EFFECTS OF ACID–BASE VARIABLES ON IN VITRO 
HEPATIC METABOLISM IN RAINBOW TROUT

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SUMMARY

The effects of hypercapnia (1% CO₂), and the independent effects of changes in extracellular pH (pHe), P₈O₂ and [HCO₃⁻] on intracellular pH (measured by the DMO method) and lactate metabolism (measured by utilization of ¹⁴C-labelled lactate), were examined in rainbow trout hepatocytes in vitro. Simulated uncompensated hypercapnia (high P₈O₂, low pHe, moderately increased [HCO₃⁻]) led to a substantial depression in the production of CO₂ (44%) and glucose (51%) from lactate. In simulated compensated hypercapnia (high P₈O₂, normal pHe, high [HCO₃⁻]), metabolism was still significantly inhibited (18–33%). Subsequent multifactorial design experiments determined that variations in P₈O₂, pH and [HCO₃⁻] independently affected metabolism; increased P₈O₂ and decreased pH inhibited metabolism, but increased [HCO₃⁻] stimulated metabolism. These results are interpreted in terms of the effects of acid–base variables on enzymatic and transport pathways, and the possible causes of decreased hepatic glycogen stores during in vivo hypercapnia are discussed.

INTRODUCTION

A variety of conditions cause acid–base disturbances in fish (e.g. exercise, hypercapnia, hypoxia, etc.). These disturbances are often complex, causing simultaneous changes in blood and tissue pH, carbon dioxide tension (P₈O₂) and bicarbonate concentration ([HCO₃⁻]). Because of the complexity of these responses, in a related paper we attempted to study respiratory acidosis in isolation by exposing trout to acute and chronic hypercapnia (Perry, Walsh, Mommsen & Moon, 1988). It was noted that external hypercapnia (1% CO₂) induced substantial effects on

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extracellular and intracellular acid–base status, and on metabolite concentrations. In particular, acute (1 h) hypercapnia depressed hepatic intracellular pH (pHi) and glycogen content, but these variables returned to normal during continued hypercapnic exposure (48 h). The effect of hypercapnia on hepatic pHi in rainbow trout is relatively well understood from a mechanistic viewpoint; intracellular acidosis is partially compensated for by Na⁺/H⁺ exchange (Walsh, 1986). However, the effects of acid–base disturbance on hepatic metabolism remain poorly described in fish, and possible mechanisms underlying such metabolic effects, as for example our observations of depressed glycogen content during hypercapnia in vivo, have not been examined.

There are several possible mechanisms causing the observed in vivo effects of hypercapnia on metabolism (Perry et al. 1988). Since hypercapnia is itself a complex state consisting of changes in pH, Pco₂, and [HCO₃⁻], any combination of changes in these three variables might initiate metabolic effects. Alternatively, changes in a single variable, such as hepatic pHi, could modulate metabolism directly. Finally, hypercapnia could affect hepatic metabolism indirectly by endocrinological or other systemic means. An evaluation of the relative roles of these potential mechanisms is extremely difficult in vivo. Therefore, we have utilized isolated hepatocyte suspensions (Moon, Walsh & Mommsen, 1985) as a model metabolic system. In this simplified in vitro system, whole-organism endocrinological effects are controlled, and the consequences of complex physiological states, such as hypercapnia, on metabolism can be analysed by multifactorial experimental design.

In this paper we report that acute hypercapnia substantially depresses in vitro hepatocyte metabolism of lactate to CO₂ or glucose. A subsequent multifactorial analysis of the acid–base variables indicates that hepatic metabolism is inhibited independently by increased Pco₂ or [H⁺] and stimulated by elevated [HCO₃⁻]. In a related paper (Mommsen, Walsh, Perry & Moon, 1988) we report on the role of catecholamines, which change during hypercapnia, in hepatic acid–base status and metabolism.

**MATERIALS AND METHODS**

*Experimental animals, acclimation regimen, isolation of hepatocytes, solutions and chemicals*

Rainbow trout were obtained from the Thistle Springs Trout Farm (Ashton, Ontario) in July and August 1986, and held in aquaria (300 l) supplied with flowing dechlorinated Ottawa tapwater at 13 ± 1°C (photoperiod = 12h:12h, L:D) for up to 1 month prior to experiments. Fish were fed a commercial trout diet (Purina) to satiation on alternate days.

Hepatocytes were isolated by the collagenase perfusion methods of French, Mommsen & Hochachka (1981) as adapted by Walsh (1986). In many cases, hepatocytes were isolated from two fish, and the hepatocytes were pooled to yield sufficient cells for the experiments. Each pooled preparation was considered as one sample for statistical purposes. Cell viability was ensured by using only preparations
Effects of hypercapnia on hepatocyte metabolism

which excluded trypan blue, or which had ATP contents, glycogen contents and metabolic rates similar to those obtained in previous studies. To obtain precise control of the P CO₂, [HCO₃⁻] and pH of the suspension media, the following protocol was used to prepare incubation solutions. Each day, solutions were prepared by diluting a 10-fold stock of Hank's salts, pH 7.5 (as in Walsh, 1986, except that [Hepes] = 5 mmol l⁻¹), and then pre-equilibrating with the appropriate gas mixture by bubbling through a plastic 'air-stone' for 30 min. CaCl₂ (1 mmol l⁻¹) and bovine serum albumin (4%, fatty acid free, Sigma no. A7030) were added and the pH was readjusted upwards with NaOH (0.1–1 mol l⁻¹). These solutions remained covered and sat blanketed with gas at 15°C during the hepatocyte isolation (30–60 min). Immediately before these solutions were used in the final washing of the hepatocytes, an appropriate amount of solid NaHCO₃ was added. The solutions were then re-equilibrated with the appropriate gas mixture, and the pH was checked again. Provided that solutions, with or without cells, were maintained on the flow-through system described below, this procedure yielded appropriate and stable pH and total carbon dioxide (CO₂) values. Pre-analysed precision gas mixtures (CO₂ in air) were purchased from Air Products (Ottawa, Ontario). Compressed air (medical grade, Air Products) was used for nominal carbon dioxide content experiments. Biochemicals were purchased from Sigma (St Louis) and isotopes were purchased from New England Nuclear (Boston). All other chemicals were reagent grade.

Measurement of metabolism

Carbon dioxide and glucose production were measured from [U-¹⁴C]L-lactate according to the methods of French et al. (1981) with the following modifications. For acceptable control of acid–base variables, vials with cell suspensions were continuously gassed as open systems. This required a modified CO₂-trapping system. The primary CO₂ trap was a filter paper soaked with hyamine hydroxide in a centre-well suspended over the cells; this trap absorbed CO₂ from suspensions that were acidified with perchloric acid at the end of an experiment. Additionally a length of polyethylene tubing (Clay-Adams, PE50) connected the incubation vial to the bottom of a vial filled with a mixture of 1 ml Carbotrap II (Baker) and 1 ml ethanol. The calculated CO₂-trapping ability of this system far exceeded the possible CO₂ available based upon the carbon dioxide content of the gas and the flow rate. In a typical experiment, more than 75% of the total radioactive CO₂ appeared in the primary trap. Combined radioactive CO₂ from both traps was used to calculate total CO₂ production. Total [lactate] was 1 mmol l⁻¹, 0.1–0.2 μCi of radioactive lactate was used, and the total volume of cells and medium was 1 ml in a 20-ml reaction vial.

Measurement of CCO₂, pH and pHi

The DMO (5,5-dimethyl-2,4-oxazolidinedione) method as previously applied to rainbow trout hepatocytes (Walsh, 1986) was used to measure pHi. pH was measured with a Radiometer PHM73 acid–base analyser and microcapillary pH electrode thermostatted to 15°C. C CO₂ was measured on a Corning 965 CO₂ analyser. Carbon dioxide partial pressures were calculated using measured C CO₂ and pHe, and
the equations and constants of Boutilier, Heming & Iwama (1984). Flasks in parallel to the metabolic experiments were set up for these acid–base measurements.

**Experimental design**

Metabolism of lactate to carbon dioxide and glucose, pHe and pHi, and suspension medium C CO₂ were measured after 1 h of incubation in the appropriate medium and gas phase at 15°C. The following experiments were performed.

(1) Hepatocytes were exposed to normal, hypercapnic and compensated hypercapnic media to simulate the control, acute exposure (decreased pHe, increased P CO₂, slightly increased [HCO₃⁻]) and compensated (control pHe, increased P CO₂, increased [HCO₃⁻]) to 1% CO₂ states of prior in vivo studies (Heisler, 1984; Perry et al. 1988). The compensated treatment effectively increased [HCO₃⁻] and carbon dioxide tension at constant pHe.

(2) Hepatocytes were exposed to normal, low, high and 'ultrahigh' pHe and [HCO₃⁻] treatments to separate the effects of the three variables observed in the first experiment. These treatments held carbon dioxide tension constant while [HCO₃⁻] and pHe were varied.

(3) Hepatocytes were exposed to normal and variable pHe (with nominal [HCO₃⁻]), and ultrahigh pHe and [HCO₃⁻] to further delineate the mechanisms involved in the first two experiments. These treatments held carbon dioxide tension and [HCO₃⁻] constant as pHe was varied.

(4) Hepatocytes were transferred from control conditions to hypercapnic medium, ultrahigh bicarbonate medium, and high and low pHe in the nominal absence of HCO₃⁻, and medium acid–base parameters and pHi were measured at 1, 5, 10, 15, 30 and 60 min after transfer. These experiments were designed to determine how rapidly acid–base disturbances and adjustments took place during the standard 1-h incubations in the first three experiments.

**Statistics**

All values are reported as means ± 1 s.e. Each measurement for each preparation was performed in duplicate, and these values were averaged to give a single sample value. Significant differences of means at the P<0.05 level were determined using a Model III, two-factor analysis of variance and Student–Newman–Keuls test. This test, which is analogous to the paired t-test used for comparing two treatment groups, is appropriate for the randomized block, multifactorial design of our experiments; in this test, between-preparation variation (which is significant in many cases) is assigned as the random second factor (Zar, 1974). In one case a linear regression and analysis of slopes was performed by the t-test (Zar, 1974).

**RESULTS**

**Hepatocyte viability**

Hepatocytes were judged viable by several measures. ATP contents were similar to in vivo freeze-clamped values (i.e. >1.6 μmol g⁻¹ cell wet mass), and pHi values
Effects of hypercapnia on hepatocyte metabolism

were consistent with other studies (Tables 1–3) (Walsh, 1986). Exclusion of trypan blue was high (>95 %), and metabolic rates were similar to those obtained previously (Tables 1–3) (e.g. French et al. 1981). The flow-through gassing experimental design allowed precise control of acid–base variables, and appeared to have no adverse effects on the actual measurements of pHi and metabolic rates (Tables 1–3).

In vitro modelling of acute and compensated hypercapnia

Compared to normocapnic conditions, exposure of hepatocytes to hypercapnic conditions, similar to acute (1 h) exposure to 1% CO₂ in vivo, caused similar depressions in pHi (0.18–0.25 units; Perry et al. 1988; Table 1), and substantial depressions in CO₂ and glucose production from lactate (44% and 51 %, respectively; Table 1). Hepatocytes exposed to conditions designed to mimic compensated hypercapnia had normal pHi values (Table 1), but still exhibited somewhat depressed rates of CO₂ and glucose production from lactate (18% and 33%, respectively; Table 1). Since pHe and pHi were normal in the compensated treatment, the data suggest that a large share of the effect of hypercapnia on metabolism is due to changes in pHe and/or pHi. However, it is clear that changes in Pₜᵣₑ₉ and/or [HCO₃⁻] also affect metabolism (Table 1).

Effects of variable pHe and [HCO₃⁻] at constant Pₐ₉

To determine the mechanisms of the Pₜᵣₑ₉ and/or HCO₃⁻ inhibition observed above, a second experiment was performed in which Pₜᵣₑ₉ was held constant, and pHe and [HCO₃⁻] were varied over a wide range of values. This combination of variables had a significant effect on metabolism and pHi (Table 2). Increasing pHe and [HCO₃⁻] at constant Pₜᵣₑ₉ enhanced rates of CO₂ and glucose production from lactate (Table 2).

Table 1. Effects of hypercapnia and compensated hypercapnia on lactate metabolism and intracellular pH in rainbow trout hepatocytes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normocapnia</th>
<th>Hypercapnia</th>
<th>Compensated</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ production* (µmol g⁻¹ h⁻¹)</td>
<td>3.23 ± 0.26</td>
<td>1.80 ± 0.21</td>
<td>2.66 ± 0.25</td>
</tr>
<tr>
<td>Glucose production* (µmol g⁻¹ h⁻¹)</td>
<td>0.86 ± 0.09</td>
<td>0.42 ± 0.06</td>
<td>0.58 ± 0.07</td>
</tr>
<tr>
<td>pHi</td>
<td>7.63 ± 0.03</td>
<td>7.38 ± 0.03</td>
<td>7.69 ± 0.03</td>
</tr>
<tr>
<td>pHe</td>
<td>7.89 ± 0.02</td>
<td>7.50 ± 0.02</td>
<td>7.96 ± 0.02</td>
</tr>
<tr>
<td>Total CO₂ (mmol l⁻¹)</td>
<td>5.3 ± 0.2</td>
<td>8.4 ± 0.5</td>
<td>25.2 ± 0.6</td>
</tr>
<tr>
<td>Pₜᵣₑ₉ (mmHg)</td>
<td>1.48 ± 0.09</td>
<td>6.06 ± 0.11</td>
<td>5.89 ± 0.21</td>
</tr>
</tbody>
</table>

Values are means ± S.E. (N = 6).

*All treatments significantly different, i.e. hypercapnia and compensated are significantly different from normocapnia and from each other, P < 0.05 (model III, two-factor ANOVA and Student–Newman–Keuls test).

† Hypercapnia significantly different from other treatments, P < 0.05 (model III, two-factor ANOVA and Student–Newman–Keuls test).
Table 2. Effect of variable pHe and [HCO$_3^-$] on lactate metabolism and intracellular pH in rainbow trout hepatocytes at constant $P_{CO_2}$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Low pHe and [HCO$_3^-$]</th>
<th>High pHe and [HCO$_3^-$]</th>
<th>Ultrahigh pHe and [HCO$_3^-$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$ production* (μmol g$^{-1}$h$^{-1}$)</td>
<td>3.90 ± 0.30</td>
<td>3.65 ± 0.47</td>
<td>4.76 ± 0.51</td>
<td>6.82 ± 0.73</td>
</tr>
<tr>
<td>Glucose production* (μmol g$^{-1}$h$^{-1}$)</td>
<td>1.37 ± 0.15</td>
<td>1.19 ± 0.14</td>
<td>1.53 ± 0.20</td>
<td>1.82 ± 0.24</td>
</tr>
<tr>
<td>pH$i$†</td>
<td>7.70 ± 0.01</td>
<td>7.44 ± 0.01</td>
<td>7.83 ± 0.04</td>
<td>8.21 ± 0.02</td>
</tr>
<tr>
<td>pHe</td>
<td>7.84 ± 0.01</td>
<td>7.41 ± 0.02</td>
<td>8.02 ± 0.01</td>
<td>8.41 ± 0.02</td>
</tr>
<tr>
<td>Total CO$_2$ (mmol l$^{-1}$)</td>
<td>5.0 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>9.4 ± 0.2</td>
<td>24.4 ± 0.4</td>
</tr>
<tr>
<td>$P_{CO_2}$ (mmHg)</td>
<td>1.57 ± 0.04</td>
<td>2.03 ± 0.12</td>
<td>1.95 ± 0.12</td>
<td>1.88 ± 0.09</td>
</tr>
</tbody>
</table>

Values are means ± s.e. ($N = 7$).

* There is a significant effect of pHe and [HCO$_3^-$], and ultrahigh is significantly different from the remaining treatments, which are not significantly different from each other, $P<0.05$ (model III, two-factor ANOVA and Student-Newman-Keuls test).

† All treatments are significantly different, $P<0.05$ (model III, two-factor ANOVA and Student-Newman-Keuls test).

(However, the average percentage decreases in metabolism for a 0·25 unit pH$i$ decrease (selected from ultrahigh and low pHe for comparison with pH$i$ change observed in Table 1) were only 15% and 11% for CO$_2$ and glucose production, respectively. These results, together with the results of the first experiment, demonstrate that increased carbon dioxide tension substantially inhibits metabolism of lactate to carbon dioxide and glucose, and that increases in pHe and pH$i$ and/or [HCO$_3^-$] slightly enhance metabolism of lactate.

Effect of variable pH$i$ at nominal carbon dioxide content

The effect of compensated hypercapnia in the first experiment and the results of the second experiment demonstrate that increased pHe and pH$i$ or [HCO$_3^-$] enhance lactate metabolism. To determine the contribution of each variable (pHe and pH$i$ versus [HCO$_3^-$]), a third experiment was designed in which pHe and pH$i$ were varied in nominal carbon dioxide content (Table 3). Variation of pHe over nearly 1 unit had a substantial effect on the production of CO$_2$ and glucose from lactate (respectively, 21% and 15% inhibition for a 0·25 unit decrease in pH$i$; Table 3). Note that the low pHe and high pHe data were statistically different (Table 3) and, in addition, that a plot of these data plus additional data obtained at pHe = 7.84 ± 0.003 and nominal total CO$_2$ = 0.5 ± 0.1 mmol l$^{-1}$ (CO$_2$ production = 3.07 ± 0.81 µmol g$^{-1}$h$^{-1}$, glucose production = 0.74 ± 0.21 µmol g$^{-1}$h$^{-1}$, $N = 3$) resulted in statistically significant regressions of metabolic rate vs pH$i$. The regression of CO$_2$ production rate vs pH$i$ yielded the equation $y = -17.52 + 2.72x$,
Effects of hypercapnia on hepatocyte metabolism

Table 3. Comparison of effects of pHe (hypocapnia = nominal bicarbonate) with effects of ultrahigh pHe and [HCO$_3^-$] on lactate metabolism and pHt in rainbow trout hepatocytes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normocapnia</th>
<th>Hypocapnia (pHe)</th>
<th>Ultrahigh pHe and [HCO$_3^-$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$ production*</td>
<td>3.50 ± 0.47</td>
<td>2.10 ± 0.26</td>
<td>3.83 ± 0.72</td>
</tr>
<tr>
<td>(µmol g$^{-1}$ h$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose production*</td>
<td>1.09 ± 0.20</td>
<td>0.73 ± 0.06</td>
<td>1.08 ± 0.11</td>
</tr>
<tr>
<td>(µmol g$^{-1}$ h$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pHt†</td>
<td>7.64 ± 0.04</td>
<td>7.35 ± 0.03</td>
<td>7.90 ± 0.03</td>
</tr>
<tr>
<td>pHe</td>
<td>7.84 ± 0.03</td>
<td>7.33 ± 0.05</td>
<td>8.20 ± 0.12</td>
</tr>
<tr>
<td>Total CO$_2$ (mmol l$^{-1}$)</td>
<td>4.7 ± 0.4</td>
<td>0.6 ± 0.2</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>$P_{CO_2}$ (mmHg)</td>
<td>1.47 ± 0.15</td>
<td>0.58 ± 0.16</td>
<td>0.21 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± s.e. ($N = 6$).

* There is a significant effect of treatments, and low pHe ≠ normocapnia = high pHe ≠ ultrahigh, $P<0.05$ (model III, two-way ANOVA and Student-Newman-Keuls test).

† There is a significant effect of treatments, and all values are significantly different, $P<0.05$ (model III, two-way ANOVA and Student-Newman-Keuls test).

$r = 0.5$, $N = 15$, and for glucose production rate vs pHt the equation was $y = -5.41 + 0.83x$, $r = 0.7$, $N = 15$; both of these slopes were significantly greater than zero ($t = 2.25$ and $3.58$, respectively, $P<0.05$). These results suggest that increased pHe and pHt ameliorate metabolism during periods of elevated $P_{CO_2}$ (e.g. compensated hypercapnia). Moreover, in these same experiments, when cells were exposed to 25 mmol l$^{-1}$ bicarbonate at high pHe and pHt, CO$_2$ and glucose production from lactate were further enhanced compared to the situation in cells exposed to high pHe and nominal [HCO$_3^-$] (Table 3). The enhancements of 42% and 33% for CO$_2$ and glucose production, respectively, are considerably greater than any enhancement attributable to the differences in pHe and pHt between these two treatments (i.e. 19% and 13%, respectively). (The effects of pHe and pHt were extrapolated using the regressions calculated above.) Furthermore, the enhancement occurred despite a slight increase in $P_{CO_2}$.

The results of the three experiments taken together indicate that (1) acute hypercapnia significantly depresses metabolism due to the combined effects of increased $P_{CO_2}$ and decreased pHe and/or pHt; (2) during compensation to hypercapnia, both increased pHe and/or pHt and increased [HCO$_3^-$] partially return metabolism to normal levels; (3) during compensation, increased $P_{CO_2}$ depresses metabolism and moderates the stimulation by increased pHe and/or pHt and [HCO$_3^-$].

Kinetics of pHt changes

When hepatocytes were rapidly transferred from normal suspension medium to hypercapnic, ultrahigh, low and high pHe (with nominal [HCO$_3^-$]) media, pHt
adjustments were rapid. Final steady-state pHi was achieved within 1–5 min of transfer.

**DISCUSSION**

Exposure of rainbow trout hepatocytes *in vitro* to acute hypercapnic conditions (low pHe, high PCO₂ and slightly increased [HCO₃⁻]), similar to those measured *in vivo* by Perry *et al.* (1988), caused a substantial depression of pHi and metabolism of lactate to CO₂ and glucose (Table 1). When hepatocytes were exposed *in vitro* to conditions designed to mimic compensated hypercapnia (normal pHe, high PCO₂, high [HCO₃⁻]), pHi was restored to control values but lactate metabolism remained significantly depressed (Table 1). These responses were analysed further by two multifactorial design experiments. In the first, carbon dioxide partial pressure was held constant as pHe and [HCO₃⁻] were varied simultaneously. The results of the first two experiments taken together clearly indicate that increasing carbon dioxide tension, independent of pH effects, markedly depresses metabolism. In a third experiment, pHe and pHi were varied, at nominal carbon dioxide content, and were shown to have a significant effect on metabolism independent of carbon dioxide content ([HCO₃⁻]); increasing pHe and pHi increased metabolic rates (Table 3). Furthermore, when bicarbonate concentration was increased at the highest pH, metabolism was further stimulated (Table 3). Analysis of all three experiments demonstrates that increased pHe and/or pHi stimulates metabolism, as does increased [bicarbonate], but increased PCO₂ depresses metabolism (Tables 1–3). Returning to the experiments designed to model acute and compensated hypercapnia *in vivo* (Table 1), variation in PCO₂ has the most pronounced effect on metabolism, and the depressing effect of increased PCO₂ is slightly offset by stimulation of metabolism by increased pH and [HCO₃⁻].

What are the possible mechanisms of hypercapnic depression of metabolism, and the independent effects of the three variables, pH, PCO₂ and [HCO₃⁻]? In hypercapnia, hepatic pHi is markedly depressed (Table 1; Perry *et al.* 1988) as a result of changes in the independent variables PCO₂ and SID (the strong ion difference; Stewart, 1981). Rainbow trout hepatocyte pHi recovers rapidly (within 10 min) from intracellular acid-base disturbances caused by exposure to increased PCO₂ (i.e. 1·07 kPa) and [HCO₃⁻] at constant pHe (Walsh, 1986). However, at least *in vitro*, SID-based decreases in pHe appear to lead to rapid and chronic reductions in pHi (Table 3; Walsh, 1986). Thus both in the present study (Table 3) and a previous one (fig. 1 of Walsh, 1986) rainbow trout hepatocyte pHi markedly depends on pHe. Therefore, one important metabolic perturbant during acute hypercapnia appears to be pHi depression through SID-based changes, and these changes in pHi could directly modulate activities of key regulatory enzymes in the metabolism of lactate (e.g. pyruvate carboxylase, 2-oxoglutarate dehydrogenase, etc.). Alternatively, is it possible that changes in pHe somehow alter rates of metabolism indirectly by changing rates of lactate transport? Lactate transport has been shown to occur by passive diffusion in toadfish hepatocytes, and this process is pHe-insensitive (Walsh,
Effects of hypercapnia on hepatocyte metabolism

1987). If a similar system is present in trout hepatocytes, it is likely that the effects of pHe and pH on metabolism are truly pH effects. The mechanisms of lactate uptake by trout hepatocytes should be examined to resolve this question.

During compensation of hypercapnia, changes in [HCO\textsubscript{3}⁻] also affect lactate metabolism independently of pH or PCO\textsubscript{2} effects. Increased [HCO\textsubscript{3}⁻] enhances rates of production of both CO\textsubscript{2} and glucose from lactate (more so on a percentage basis for CO\textsubscript{2} production; Table 3). A potential explanation for enhanced glucose production is as follows. Pyruvate carboxylase is believed to be an important regulatory site for control of hepatic gluconeogenesis in mammals (Kraus-Friedman, 1984) and fish (Suarez & Hochachka, 1981). Since bicarbonate is a substrate for trout liver pyruvate carboxylase (K\textsubscript{m} = 3.2 mmolL\textsuperscript{-1}; Suarez & Hochachka, 1981), increased [HCO\textsubscript{3}⁻] may lead to increased flux through this step. Similarly, Robinson, Oei, Cheema-Dhadli & Halperin (1977) have demonstrated that bicarbonate enhances pyruvate dehydrogenase activity in rat kidney mitochondria. If the trout liver enzyme has similar sensitivities to [HCO\textsubscript{3}⁻], this molecular mechanism may account for our observations of bicarbonate-enhanced CO\textsubscript{2} production. An alternative explanation for enhancement of glucose production and lactate oxidation is that bicarbonate affects the appropriate mitochondrial transport systems. Effects of bicarbonate on citrate and phosphate transport by rat kidney mitochondria have been observed (Robinson \textit{et al.} 1977), but these processes have not been examined in fish mitochondria.

In addition to these pronounced effects of pH and/or pHe and [HCO\textsubscript{3}⁻] on hepatic metabolism, our studies are the first to demonstrate that changes in PCO\textsubscript{2} directly and independently affect metabolism. A possible mechanism for this effect is that CO\textsubscript{2} inhibits reactions in which it is a product (e.g., phosphoenolpyruvate carboxykinase in gluconeogenesis, and decarboxylations of the Krebs cycle) by mass action effects. Clearly, additional experiments are required to explain our observations more fully in terms of molecular mechanisms. Studies of enzyme kinetics using similar types of conditions, in which all three acid–base variables are well-controlled, could be used to test our hypotheses.

Can our \textit{in vitro} observations account for the observations by Perry \textit{et al.} (1988) of depressed hepatic glycogen levels during acute hypercapnia and subsequent recovery during chronic hypercapnia? The observed \textit{in vivo} changes could result from enhanced breakdown of glycogen, depressed synthesis of glucose/glycogen, or both. In this regard, Perry \textit{et al.} (1988) have demonstrated that glycogen phosphorylase is in the active form during both normocapnia and hypercapnia, and Mommsen \textit{et al.} (1988) did not observe enhanced breakdown of glycogen in \textit{in vitro} experiments designed to simulate these states. Experiments in the present study, however, clearly demonstrate a diminished capacity for gluconeogenesis in acute hypercapnia and even in compensated hypercapnia (Table 1), which would certainly contribute to decreased liver glycogen content. Interestingly, glucose synthesis continued to be depressed in compensated hypercapnia \textit{in vitro} (Table 1), whereas glycogen levels \textit{in vivo} were not as depressed during compensated hypercapnia (Perry \textit{et al.} 1988). This discrepancy between \textit{in vitro} and \textit{in vivo} experimental results might reflect a...
systemic (hormonally?)-mediated increase in gluconeogenic capacity in vivo. This possibility is considered further by Mommsen et al. (1988).

The present studies were initiated, in part, to dissect the complex response of fish to acid–base disturbances including exhaustive exercise. During recovery from exhaustive exercise in rainbow trout, liver intracellular pH is depressed by about 0·2 units, but recovers rapidly (within 30 min) and even increases by 0·15 units after 8 h (Milligan & Wood, 1986). Liver lactate concentration closely mirrors blood lactate concentration and slowly rises to a peak of about 22 mmol l⁻¹ at 2 h (Milligan & Wood, 1986). Liver glycogen content varies markedly, with a possible decrease at about 2 h post-exercise (these changes were not statistically significant due to large variability in the response; Milligan & Wood, 1986). The results of the present study and of Perry et al. (1988) suggest that the hypercapnia associated with exercise will act to depress liver glycogen levels at basal lactate levels (i.e. 1 mmol l⁻¹). However, the variable post-exercise response of liver glycogen noted in vivo (Milligan & Wood, 1986) suggests that other factors (e.g. increased [lactate]) may operate to maintain liver glycogen levels.

Finally, the results of this study demonstrate the importance of modelling in vitro experiments very closely on in vivo conditions, especially in the case of acid–base variables. For example, in a prior study by one of us, one preliminary observation, made when acid–base conditions were less strictly controlled, suggested that high [bicarbonate] decreased rates of lactate metabolism (Mommsen & Suarez, 1984). This preliminary conclusion is exactly opposite to that reached in the present study.

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Effects of hypercapnia on hepatocyte metabolism


