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

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SUMMARY

The effects of hypercapnia (1 % CO_2), and the independent effects of changes in extracellular pH (pHe), P_{CO_2} and [HCO_3^-] on intracellular pH (measured by the DMO method) and lactate metabolism (measured by utilization of ^{14}C-labelled lactate), were examined in rainbow trout hepatocytes *in vitro*. Simulated uncompensated hypercapnia (high P_{CO_2}, low pHe, moderately increased [HCO_3^-] led to a substantial depression in the production of CO_2 (44 %) and glucose (51 %) from lactate. In simulated compensated hypercapnia (high P_{CO_2}, normal pHe, high [HCO_3^-], metabolism was still significantly inhibited (18–33 %). Subsequent multifactorial design experiments determined that variations in P_{CO_2}, pH and [HCO_3^-] *independently* affected metabolism; increased P_{CO_2} and decreased pH inhibited metabolism, but increased [HCO_3^-] 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 (P_{CO_2}) and bicarbonate concentration ([HCO_3^-]). 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 % CO_2) 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, P_{CO_2} and $[HCO_3^-]$, 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 CO_2 or glucose. A subsequent multifactorial analysis of the acid–base variables indicates that hepatic metabolism is inhibited *independently* by increased P_{CO_2} or $[H^+]$ and stimulated by elevated $[HCO_3^-]$. 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 = 12 h:12 h, 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

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_{CO_2}, $[HCO_3^-]$ 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. $CaCl_2$ (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 $NaHCO_3$ 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 (C_{CO_2}) values. Pre-analysed precision gas mixtures (CO_2 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-^{14}C]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 CO_2-trapping system. The primary CO_2 trap was a filter paper soaked with hyamine hydroxide in a centre-well suspended over the cells; this trap absorbed CO_2 from suspensions that were acidified with perchloric acid at the end of an experiment. Additionally a length of polyethylene tubing (Clay-Adams, PE 50) 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 CO_2-trapping ability of this system far exceeded the possible CO_2 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 CO_2 appeared in the primary trap. Combined *radioactive* CO_2 from both traps was used to calculate *total* CO_2 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 C_{CO_2}, pHe 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. pHe was measured with a Radiometer PHM 73 acid–base analyser and microcapillary pH electrode thermostatted to 15°C. C_{CO_2} was measured on a Corning 965 CO_2 analyser. Carbon dioxide partial pressures were calculated using measured C_{CO_2} 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_{CO_2} 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_{CO_2}, slightly increased $[HCO_3^-]$) and compensated (control pHe, increased P_{CO_2}, increased $[HCO_3^-]$) to 1 % CO_2 states of prior *in vivo* studies (Heisler, 1984; Perry *et al.* 1988). The compensated treatment effectively increased $[HCO_3^-]$ and carbon dioxide tension at constant pHe.

(2) Hepatocytes were exposed to normal, low, high and 'ultrahigh' pHe and $[HCO_3^-]$ treatments to separate the effects of the three variables observed in the first experiment. These treatments held carbon dioxide tension constant while $[HCO_3^-]$ and pHe were varied.

(3) Hepatocytes were exposed to normal and variable pHe (with nominal $[HCO_3^-]$), and ultrahigh pHe and $[HCO_3^-]$ to further delineate the mechanisms involved in the first two experiments. These treatments held carbon dioxide tension and $[HCO_3^-]$ 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 HCO_3^-, 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 $P < 0{\cdot}05$ 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. $>1{\cdot}6\,\mu mol\,g^{-1}$ cell wet mass), and pHi values

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 % CO_2 *in vivo*, caused similar depressions in pHi (0·18–0·25 units; Perry *et al.* 1988; Table 1), and substantial depressions in CO_2 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 CO_2 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 P_{CO_2} and/or $[HCO_3^-]$ also affect metabolism (Table 1).

Effects of variable pHe and $[HCO_3^-]$ at constant P_{CO_2}

To determine the mechanisms of the P_{CO_2} and/or HCO_3^- inhibition observed above, a second experiment was performed in which P_{CO_2} was held constant, and pHe and $[HCO_3^-]$ 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 $[HCO_3^-]$ at constant P_{CO_2} enhanced rates of CO_2 and glucose production

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

Parameter	Experimental group		
	Normocapnia	Hypercapnia	Compensated
CO_2 production* (μmol g^{-1} h^{-1})	3·23 ± 0·26	1·80 ± 0·21	2·66 ± 0·25
Glucose production* (μmol g^{-1} h^{-1})	0·86 ± 0·09	0·42 ± 0·06	0·58 ± 0·07
pHi	7·63 ± 0·03	7·38 ± 0·03†	7·69 ± 0·03
pHe	7·89 ± 0·02	7·50 ± 0·02	7·96 ± 0·02
Total CO_2 (mmol l^{-1})	5·3 ± 0·2	8·4 ± 0·5	25·2 ± 0·6
P_{CO_2} (mmHg)	1·48 ± 0·09	6·06 ± 0·11	5·89 ± 0·21

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}*

Parameter	Treatment			
	Normal	Low pHe and [HCO_3^-]	High pHe and [HCO_3^-]	Ultrahigh pHe and [HCO_3^-]
CO_2 production* (μmol g^{-1} h^{-1})	3·90 ± 0·30	3·65 ± 0·47	4·76 ± 0·51	6·82 ± 0·73
Glucose production* (μmol g^{-1} h^{-1})	1·37 ± 0·15	1·19 ± 0·14	1·53 ± 0·20	1·82 ± 0·24
pHi†	7·70 ± 0·01	7·44 ± 0·01	7·83 ± 0·04	8·21 ± 0·02
pHe	7·84 ± 0·01	7·41 ± 0·02	8·02 ± 0·01	8·41 ± 0·02
Total CO_2 (mmol l^{-1})	5·0 ± 0·2	2·3 ± 0·1	9·4 ± 0·2	24·4 ± 0·4
P_{CO_2} (mmHg)	1·57 ± 0·04	2·03 ± 0·12	1·95 ± 0·12	1·88 ± 0·09

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).

(Table 2). However, the average percentage decreases in metabolism for a 0·25 unit pHi decrease (selected from ultrahigh and low pHe for comparison with pHi 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 pHi and/or [HCO_3^-] slightly enhance metabolism of lactate.

Effect of variable pHe 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 pHi or [HCO_3^-] enhance lactate metabolism. To determine the contribution of each variable (pHe and pHi *versus* [HCO_3^-]), a third experiment was designed in which pHe and pHi 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 pHi; 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* pHi. The regression of CO_2 production rate *vs* pHi yielded the equation $y = -17·52 + 2·72x$,

Table 3. *Comparison of effects of pHe (hypocapnia = nominal bicarbonate) with effects of ultrahigh pHe and [HCO_3^-] on lactate metabolism and pHi in rainbow trout hepatocytes*

Parameter	Normocapnia	Hypocapnia		Ultrahigh pHe and [HCO_3^-]
		Low pHe	High pHe	
CO_2 production* (μmol g^{-1} h^{-1})	3·50 ± 0·47	2·10 ± 0·26	3·83 ± 0·72	5·42 ± 0·64
Glucose production* (μmol g^{-1} h^{-1})	1·09 ± 0·20	0·73 ± 0·06	1·08 ± 0·11	1·44 ± 0·18
pHi†	7·64 ± 0·04	7·35 ± 0·03	7·90 ± 0·03	8·12 ± 0·03
pHe	7·84 ± 0·03	7·33 ± 0·05	8·20 ± 0·12	8·30 ± 0·06
Total CO_2 (mmol l^{-1})	4·7 ± 0·4	0·6 ± 0·2	1·6 ± 0·2	24·6 ± 0·3
P_{CO_2} (mmHg)	1·47 ± 0·15	0·58 ± 0·16	0·21 ± 0·05	2·48 ± 0·18

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* pHi 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 pHi 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 pHi, 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 pHi between these two treatments (i.e. 19% and 13%, respectively). (The effects of pHe and pHi 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 pHi; (2) during compensation to hypercapnia, both increased pHe and/or pHi 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 pHi and [HCO_3^-].

Kinetics of pHi changes

When hepatocytes were rapidly transferred from normal suspension medium to hypercapnic, ultrahigh, low and high pHe (with nominal [HCO_3^-]) media, pHi

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 P_{CO_2} and slightly increased $[HCO_3^-]$), similar to those measured *in vivo* by Perry *et al.* (1988), caused a substantial depression of pHi and metabolism of lactate to CO_2 and glucose (Table 1). When hepatocytes were exposed *in vitro* to conditions designed to mimic compensated hypercapnia (normal pHe, high P_{CO_2}, high $[HCO_3^-]$), 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 $[HCO_3^-]$ 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 ($[HCO_3^-]$); 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 P_{CO_2} depresses metabolism (Tables 1–3). Returning to the experiments designed to model acute and compensated hypercapnia *in vivo* (Table 1), variation in P_{CO_2} has the most pronounced effect on metabolism, and the depressing effect of increased P_{CO_2} is slightly offset by stimulation of metabolism by increased pH and $[HCO_3^-]$.

What are the possible mechanisms of hypercapnic depression of metabolism, and the independent effects of the three variables, pH, P_{CO_2} and $[HCO_3^-]$? In hypercapnia, hepatic pHi is markedly depressed (Table 1; Perry *et al.* 1988) as a result of changes in the independent variables P_{CO_2} 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 P_{CO_2} (i.e. 1·07 kPa) and $[HCO_3^-]$ 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,

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

During compensation of hypercapnia, changes in $[HCO_3^-]$ also affect lactate metabolism independently of pH or P_{CO_2} effects. Increased $[HCO_3^-]$ enhances rates of production of both CO_2 and glucose from lactate (more so on a percentage basis for CO_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 ($K_m = 3{\cdot}2\,mmol\,l^{-1}$; Suarez & Hochachka, 1981), increased $[HCO_3^-]$ 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 $[HCO_3^-]$, this molecular mechanism may account for our observations of bicarbonate-enhanced CO_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 *et al.* 1977), but these processes have not been examined in fish mitochondria.

In addition to these pronounced effects of pHi and/or pHe and $[HCO_3^-]$ on hepatic metabolism, our studies are the first to demonstrate that changes in P_{CO_2} directly and independently affect metabolism. A possible mechanism for this effect is that CO_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 *in vitro* observations account for the observations by Perry *et al.* (1988) of depressed hepatic glycogen levels during acute hypercapnia and subsequent recovery during chronic hypercapnia? The observed *in vivo* changes could result from enhanced breakdown of glycogen, depressed synthesis of glucose/glycogen, or both. In this regard, Perry *et al.* (1988) have demonstrated that glycogen phosphorylase is in the active form during both normocapnia and hypercapnia, and Mommsen *et al.* (1988) did not observe enhanced breakdown of glycogen in *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 *in vitro* (Table 1), whereas glycogen levels *in vivo* were not as depressed during compensated hypercapnia (Perry *et al.* 1988). This discrepancy between *in vitro* and *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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REFERENCES

BOUTILIER, R. G., HEMING, T. A. & IWAMA, G. K. (1984). Physicochemical parameters for use in fish respiratory physiology. In *Fish Physiology*, vol. XA (ed. W. Hoar & D. Randall), pp. 401–429. New York: Academic Press.

FRENCH, C. J., MOMMSEN, T. P. & HOCHACHKA, P. W. (1981). Amino acid utilization in isolated hepatocytes from rainbow trout. *Eur. J. Biochem.* **113**, 311–317.

HEISLER, N. (1984). Acid–base regulation in fish. In *Fish Physiology*, vol. XA (ed. W. Hoar & D. Randall), pp. 315–401. New York: Academic Press.

KRAUS-FRIEDMANN, N. (1984). Hormonal regulation of hepatic gluconeogenesis. *Physiol. Rev.* **64**, 170–259.

MILLIGAN, C. L. & WOOD, C. M. (1986). Tissue intracellular acid–base status and the fate of lactate after exhaustive exercise in the rainbow trout. *J. exp. Biol.* **123**, 123–144.

MOMMSEN, T. P. & SUAREZ, R. K. (1984). Control of gluconeogenesis in rainbow trout hepatocytes: role of pyruvate branchpoint and phosphoenolpyruvate–pyruvate cycle. *Molec. Physiol.* **6**, 9–18.

MOMMSEN, T. P., WALSH, P. J., PERRY, S. F. & MOON, T. W. (1988). Interactive effects of catecholamines and hypercapnia on glucose production and intracellular pH in isolated trout hepatocytes. *Gen. comp. Endocr.* (in press).

MOON, T. W., WALSH, P. J. & MOMMSEN, T. P. (1985). Fish hepatocytes: a model metabolic system. *Can. J. Fish. aquat. Sci.* **42**, 1772–1782.

PERRY, S. F., WALSH, P. J., MOMMSEN, T. P. & MOON, T. W. (1988). Metabolic consequences of hypercapnia in the rainbow trout, *Salmo gairdneri*: beta-adrenergic effects. *Gen. comp. Endrocr.* (in press).

ROBINSON, B. H., OEI, J., CHEEMA-DHADLI, S. & HALPERIN, H. L. (1977). Regulation of citrate transport and pyruvate dehydrogenase in rat kidney cortex mitochondria by bicarbonate. *J. biol. Chem.* **252**, 5661–5665.

STEWART, P. A. (1981). *How to Understand Acid–Base*. New York: Elsevier.

SUAREZ, R. K. & HOCHACHKA, P. W. (1981). Pyruvate carboxylase from rainbow trout liver. *J. comp. Physiol.* **143**, 281–288.

WALSH, P. J. (1986). Ionic requirements for intracellular pH regulation in rainbow trout hepatocytes. *Am. J. Physiol.* **250** (*Reg. integr. comp. Physiol.* **19**), R24–R29.

WALSH, P. J. (1987). Lactate uptake by toadfish hepatocytes: passive diffusion is sufficient. *J. exp. Biol.* **130**, 295–304.

ZAR, J. H. (1974). *Biostatistical Analysis*. Englewood Cliffs: Prentice-Hall.