MECHANISMS OF ACID-BASE ADJUSTMENT IN DOGFISH (*SCYLIORHINUS STELLARIS*) SUBJECTED TO LONG-TERM TEMPERATURE ACCLIMATION

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SUMMARY

Specimens of Larger Spotted Dogfish (*Scyliorhinus stellaris*) were acclimated to a range of temperatures for 22–28 days. The relationships of pH to acclimation temperature in the extracellular space and in three intracellular compartments were essentially the same as previously observed in 24 h acclimated dogfish (Heisler, Weitz & Weitz, 1976).

In dogfish subjected to elevated inspired P_{CO_2} during acclimation to low temperature, there was an inversion in the relationship of acclimation temperature to plasma P_{CO_2}, and in that to plasma bicarbonate, whereas the relationships to pH values were unchanged.

It is concluded that the temperature adaptation of the acid-base parameters is complete after 24 h of temperature acclimation. At least the following mechanisms are involved in the adjustment of pH with changes of temperature: changes of P_{CO_2}, changes of buffer pK values, and transmembrane transfer of bicarbonate. Apparently only one of them, the transmembrane transfer of bicarbonate, can be modulated in a sufficient range and is responsible for the final pH adjustment.

INTRODUCTION

The acid-base regulation observed in the Larger Spotted Dogfish with change of acclimation temperature is peculiar in comparison with that observed in air-breathing ectothermal vertebrates (Heisler *et al.* 1976). Most of the studied air-breathing ectotherms maintain a constant relative alkalinity (i.e. a constant $[OH^-]/[H^+]$ ratio) and thus a constant dissociation of imidazole in the extracellular compartment, by adjustment of P_{CO_2} while the bicarbonate concentration remains constant (Baumgardner & Rahn, 1967; Howell *et al.* 1970; Reeves, 1972; Crawford & Gatz, 1974; Jackson & Kagen, 1976). Also, intracellular pH was found in two air-breathing species to be maintained more or less at a constant relative alkalinity (Reeves & Wilson, 1970; Malan & Reeves, 1973; Malan, Wilson & Reeves, 1976). In dogfish, acclimation to a new temperature for 24 h has been shown to result in a change of extracellular pH

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that was produced by changes of both P_{CO_2} and extracellular bicarbonate concentration (Heisler *et al.* 1976), and was smaller than that to be expected on the basis of the rule of constant relative alkalinity (Rahn, 1966, 1967). Also, in two of three studied dogfish cell types pH changed with temperature much more (red muscle) or much less (heart muscle) than expected according to the rule of constant relative alkalinity.

There are, however, some doubts whether the observations of Heisler *et al.* were made in equilibrium conditions or whether the differences observed in comparison with other species can be attributed to a non-steady-state due to the relatively short acclimation period of only 24 h. To resolve this problem, we here report the effect upon acid-base parameters of acclimation for the relatively long time period, of more than 3 weeks, to three different temperatures.

In this paper we also investigated the relative importance of changes in P_{CO_2} and bicarbonate concentration in pH regulation. For this we raised plasma P_{CO_2}, by elevation of inspired P_{CO_2}, in dogfish acclimated to low temperature.

MATERIALS AND METHODS

Specimens of Larger Spotted Dogfish (*Scyliorhinus stellaris*) were caught in the Bay of Naples and kept at temperatures of 14–17 °C in large, well aerated seawater tanks for two weeks to several months prior to the experiments. Then groups of 4–6 were acclimated to the desired temperatures for 22–28 days in thermostatted 400 l PVC tanks. The tanks were flushed with fresh sea water at a rate of 40–80 l/h and the water was bubbled with air or air/CO_2 mixture at a rate of 10–15 l/min. Circulation of the water in the tanks was provided by mixing pumps immersed into the water of the tanks (20–30 l/min). The animals were fed daily during the adaptation time. Two experimental series were conducted:

1. Long-term acclimation

Groups of four juvenile (0·25–1 kg body weight) and five adults (1·8–3·2 kg) were acclimated to 10, 15 or 20 °C. The tanks were aerated with room air. (Series performed during April and May 1976.)

2. Long-term acclimation with hypercapnia at low temperature

Six adults (1·7–3·5 kg) were acclimated to 20 °C, in a tank aerated with room air. Five others (1·9–3·4 kg) were acclimated to 10 °C, in a tank aerated with a mixture of air and CO_2. The CO_2 concentration of the mixture was adjusted by an electronic regulation circuit, consisting of a P_{CO_2}-electrode in the tank water, a high impedance amplifier, a comparator and a solenoid valve, to produce a constant P_{CO_2} in the tank of about 1·9 mmHg. The regulation circuit was recalibrated twice a day and test measurements in the water showed that P_{CO_2} varied less than ±0·2 mmHg. (Series performed during April and May 1978.)

Two days prior to the measurements indwelling catheters were placed in the dorsal aorta (see Heisler *et al.* 1976) and [¹⁴C]DMO (5,5-dimethyl-2,4-oxazolidinedione) and [³H]inulin were injected for the determination of intracellular pH (Waddell & Butler, 1959) in white muscle (from fillets of the medium third of the body length).
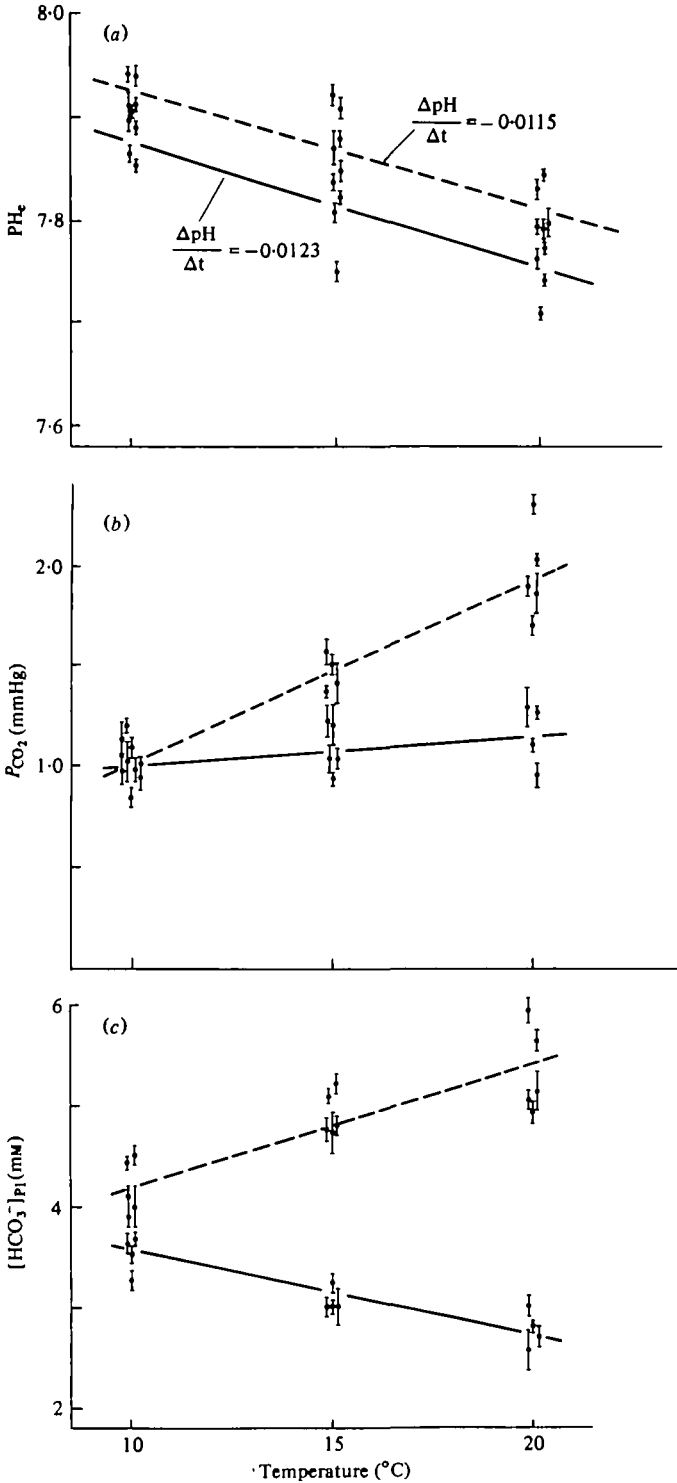
Fig. 1. Extracellular pH (*a*), arterial P_{CO_2} (*b*) and arterial bicarbonate concentration (*c*) in long-term temperature-acclimated juvenile (solid lines, closed circles) and adult dogfish (dashed lines, open circles) as a function of environmental temperature. Each circle represents the mean of 4–6 measurements in an individual animal ±2 S.E.

Table 1. *Fractional water content (F_{H_2O}) and fractional extracellular volume (Q_e)*
of white, red and heart muscle (mean $\pm$ 2 s.e., n = number of determinations)

	F_{H_2O}	Q_e	n
White muscle	0·779 ± 0·003	0·112 ± 0·004	810
Red muscle	0·781 ± 0·004	0·252 ± 0·006	405
Heart muscle	0·780 ± 0·008	0·336 ± 0·018	106

red muscle (from the side line red muscle layer, hind two thirds of body length) and heart muscle. Measurements and calculations of plasma pH, P_{CO_2}, bicarbonate concentration, and intracellular pH were carried out as described earlier (Heisler *et al.* 1976).
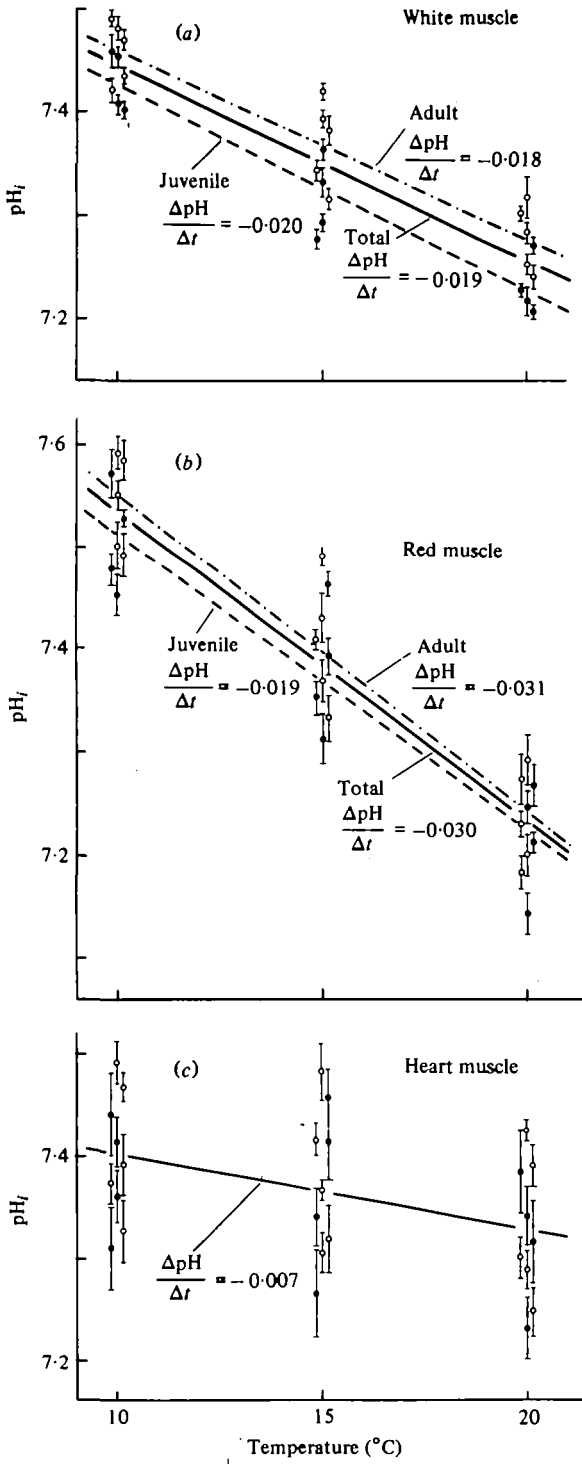
RESULTS

1. *Long-term acclimation*

The mean extracellular pH values determined in 12 juvenile and 15 adult specimens acclimated to 10, 15 and 20 °C are presented in Fig. 1 (*a*). The values for change of pH with acclimation temperature ($\Delta pH/\Delta t$, Fig. 1) are not significantly different from those determined earlier in 24 h temperature-acclimated specimens (Heisler *et al.* 1976). However, they were significantly different from those expected on the basis of a constant relative alkalinity ($\Delta pH/\Delta t = -0.0184$, *Handbook of Chemistry and Physics*, 1975).

The slight increase of P_{CO_2} with temperature (Fig. 1 *b*) in juvenile fish was the same as in 24 h acclimated fish, but the increase in adult fish was slightly smaller than with short-term acclimation (Heisler *et al.* 1976). The decrease of calculated bicarbonate in juvenile fish and the increase in adults (Fig. 1 *c*) was again very similar to the pattern observed in 24 h acclimated fish (Heisler *et al.* 1976).

The mean intracellular pH values measured in white muscle, red muscle and heart muscle in the 27 specimens of this series are presented in Fig. 2, together with the determined temperature coefficients. None of the coefficients is significantly different from the corresponding value in 24 h temperature-acclimated fish (Heisler *et al.* 1976). The values for red muscle and heart muscle, however, are significantly different from that anticipated on the basis of constant relative alkalinity ($\Delta pN/\Delta t = 0.0184$, *Handbook of Chemistry and Physics*, 1975).

The fractional water contents (F_{H_2O}) and the fractional extracellular volumes (Q_e) of the three muscle types, determined by drying tissue samples and by application of the inulin distribution method respectively, are summarized in Table 1. None of

Fig. 2. Intracellular pH of (*a*) white, (*b*) red and (*c*) heart muscle of juvenile (closed circles, dashed lines) and adult dogfish (open circles, dashed-dotted lines) acclimated to environmental temperatures of 10, 15 and 20 °C for 22–25 days. Each circle represents the mean of 30 determinations in white muscle, 15 determinations in red muscle or 3–5 determinations in heart muscle of an individual animal ±2 s.e. The solid lines represent the regression line of all determinations in red muscle and 3–5 determinations in heart muscle of an individual animal ±2 s.e. The solid lines represent the regression line of all determinations in juvenile and adult specimens.

For legend see opposite.
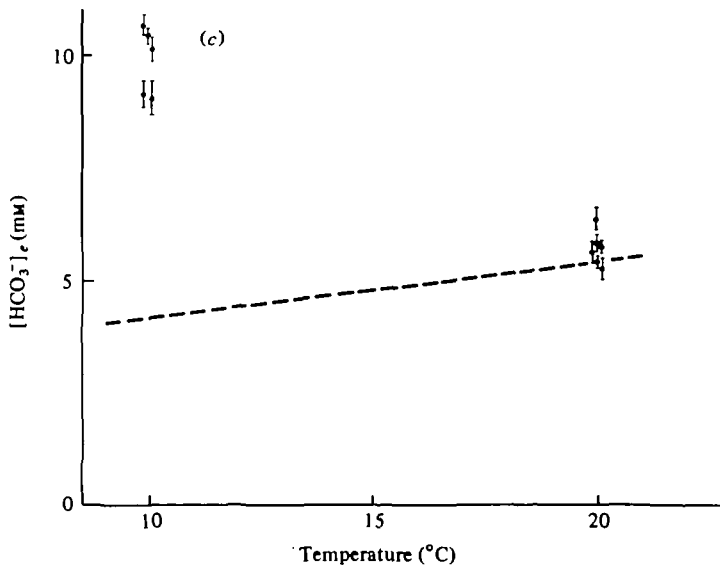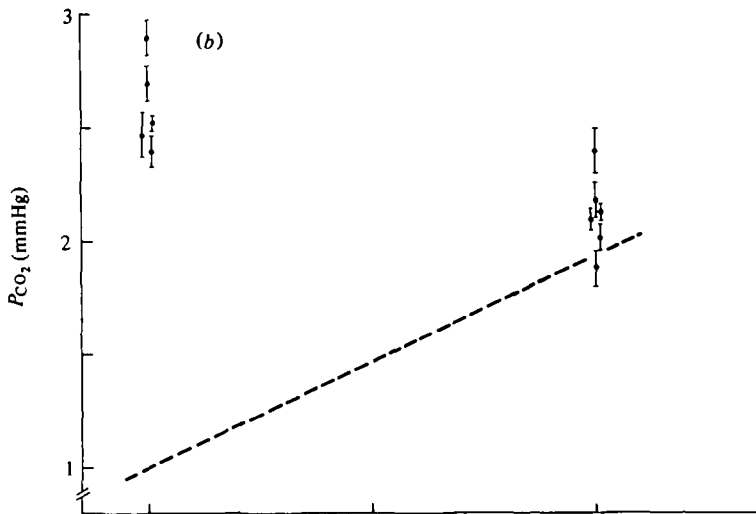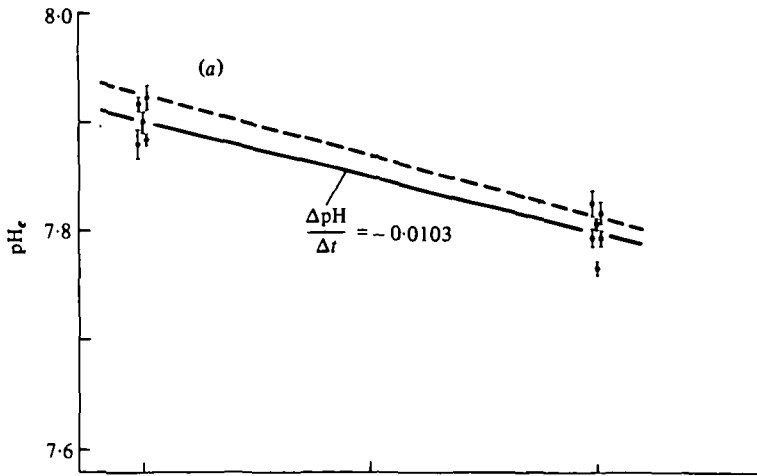
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the values is significantly different from the corresponding value measured in 24 h temperature-acclimated fish.

2. *Long-term acclimation with hypercapnia at low temperature*

The results obtained in the 11 adult specimens of this series are presented in Figs. 3 and 4. Plasma P_{CO_2} (Fig. 3b) in specimens acclimated to 10 °C at elevated inspired P_{CO_2} is, at about 2·7 mmHg, markedly above the normal value of about 1 mmHg (series 1). This, however, does not influence the extracellular pH (Fig. 3a) as the bicarbonate concentration (Fig. 3c) is also markedly increased (from about 4 mM under normal conditions to about 10 mM).

The intracellular pH of white muscle and heart muscle (Fig. 4) at 10 and 20 °C was slightly lower than in series 1, but the temperature coefficients ($\Delta pH_i/\Delta t$) of all three muscle types was not significantly affected by the P_{CO_2} manipulation.

DISCUSSION

The acid-base equilibrium observed in 22–28 days acclimated dogfish was almost the same as in 24 h acclimated specimens (Heisler *et al.* 1976). Apparently the temperature adaptation of the acid-base parameters is largely complete after 24 h. Consequently the peculiar pH/temperature regulation reported earlier (Heisler *et al.* 1976) is not the expression of non-steady-state conditions.

Relatively little is known about the regulatory mechanisms responsible for the temperature-dependent extracellular and intracellular acid-base regulation in poikilothermic animals. The following mechanisms could be involved:

(1) Changes of P_{CO_2} due to changes in ventilation, changes of metabolic rate and/or limitations of the CO_2 elimination.

(2) Changes in pK values of buffer substances (substances of special interest: imidazole-like and phosphate-like compounds, which are, according to their pK values, known to be almost exclusively responsible for non-bicarbonate buffering in the physiological pH range).

(3) Changes in the concentration of substrates or metabolites according to changes of temperature-dependent reaction constants associated with release of H^+ or OH^-.
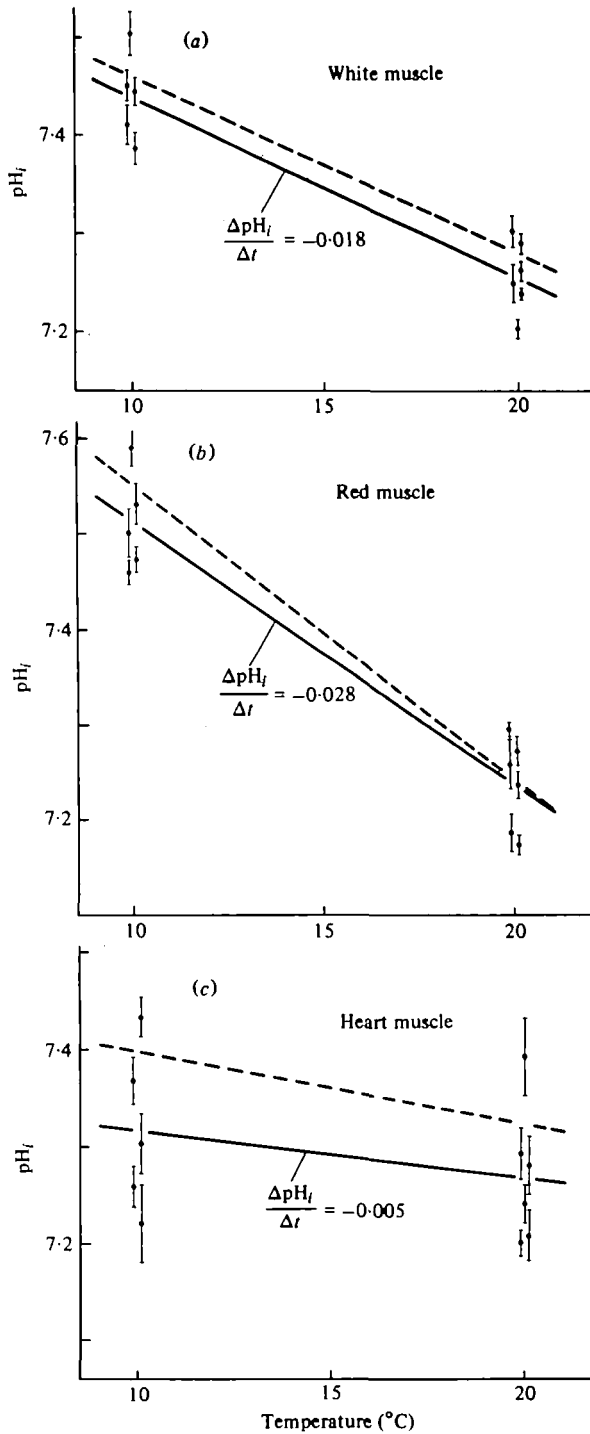
(4) Changes of the steady-state output of H^+ or OH^- in the metabolism according to temperature-dependent changes of metabolic rate and pathways.

(5) Exchange of either HCO_3^-, H^+ or OH^- between body compartments and/or with the environment

(*a*) passively due to changes in membrane permeabilities, chemical or electrical gradients,

(*b*) actively by pump mechanisms for the adjustment of pH or of other parameters.

Fig. 3. Extracellular pH (*a*), arterial P_{CO_2} (*b*) and arterial bicarbonate concentration (*c*) in long-term temperature-acclimated dogfish (series 2). The inspired P_{CO_2} of the specimens at 10 °C was artificially elevated to about 1·9 mmHg. The solid line is the regression line of the extracellular pH values, the dashed lines are the corresponding regression lines shown in Fig. 1 (series 1). Each circle represents the mean of 4–6 measurements in an individual animal ±2 S.E.

(a)

White muscle

$$\frac{\Delta pH_i}{\Delta t} = -0.018$$

(b)

Red muscle

$$\frac{\Delta pH_i}{\Delta t} = -0.028$$

(c)

Heart muscle

$$\frac{\Delta pH_i}{\Delta t} = -0.005$$

Temperature (°C)

For legend see opposite.

The imidazole alphastat hypothesis (Reeves, 1972), a model for acid-base regulation with temperature changes, seems to explain completely the observed extracellular and intracellular regulation in frog and turtle (Malan *et al.* 1976) based only on mechanisms (1) and (2) (Reeves & Malan, 1976). Also the majority of the other studied air breathing ectotherms fits reasonably well the proposed imidazole alphastat regulation (see Introduction). The essence of this model is that P_{CO_2} is regulated in such manner that the dissociation (alpha) of imidazole is kept constant. As imidazole-histidine is the predominant nonbicarbonate buffer in the extracellular compartment and the concentration of imidazole is considered to be fairly large also in the intra-cellular compartments (Reeves & Malan, 1976), the maintenance of a constant imidazole dissociation results in more or less constant bicarbonate concentrations with no additional mechanisms required.

This model, however, does not fit the obtained data in dogfish. Extracellular pH ($\Delta pH_e/\Delta t = -0.012$) and intracellular pH in heart muscle ($\Delta pH_i/\Delta t = -0.007$) change with temperature much less, and in red muscle ($\Delta pH_i/\Delta t = -0.03$) much more, than required for a constant dissociation of imidazole ($\Delta pK_{1m}/\Delta t = -0.018$ to -0.024, dependent on the type of imidazole compound; Edsall & Wyman, 1958). This complex pattern of pH regulation with different relationships of pH to acclimation temperature in all four studied compartments cannot be achieved by changes of only buffer pK values and P_{CO_2}. Actually, the bicarbonate concentrations in the extracellular space of both adult and juvenile specimens (Fig. 1c) change considerably when temperature is changed. As recently shown, these changes in bicarbonate concentration have to be attributed to exchange processes between extracellular space, intracellular space and environmental sea water (Heisler, 1978). Thus at least mechanisms (1), (2) and (5) are involved in the acid-base regulation of dogfish.

It is quite evident from the results of the second series of experiments in the present study that changes of P_{CO_2}, artificially produced by increasing the inspired P_{CO_2}, have little influence on pH in extracellular and intracellular compartments. These data suggest that P_{CO_2} is not of major importance for the pH regulation in dogfish. This is also supported by the observation that even larger increases of P_{CO_2} in dogfish result only in very transient increases of gill ventilation (Randall, Heisler & Drees, 1976). In this context it must be realized that ventilation and thus P_{CO_2} can be varied in water breathing animals only within very narrow limits, because of the low oxygen content of water in comparison with air, while the bicarbonate concentration in the extracellular space has been shown to be increased by a factor of 4; from 5 to more than 20 mM (Heisler *et al.* 1976).

It has to be concluded that the acid-base equilibrium in dogfish is not characterized by imidazole alphastat regulation. Of the three mechanisms proposed to be involved, (1), (2) and (5), the third will be the most significant. The changes of buffer pK values are inevitably constant for a given change in temperature. The changes of P_{CO_2} are more or less predetermined according to metabolic rate and the gas exchange con-

Fig. 4. Intracellular pH of (*a*) white, (*b*) red and (*c*) heart muscle (series 2). The animals acclimated to 10 °C were subjected to an increased inspiratory P_{CO_2} of about 1·9 mmHg. Each circle represents the mean of 30 determinations in white muscle, 15 in red muscle and 3–5 determinations in heart muscle of an individual animal ±2 S.E. The dashed line is the corre-sponding regression line shown in Fig. 2 (series 1).

ditions and can only be modulated in a small range. Thus only the third mechanism, the bicarbonate transfer between body compartments and environment, is variable within sufficiently wide limits to account for the ultimate acid-base regulation in dogfish.

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