constant P_{CO_2} causes an increase of λ and a decrease of the $\Delta\text{H}^+/\Delta\text{O}_2$ ratio [6, 10]. Thus, it is interesting to determine these variables in fetal blood at different levels of [BE]. In this study the fetal blood was alkalized in order to obtain [BE] = 0 and +10 mEq/liter and the λ and the $-\Delta\text{H}^+/\Delta\text{O}_2$ ratio was determined at three different P_{CO_2} and compared with the adult values obtained under the same conditions. This comparison brings out the following differences between adult and fetal blood: (1) the pH-dependent variation of λ is more marked, at low P_{CO_2} , in fetal blood than in adult blood; (2) the relation between the plasma pH and the Bohr effect is different in fetal blood compared with adult blood.

Methods

Blood from full term fetuses was obtained at delivery by puncture of a cord vessel before separation of the placenta; this blood was immediately alkalized by addition of isotonic NaHCO_3 . In order to obtain a [BE]

of 0 mEq/liter, 1 ml solution containing 160 mEq/liter NaHCO_3 (301 mOsmol/liter) was slowly mixed *in vitro* with 15 ml fresh heparinized fetal blood. Two milliliters of the same solution were added in the same manner to 15 ml fetal blood in order to obtain a [BE] of +10 mEq/liter. After this alkalization, each blood sample was immediately handled as previously described [3]. All procedures were identical with those previously reported [2, 3].

Results

Ten experiments were performed on fetal blood *in vitro* at a [BE] of 0 mEq/liter and 10 at a [BE] of +10 mEq/liter. For each [BE] and at three different P_{CO_2} levels (30, 40, and 60 mm Hg), the following variables were studied: (1) the pH of saturated blood at each P_{CO_2} (pH_{ox}), (2) the change of pH caused by the complete oxygenation of a desaturated blood sample at a constant P_{CO_2} ($\Delta\text{pH}_{\text{ox-red}}$), (3) the metabolic buffer value (λ), (4) the Bohr effect ($-\Delta\text{H}^+/\Delta\text{O}_2$). The mean values ($\pm\text{SEM}$) are represented on Table I; for the purpose of comparison, the mean adult values ob-

Table I. Mean values ($\pm\text{SEM}$) for fetal blood ($n = 10$) and for adult blood ($n = 9$)

P_{CO_2}	[BE], mEq/liter	[Hb], g/100 ml	pH_{ox}	$\Delta\text{pH}_{\text{ox-red}}$	λ mEq/liter·pH	$-\Delta\text{H}^+/\Delta\text{O}_2$ mEq/mM
30 mm Hg	- 0.46 $\pm$ 0.77	15.69 $\pm$ 0.46	7.484 $\pm$ 0.014	0.048 $\pm$ 0.003	74.00 $\pm$ 2.01 ¹	0.447 $\pm$ 0.021 ²
	- 3.40 $\pm$ 0.42	16.31 $\pm$ 0.30	7.404 $\pm$ 0.006	0.047 $\pm$ 0.004	68.20 $\pm$ 4.66	0.354 $\pm$ 0.025 ¹
	+10.13 $\pm$ 0.54	14.88 $\pm$ 0.29	7.630 $\pm$ 0.008	0.028 $\pm$ 0.003	96.75 $\pm$ 3.24 ¹	0.361 $\pm$ 0.033 ²
	+10.04 $\pm$ 0.47	16.31 $\pm$ 0.29	7.627 $\pm$ 0.007	0.022 $\pm$ 0.002	78.66 $\pm$ 3.95	0.205 $\pm$ 0.012 ¹
40 mm Hg	- 0.46 $\pm$ 0.77	15.69 $\pm$ 0.46	7.401 $\pm$ 0.013	0.047 $\pm$ 0.003	79.00 $\pm$ 1.35 ¹	0.469 $\pm$ 0.023
	- 3.40 $\pm$ 0.42	16.31 $\pm$ 0.30	7.347 $\pm$ 0.006	0.045 $\pm$ 0.004	70.03 $\pm$ 4.69 ²	0.350 $\pm$ 0.022 ¹
	+ 10.13 $\pm$ 0.54	14.88 $\pm$ 0.29	7.534 $\pm$ 0.007	0.031 $\pm$ 0.002	97.50 $\pm$ 3.85 ¹	0.395 $\pm$ 0.032
	+ 10.04 $\pm$ 0.47	16.31 $\pm$ 0.29	7.535 $\pm$ 0.006	0.022 $\pm$ 0.002	84.88 $\pm$ 4.44 ²	0.227 $\pm$ 0.012 ¹
60 mm Hg	- 0.46 $\pm$ 0.77	15.69 $\pm$ 0.46	7.284 $\pm$ 0.011	0.046 $\pm$ 0.004	87.75 $\pm$ 2.42	0.505 $\pm$ 0.027
	- 3.40 $\pm$ 0.42	16.31 $\pm$ 0.30	7.234 $\pm$ 0.005	0.043 $\pm$ 0.003	75.13 $\pm$ 4.48 ³	0.357 $\pm$ 0.020 ³
	+ 10.13 $\pm$ 0.54	14.88 $\pm$ 0.29	7.399 $\pm$ 0.006	0.034 $\pm$ 0.003	103.75 $\pm$ 8.17	0.467 $\pm$ 0.051
	+ 10.04 $\pm$ 0.47	16.31 $\pm$ 0.29	7.406 $\pm$ 0.004	0.022 $\pm$ 0.002	101.55 $\pm$ 7.51 ⁵	0.273 $\pm$ 0.025 ²

¹ Student *t* test: [BE]0 - [BE] + 10: $P < 0.001$.

² Student *t* test: [BE]0 - [BE] + 10: $P < 0.05$.

³ Student *t* test: adult-fetal: $P < 0.05$.

⁴ Student *t* test: adult-fetal: $P < 0.005$.

⁵ Student *t* test: [BE]0 - [BE] + 10: $P < 0.01$.

Fig. 1. Relation between the metabolic buffer value (λ) and the pH at three different P_{CO_2} in adult (---) and fetal (—) blood.

tained in previous studies in the same conditions [2, 6] are set in *italics*.

Figure 1 shows the relation between the mean λ and the pH at P_{CO_2} 30, 40, and 60 mm Hg. The values obtained previously for adult and fetal blood [3] at $[BE] = -9$ mEq/liter are included on this graph together with the values of Table I. This diagram makes apparent the following. At P_{CO_2} 30 mm Hg the fetal λ is significantly lower than the adult λ in an acid pH range, whereas fetal λ becomes significantly greater than the adult λ in an alkaline pH range. At P_{CO_2} 40 mm Hg no differences can be demonstrated between fetal and adult λ in acid pH range, but the fetal λ is also greater than the adult λ in an alkaline pH range. At P_{CO_2} 60 mm Hg, the fetal and adult λ are not significantly different at each pH level.

Figure 2 represents the mean change of $[BE]$ ($\Delta[BE]$) after the addition of known amounts of base to 10 fetal blood samples *in vitro*, at an initial $[BE]$ of either 0 or +10 mEq/liter. The $\Delta[BE]$ was read on the base excess scale of the Siggaard-Andersen nomogram [9]. Figure 2 indicates that the $\Delta[BE]$ is lower than the true amount of base added. This relation can be expressed by the following equation: $\Delta[BE]$ (milliequivalents per liter) = $0.88 \cdot$ base added (milliequivalents per liter). The coefficient of 0.88 is valid for an initial $[BE]$ of either 0 or +10 mEq/liter.

The relation between the pH and the Bohr effect is shown in Figure 3 for adult and fetal blood at P_{CO_2} 30, 40, and 60 mm Hg. As in Figure 1, the adult and fetal values for $[BE] = -9$ mEq/liter are included from the

previous study [3]. The values obtained at three different $[BE]$ at constant P_{CO_2} were linked by dashed straight lines (---). This graph demonstrates several differences between the adult and the fetal Bohr effect. (1) At any constant P_{CO_2} , the adult $-\Delta H^+/\Delta O_2$ ratio decreases regularly when the pH increases. In fetal blood, the $-\Delta H^+/\Delta O_2$ ratio first increases when the pH increases to a given value for each P_{CO_2} . A further increase of pH then results in a decrease of the ratio.

Fig. 2. Relation between the change of BE obtained on the Siggaard-Andersen nomogram and the actual amount of base added in fetal blood. ● $[BE]$ initial: 0 mEq/liter; ◇ $[BE]$ initial: +10 mEq/liter.

Fig. 3. Relation between the Bohr effect and the pH at three different P_{CO_2} in adult (---) and fetal (—) blood.

(2) At $[\text{BE}] = -9$ mEq/liter, the fetal and adult Bohr effects are similar at each P_{CO_2} , whereas at $[\text{BE}] = 0$ and $+10$ mEq/liter, the fetal ratios are significantly greater than the adult ratios at each P_{CO_2} (cf. Table I). (3) At any constant pH, adult Bohr effect is greater at low P_{CO_2} than at high P_{CO_2} . In contrast, fetal Bohr effect at constant pH is lower at low P_{CO_2} than at high P_{CO_2} , at least in the pH range below 7.4.

Discussion

Metabolic Buffer Value

Although the fetal λ value was similar or even smaller than the adult λ in an acidotic range ($[\text{BE}] = -9$ mEq/liter) [3], it appears from the present results that the fetal λ becomes significantly greater than the adult λ in alkaline conditions ($[\text{BE}] = +10$ mEq/liter). Figure 1 shows clearly the difference of evolution of the λ according to the pH in fetal blood compared with adult blood. This dissimilarity between adult and fetal λ in the alkaline range can be due to differences in both buffer systems of whole blood, *i.e.*, the bicarbonate-carbonic acid system or the noncarbonic buffers. The difference in fetal λ compared with adult λ observed at P_{CO_2} 60 mm Hg for a respective $[\text{BE}]$ of 0 and -3.4 mEq/liter may be an artifact, since the fetal plasma pH (pH_p) was greater than the adult pH_p (Table I). Thus, the concentration of plasma HCO_3^- is greater in fetal than in adult blood and this fact may explain the greater fetal λ . At a $[\text{BE}]$ of $+10$ mEq/liter, this explanation cannot account for the greater λ in fetal blood than in adult blood observed at P_{CO_2} 30 and 40 mm Hg. As pH_p is the same in adult and fetal blood at each P_{CO_2} , the plasma bicarbonate concentration is identical in both. A greater amount of hemoglobin (Hb) in fetal blood than in adult blood could be responsible for the greater fetal λ , but as shown on Table I, the Hb concentration is lower in fetal blood than in adult blood because of the dilution caused by the alkalization. This difference in hematocrit is not sufficient to cause a blood HCO_3^- concentration greater in fetal than in adult blood. Because the carbonic buffers are not responsible for the difference between fetal λ and adult λ , a different quality of the noncarbonic buffers in fetal blood must be postulated. Inasmuch as the λ was measured on fully oxygenated blood, one can propose that the oxygenated fetal Hb (HbF) is a better buffer than the oxygenated adult Hb (HbA), at least at low P_{CO_2} in pH range above 7.5. The superior buffering capacity of HbF could be due to differences in the pK of some groups of the γ -globin

chain of HbF in regard to the β -globin chain of HbA. Differences in carbamino compounds do not seem to be involved, because Bauer and Schröder [5] determined that the same amount of CO_2 was bound to fetal HbO_2 as to adult HbO_2 . Studies of the buffer value of HbF in solutions at various P_{CO_2} and 2,3-diphosphoglycerate levels would be useful in order to explain the present results. Although the underlying causes of this high λ in fetal blood in alkaline conditions cannot be elucidated with the available data, a practical consequence arises from this difference between adult and fetal buffering properties. As shown on Figure 2, a discrepancy exists between the change of $[\text{BE}]$ obtained from the BE scale of the Siggaard-Andersen nomogram and the actual amount of base added, when the fetal blood is titrated in an alkaline pH range. The BE scale was constructed from values of adult blood [8]. Therefore, the greater λ in fetal blood compared with adult blood in alkaline range results in nomogram $[\text{BE}]$ values lower than the true $[\text{BE}]$. As previously reported [3], the BE scale is accurate for fetal blood in an acid pH range.

Bohr Effect

The present study demonstrates some differences between the Bohr effects of fetal and adult whole blood, which were similar in an acid pH range. Indeed as in adult blood, the fetal $-\Delta\text{H}^+/\Delta\text{O}_2$ ratio is pH- and P_{CO_2} -dependent but the shapes of the dependence are unlike in fetal and in adult blood (Fig. 3). It may be emphasized that the adult values are, in the range of pH studied, in good agreement with the results obtained under similar conditions by Siggaard-Andersen [10]. Thus, the magnitude of the Bohr effect in adult whole blood and its relation with P_{CO_2} and pH could be explained at least qualitatively by the effects of the carbamino compounds in Hb solutions [7] and by the simultaneous effects of 2,3-diphosphoglycerate reported by several authors in HbA solutions or in whole blood [4, 10].

In fetal blood the interpretation of the present results in accordance with the facts observed in HbF solutions or in fetal erythrocytes is difficult. According to the work of Antonini *et al.* [1] on Hb solutions in absence of CO_2 , the $-\Delta\text{H}^+/\Delta\text{O}_2$ ratio is the same in HbF solutions as in HbA solutions, at least for the pH range from 6.5 to 9.0. Bauer and Schröder [5] demonstrated that the amount of oxylabile carbamino compounds is higher in fetal than in adult erythrocytes at intraerythrocytic pH (pH_i) from 7.0 to 7.5 [5]. Thus the fetal $\Delta\text{H}^+/\Delta\text{O}_2$ ratio should be smaller than the

adult ratio in this range of pH_i , at least when the Bohr effect is measured inside the erythrocyte. When the determinations are made in whole blood at constant pH_p , the variations of pH_i which occur during oxygenation influence the measured values [6, 10]. Indeed, a decrease of pH_i at constant pH_p means that a given amount of protons has been titrated inside the erythrocyte. This amount depends on the buffer value of the erythrocyte content and on the pH_i according to the classic equation: $\Delta\text{H}^+ = \Delta\text{pH}_i \cdot \text{buffer value } (\lambda)$. According to the data of Bauer and Schröder [5], the pH_i -decrease occurring during oxygenation at a constant pH_p of 7.4 and at a P_{CO_2} of 40 mm Hg, is 0.047 for adult erythrocytes and 0.038 for fetal erythrocytes. For an intraerythrocytic buffer value which corresponds to the conditions mentioned above and an Hb concentration of 10 mm/liter blood, it can be calculated that these pH_i changes correspond to 0.21 mEq $\text{H}^+/\text{mm O}_2$ in adult erythrocytes and to 0.17 mEq $\text{H}^+/\text{mm O}_2$ in fetal erythrocytes. These intracellular amounts of protons are not "seen" by measurements of the $\Delta\text{H}^+/\Delta\text{O}_2$ ratio in whole blood at constant pH_p . Thus, the pH_i shift caused by oxygenation at constant pH_p influences largely the $\Delta\text{H}^+/\Delta\text{O}_2$ ratio obtained in whole blood and could account by itself for the discrepancy between the adult whole blood Bohr effect and the HbA solutions Bohr effect. These figures are perhaps not strictly accurate because of the uncertainties associated with the pH_i determinations and the buffer value of the Hb molecule inside the erythrocyte. Nevertheless, it is evident that the value of the Bohr effect obtained by measurements in whole blood does not permit inferences about the quantitative role of the various intraerythrocytic components on the Bohr effect.

However, this empiric $\Delta\text{H}^+/\Delta\text{O}_2$ ratio obtained in whole blood has a practical implication, because it represents the magnitude of the correction needed when partly desaturated blood is analyzed by the Astrup method [9]. According to the present results, this correction depends on the P_{CO_2} and on the pH_p , the extreme values being between 0.33 and 0.50 mEq $\text{H}^+/\text{mm O}_2$ in the ranges studied. When an unique correction factor of 0.50 mEq $\text{H}^+/\text{mm O}_2$ is used as proposed by Siggaard-Andersen for adult blood [9], the maximal error involved is 0.17 mEq $\text{H}^+/\text{mm O}_2$, in terms of P_{CO_2} at the most 2 mm Hg at an initial oxygen saturation level (S_{O_2}) of 60% and a Hb concentration of 10 mm/liter. This error is of minor importance for the determinations of the blood acid-base status in

clinical routine. However, when a scientific aim is pursued, it may be useful to remember that the fetal $\Delta\text{H}^+/\Delta\text{O}_2$ ratio differs from the adult one under some conditions.

Summary

This study shows that, at $[\text{BE}]$ of 0 and +10 mEq/liter, the fetal metabolic buffer value and the fetal Bohr effect differ from the adult ones. The causes and the practical implications of these differences between fetal and adult blood are discussed.

References and Notes

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