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Haemolymph Gas Transport, Acid-Base Regulation, and Anaerobic Metabolism During Exercise in the Land Crab *(Cardisoma carnifex)*

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ABSTRACT Haemolymph gases, acid-base status, and metabolite levels were studied in *Cardisoma carnifex* at rest and after 10 minutes of mild (0.2 body lengths/second) or severe (exhausting, 0.5 BL/second) exercise. O_2 transport is very similar to that in aquatic crabs. At rest, arterial haemocyanin saturation is ≈ 87%, venous saturation is ≈45%, and tissue utilization is ≈57%. During exercise, $\dot{M}_{O_2}$ rises 2–3-fold. P_{O_2}s fall so that O_2 transport is shifted onto the steep part of the dissociation curve, venous saturation decreases markedly while arterial saturation remains high, and cardiac output rises. These adjustments raise the P_{O_2} gradient at the respiratory surface, tap the haemolymph O_2 store, and maintain or increase the a–v O_2 difference so that utilization reaches ≈82%. Postexercise acidosis augments these effects via the Bohr shift. Resting P_{CO_2}'s are low (≈15 torr) for an air-breather, and Pa_{CO_2} changes minimally despite 2.5–5-fold evaluations in $\dot{M}_{CO_2}$. All haemolymph gas levels return to normal within 0.5–1.0 hours. Postexercise acidosis is largely metabolic, and smaller than in aquatic crabs. Lactate anions and protons enter the haemolymph in equivalent amounts and totally account for the metabolic acidosis. Elevated NH_3 and pyruvic acid levels have negligible influence. During recovery, the metabolic acid load is reduced faster than the lactate load, resulting in alkalosis, possibly because of $CaCO_3$ mobilization from the carapace. Exercise metabolism appears largely anaerobic, but changes in haemolymph lactate levels do not correlate with the O_2 debt. However, the "excess lactate" concept which compensates for pyruvate elevation gives a good index of the debt. All changes are more marked after severe than after mild exercise, but the patterns are similar.

In the previous paper (Wood and Randall, '81), we have shown that the land crab *Cardisoma carnifex* has considerable running ability. Exercise is facilitated by marked increases in ventilation, O_2 consumption, CO_2 production, and the acquisition of a substantial O_2 debt. Basically similar effects were seen in *Cardisoma guanhami* (Herreid et al.,'79). Very little is known about the role of haemolymph gas transport in these events, about the nature of the O_2 debt, or about the influence of the increased CO_2 flux and anaerobic metabolism on acid-base regulation in land crabs, though these areas have been extensively studied in marine brachyurans (Johansen et al., '70; Mangum and Weiland, '75; McMahon et al., '79; McDonald et al., '79). Comparison of the very limited information available on land crabs with these observations on marine crabs raises a number of issues.

Increased O_2 delivery to the tissues during exercise in aquatic crabs is facilitated by reductions in both $P_{a_{O_2}}$ and $P_{v_{O_2}}$. This taps the haemolymph O_2 store, increases the diffusion gradient for O_2 loading at the gills, and at the same time takes maximum advantage of the O_2 transport properties of the respiratory pigment, haemocyanin. The extremely low in vivo P_{O_2}'s, arterial saturations, and in vitro P_{50}'s reported for *Cardisoma guanhami* at rest (Redmond, '62, '68a; Young, '73) make it questionable whether this strategy can be implemented by the exercising land crab.

Despite the relative ease of CO_2 excretion in water, *Cancer magister* suffers a significant rise in $P_{a_{CO_2}}$ and associated respiratory acidosis

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after exercise, as well as a substantial metabolic acidosis (McDonald et al., '79). This problem may well be much more serious in air, where CO_2 levels are often elevated. Smatresk et al. ('79) calculated P_{vCO_2} indirectly in the terrestrial crab *Gecarcinus lateralis,* and found that it more than doubled after exhaustive exercise.

Cancer accumulates extremely high haemolymph lactate concentrations after exhausting exercise (≈11.5 mEq/L), and these levels more than account for the metabolic acid load (McDonald et al., '79). Indeed, protons seem to be retained in the tissues and released at a slower rate than lactate anions. In contrast, *Gecarcinus lateralis* accumulates minimal haemolymph lactate concentrations (≈1.5 mEq/L) after exhausting execise, and the metabolic acid load greatly exceeds the lactate load (Smatresk et al., '79), indicating either very different lactate versus proton release kinetics from those in *Cancer,* or the accumulation of unknown acidic end products of metabolism. As both McMahon et al. ('79) and Herreid et al. ('79) have pointed out, the whole area of anaerobic metabolism, lactate dynamics, and O_2 debt is poorly understood in crabs.

Finally, marine crabs can theoretically exchange acid or base with the external seawater; Truchot ('79) has recently demonstrated that the marine crab *Carcinus maenas* employs this mechanism to help correct an experimental acid or base load. McDonald et al. ('79) have suggested that *Cancer* also uses this mechanism to compensate a postexercise acidosis. This option may not be available to land crabs.

The aim of the present study was to address some aspects of these problems by investigating haemolymph gas transport, acid-base regulation, and anaerobic metabolism in the land crab *Cardisoma carnifex* at rest and after forced running activity on a treadmill.

MATERIALS AND METHODS

All experimental methods and symbols employed are described in detail in Wood and Randall ('81). The present study deals with in vivo haemolymph gas, acid-base, pyruvate, lactate, ammonia, and inorganic ion measurements from crabs subjected to 10 minutes exercise at 0.2 and 0.5 BL/second (approximately 4.5 and 11.0 cm/second, respectively) on the treadmill, and with the in vitro characteristics of the haemolymph.

All data are reported as means ± 1 standard error (N) where N represents the number of different animals contributing to the mean. The significance ($P \leq 0.05$) of changes within an experimental group was determined by Student's paired two-tailed t-test with each crab as its own control. Differences between groups ($P \leq 0.05$) were assessed by Student's unpaired two-tailed t-test. Where linear regression relationships are employed, the significance level of the correlation coefficient is given.

RESULTS

Haemolymph properties in vitro

The mean O_2 capacity of the haemolymph ($C_{O_2}^{max}$) in our experimental animals was 0.90 ± 0.05 (17) mM/L (2.02 vol %), while that for haemolymph haemocyanin alone ($C_{Hcy\ O_2}^{max}$) was 0.67 ± 0.05 (17) mM/L (1.51 vol %). These values slightly underestimate the true values in unoperated crabs because of blood loss during surgery. Typical haemolymph buffer curves are presented by Randall and Wood ('81). The nonbicarbonate buffer capacity (β, slykes) of the haemolymph was directly proportional to $C_{Hcy\ O_2}^{max}$ (mM/L) (Fig. 1):

$$\beta = 15.67\ (C_{Hcy\ O_2}^{max}) + 1.56$$
$$(r = 0.95,\ P < 0.001)$$

Mean β in the experimental crabs was 12.10 ± 0.81 (17) slykes, again a slight underestimate of the value in intact animals because of blood loss. At approximately resting arterial values of pH (7,420) and P_{CO_2} (14.6 torr), the P_{50} of the haemocyanin in vitro was about 13.5 torr P_{O_2}, and the magnitude of the Bohr effect ($\Delta \log P_{50}/\Delta pH$) was −0.68 (W.W. Burggren and B.R. McMahon, personal communication).

Oxygen transport in the haemolymph

At rest, P_{aO_2} averaged about 55 torr, but was extremely variable (Fig. 2A, B). However, most of this scatter occurred over the linear region of the haemolymph O_2 dissociation curve where the pigment is close to saturation and additional O_2 is carried only in physical solution. Overall, arterial haemocyanin saturation (S_{aO_2}) as calculated from $C_{O_2}^{max}$, P_{O_2}, pH, and the haemolymph O_2 dissociation curve (cf. Wood and Randall '81) was 87.0 ± 6.4 (14)%. P_{vO_2} was much more uniform, averaging about 13 torr or 44.6 ± 5.2 (14)% saturation. Both arterial and venous O_2 levels tended to fall after mild exercise, but variability was large. Consequently none of these changes, whether expressed as P_{O_2} (Fig. 2A, B), S_{O_2}, or C_{O_2} (Fig. 2C, D) were significant ($P > 0.05$) with mild

exercise (0.2 BL/second). With exhausting activity (0.5 BL/second), the venous changes were highly significant ($P < 0.001$), P_{vO_2} dropping to 8.0 ± 1.4 (8) torr or 8.8 ± 2.3 (8)% saturation. About two-thirds of the drop in S_{vO_2} was directly attributable to the fall in P_{vO_2}, and the other one-third to the Bohr shift caused by postexercise acidosis (Fig. 4B). S_{aO_2} remained relatively high, 70.6 ± 10.5 (7) %. O_2 transport by physical solution dropped from 18 to 10% of the total. The utilization of O_2 from the haemolymph by the tissues, defined as the percentage of total O_2 removed from arterial blood, increased from 57.3 ± 6.7 (8)% at rest to 82.3 ± 6.4 (7)% after severe exercise. Haemolymph O_2 levels had returned to normal by 30–60 minutes postexercise (Fig. 2).

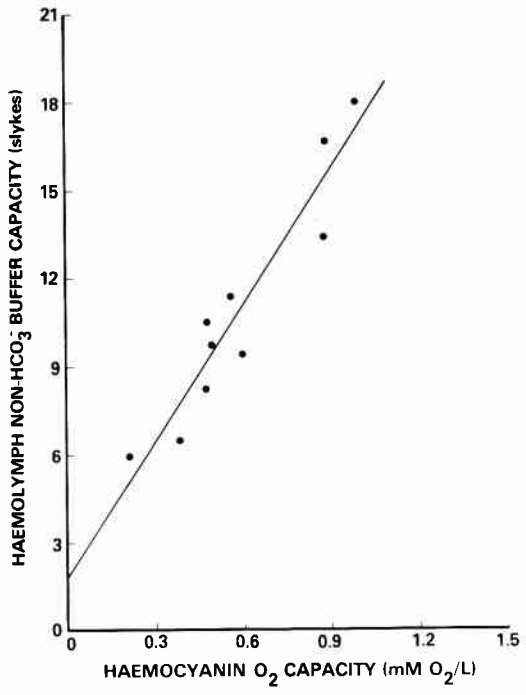

Fig. 1. The relationship in *Cardisoma* between haemolymph nonbicarbonate buffer capacity (β, slykes) and haemocyanin O_2 capacity ($C^{max}_{Hcy\ O_2}$, mM/L) in vitro at 25°C. The regression relationship is $\beta = 15.67\ C^{max}_{Hcy\ O_2} + 1.56$ ($r = 0.95$, $P < 0.001$).

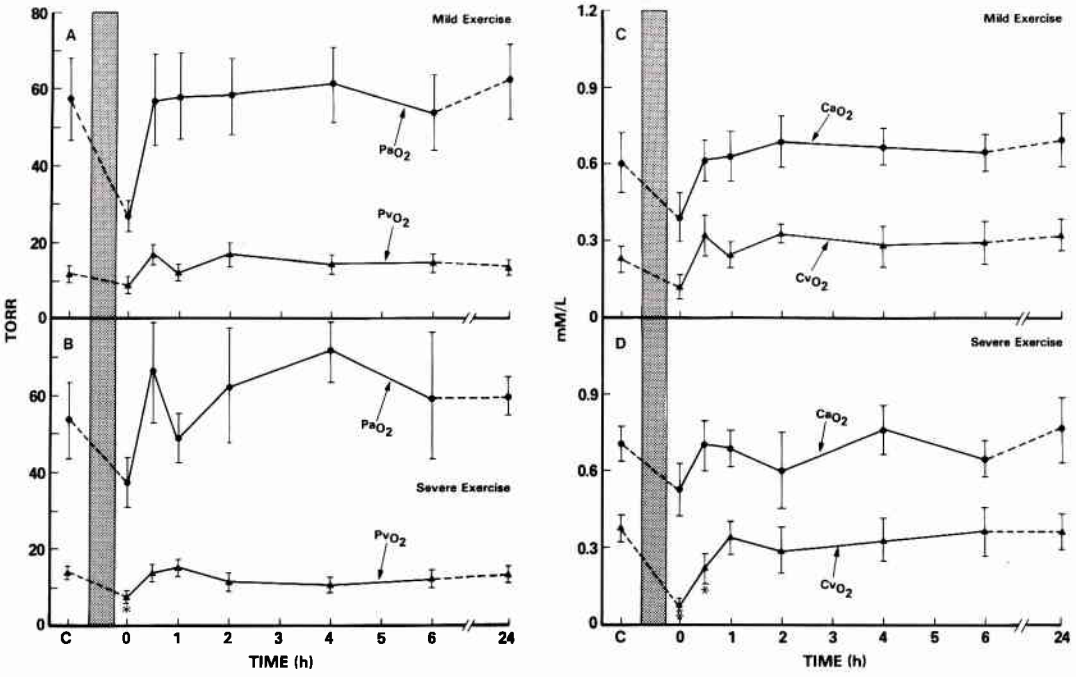

Fig. 2. A, B) Changes in arterial (P_{aO_2}) and venous (P_{vO_2}) oxygen tensions in *Cardisoma* at 25°C after 10 minutes of either (A) mild or (B) severe exercise. C, D) Changes in calculated arterial (C_{aO_2}) and venous (C_{vO_2}) oxygen contents in whole haemolymph of *Cardisoma* at 25°C after 10 minutes of either (C) mild or (D) severe exercise. The exercise period is indicated by the stippled bar. Means ± 1 S.E. * = significantly different from preexercise control value. In A, C, N = 5–6. In B, D, N = 5–8.

Carbon dioxide transport and acid-base regulation in the haemolymph

At rest, P_{CO_2}'s in the haemolymph were about 15 torr (Fig. 3A, B), C_{CO_2}'s about 19 mM/L (Fig. 3C, D), and pHs about 7.5 (Fig. 4). Arterial–venous differences in these parameters were negligible. This is not surprising in view of the fact that there is a small but definite Haldane effect in *Cardisoma* haemolymph (Randall and Wood, '81) so that at any given P_{CO_2}, deoxygenated haemolymph holds more CO_2 and has a higher pH than oxygenated haemolymph. Furthermore, $C_{aO_2} - C_{vO_2}$ at rest was only ≃0.35 mM/L (Fig. 2C, D) and R was ≃0.60 (Wood and Randall, '81), so the expected $C_{vCO_2} - C_{aCO_2}$ would be only ≃0.21 mM/L, virtually undetectable against a background of 19 mM/L.

Mild exercise did not increase either P_{aCO_2} or P_{vCO_2} (Fig. 3A); indeed, if anything, these parameters tended to drop slightly below control level during the recovery period. The combined effects of increases in the CO_2 diffusing capacity of the respiratory epithelium, hyperventilation, and increased cardiac output, $\dot{V}_b$ (cf. Wood and Randall, '81, for details) were therefore sufficient to deal with a 2.5-fold rise in $\dot{M}_{CO_2}$ without disturbance of CO_2 tensions. After severe exercise, both P_{aCO_2} and P_{vCO_2} rose significantly (Fig. 3B). However, the venous increase was only about 5.5 torr, while the arterial change (+ 2.7 torr) was of borderline significance ($P \simeq 0.05$). These data illustrate the efficiency of the system for dealing with elevated CO_2 fluxes, for here $\dot{M}_{CO_2}$ increased greater than 5-fold (cf. Fig. 10B of Wood and Randall, '81). At 30 minutes postexercise, CO_2 tensions had returned to normal (Fig. 3B) despite the fact that $\dot{M}_{CO_2}$ was still elevated about 3.5-fold. Thereafter, P_{CO_2}'s tended to fall slightly below resting levels as elevated ventilation and heart rate persisted (cf. Fig. 8B of Wood and Randall, '81).

Haemolymph C_{CO_2} levels fell significantly ($P < 0.05$) after both mild and severe exercise (Fig. 3C, D), though the changes were more marked in the latter. These decreases reflected the buffering of metabolic acids (see *Haemolymph metabolite levels* below) by haemolymph HCO_3^- and the subsequent elimination of the resulting CO_2. By 2 hours postexercise, C_{CO_2} levels had been restored to normal. However, in the severe exercise treatment (Fig. 3D),

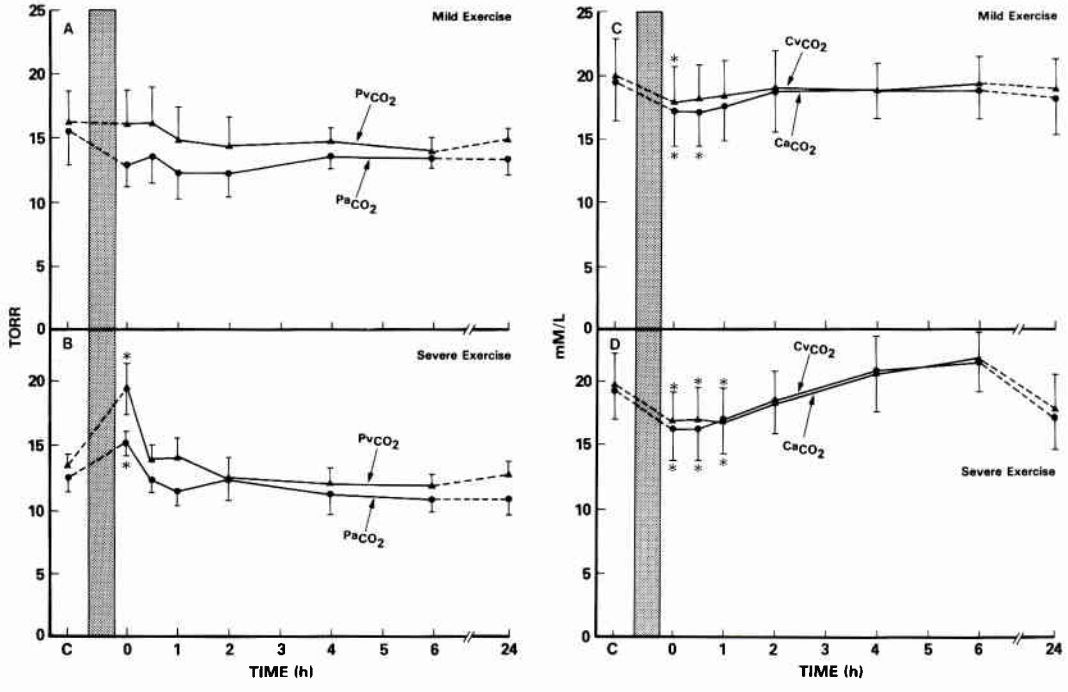

Fig. 3. A, B) Changes in arterial (P_{aCO_2}) and (P_{vCO_2}) carbon dioxide tensions in *Cardisoma* at 25°C after 10 minutes of either (A) mild or (B) severe exercise. C, D) Changes in arterial (C_{aCO_2}) and venous (C_{vCO_2}) carbon dioxide contents in *Cardisoma* at 25°C after 10 minutes of either (C) mild or (D) severe exercise. Other details as in Figure 2.

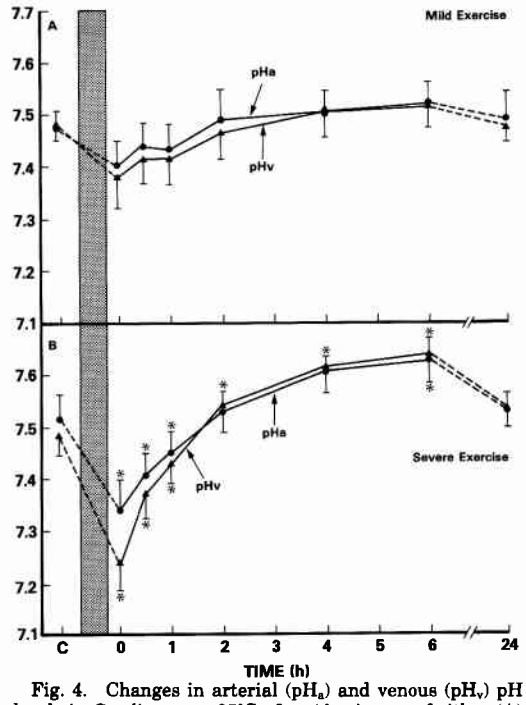

Fig. 4. Changes in arterial (pH_a) and venous (pH_v) pH levels in *Cardisoma* at 25°C after 10 minutes of either (A) mild or (B) severe exercise. Other details as in Figure 2.

C_{CO_2}'s continued to rise to levels about 2mM/L above control at 6 hours before returning to normal at 24 hours. This effect was not significant ($0.1 < P > 0.05$) because of great variablity in the data.

In concert with the immediate postexercise decrease in C_{CO_2} (Fig. 3C), haemolymph pH levels declined slightly after mild exercise (Fig. 4A), but the changes were not significant because of variability and simultaneous slight decreases in P_{CO_2} levels (Fig. 3A). More marked pH depressions (≃0.2 pH units) occurred after severe exercise (Fig. 4B) in association with elevated P_{CO_2} levels (Fig. 3B), depressed C_{CO_2} levels (Fig. 3D), and elevated concentrations of metabolic acids in the haemolymph (see *Haemolymph metabolite levels* below). The arterial–venous pH difference also increased. These effects remained significant at 1 hour postexercise but, by 2 hours, pH's had started to rise significantly above control values. This trend became progressively greater at 4 and 6 hours (Fig. 4B). The values at 24 hours were similar to controls.

Haemolymph metabolite levels

In resting animals, lactate concentrations were 1–2 mM/L (Fig. 5A, B), and pyruvate lev-

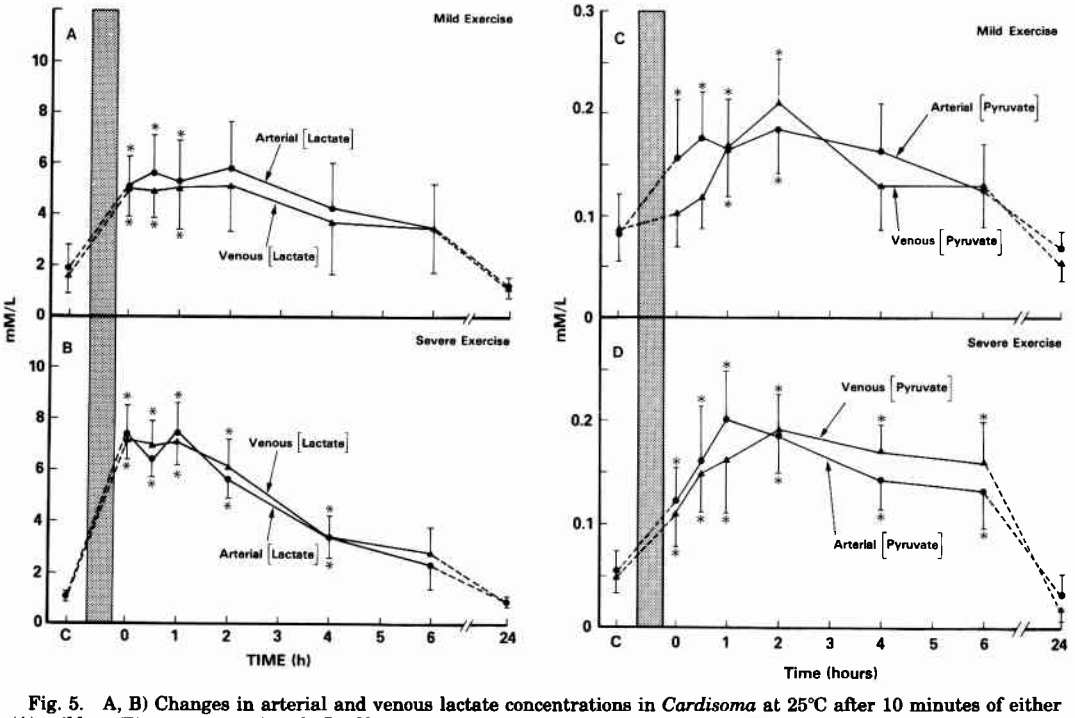

Fig. 5. A, B) Changes in arterial and venous lactate concentrations in *Cardisoma* at 25°C after 10 minutes of either (A) mild or (B) severe exercise. C, D) Changes in arterial and venous pyruvate concentrations in *Cardisoma* after 10 minutes of either (C) mild or (D) severe exercise. Other details as in Figure 2.

*TABLE 1. Lactate: pyruvate ratios in the haemolymph before and after mild and severe exercise**

		Mild Exercise	Severe Exercise
Control		20.8	23.2
Postexercise:	0 h	38.9	62.9
	0.5 h	35.5	42.9
	1 h	32.0	39.8
	2 h	27.9	37.2
	4 h	27.2	21.3
	6 h	27.4	17.1
	24 h	19.0	22.8

* Arterial and venous values averaged.

els 0.04–0.09 mM/L (Fig. 5C, D). There were no detectable arterial–venous differences. The control lactate:pyruvate ratio in both groups was about 22:1 (Table 1). Activity caused large increases in both parameters, with greater changes in the severely exercised group (Fig. 5). Lactate peaks (≈5.5 mM/L at 0.2 BL/second, ≈7.5 mM/L at 0.5 BL/second) occurred immediately postexercise followed by maintained plateaus until at least 2 hours (Fig. 5A, B). Lactate was still elevated at 6 hours, but a complete return to control levels was seen in the 24-hour samples. Pyruvate levels increased more gradually, peaking at 1–2 hours postexercise and returning toward resting levels with a somewhat slower time course than lactate (Fig. 5C, D). There was also a tendency for venous pyruvate levels to increase more gradually than arterial values, and then fall more slowly thereafter, though there was too much scatter in the data to determine if this was a real effect. Therefore averaged arterial and venous values have been used in calculating the lactate:pyruvate ratio (Table 1), which is thought to reflect the ratio of reduced to oxidized NAD and therefore serve as an index of the intensity of anaerobic glycolysis (Harris, '69). The ratio was greatest immediately after exercise, and about 60% higher at 0.5 BL/second than at 0.2 BL/second. It declined steadily thereafter, indicating a shift away from anaerobiosis.

Haemolymph ammonia levels (C_{NH_3}) were measured only in the severely exercised group. Resting levels were generally low but quite variable with C_{vNH_3} (0.20 ± 0.04 (5) mM/L), consistently greater than C_{aNH_3} (0.14 ± 0.02 (5) mM/L). These levels approximately doubled after severe exercise, reaching a peak at 1 hour and thereafter declining (Fig. 6). The arterial–venous difference was maintained throughout the recovery period.

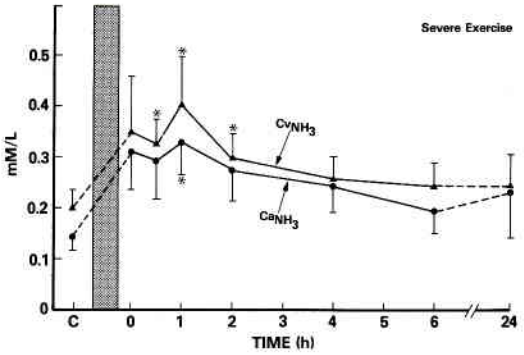

Fig. 6. Changes in arterial (C_{aNH_3}) and venous (C_{vNH_3}) ammonia contents in *Cardisoma* at 25°C after 10 minutes of severe exercise. N = 5 except at 24h where N = 3. Other details as in Figure 2.

Inorganic ion levels in the haemolymph

Ion levels were determined only in the severely exercised group. As there were no detectable arterial–venous differences, the data have been pooled. Sodium (366 ± 24 (5) mEq/L) and chloride (374 ± 26 (5) mEq/L) concentrations were essentially identical at rest and did not change during exercise or recovery (Fig. 7), suggesting that there were no major changes in water balance of the animals over the experimental period. However, calcium increased significantly from a resting level of 22.2 ± 1.4 (5) mEq/L at rest to a peak of 25.9 ± 1.5 mEq/L at 1 hour's recovery (Fig. 7), suggesting a mobilization of calcium salts from the carapace.

DISCUSSION

In vitro properties of the haemolymph

Haemolymph O_2 capacities in *Cardisoma carnifex* were similar to those reported for other *Gecarcinidae* (Redmond, '62, '68a, b) and

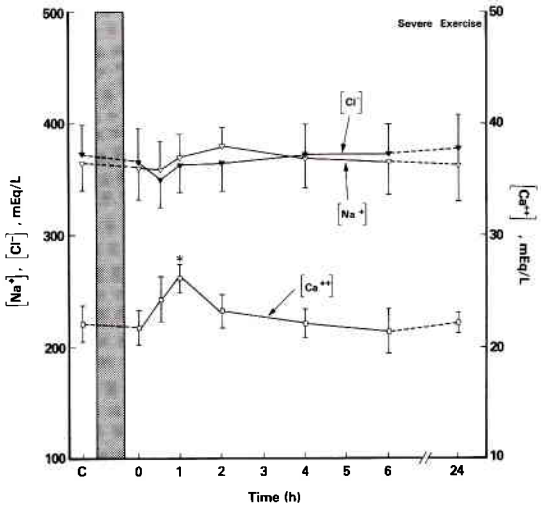

Fig. 7. Changes in the concentrations of sodium, chloride, and calcium ions in the haemolymph of *Cardisoma* at 25°C (means of arterial and venous values) after 10 minutes of severe exercise. Note the different scales for Na^+/Cl^- and Ca^{++}. N = 5 except at 24h where N = 3. Other details as in Figure 2.

agree with the general trend of higher haemocyanin concentrations accompanying terrestriality (McMahon and Burggren, '79). $C^{max}_{Hcy\ O_2}$ in the aquatic *Cancer magister*, for example, was only about half that in *Cardisoma carnifex* (McMahon et al., '79). The Bohr shift ($\Delta \log P_{50}/\Delta pH = -0.68$) in *Cardisoma carnifex* was similar to that in other terrestrial (Redmond, '62; McMahon and Burggren, '79; Burnett, '79) and aquatic crabs (Truchot, '75; McMahon et al., '79; Burnett '79) but very different from that reported for *Gecarcinus lateralis* (−0.37; Redmond, '68b). While it is claimed that the magnitude of the Bohr shift decreases with increasing terrestriality (Young, '73; McMahon and Burggren, '79), the argument seems to hinge only on this one possibly abnormal value for *Gecarcinus*. As both bicarbonate and nonbicarbonate buffer capacities rise with terrestriality (see below), there is no reason to believe that haemolymph pH fluctuations should be any greater in land crabs than in aquatic ones. Indeed if the present results are any guide, they may in fact be smaller (see below).

The linear relationship between β and $C^{max}_{Hcy\ O_2}$ in *Cardisoma* haemolymph (Fig. 1) illustrates that buffering is a direct function of the haemocyanin concentration. The intercept at $C^{max}_{Hcy\ O_2} = O$ was only 1.56 slykes, indicating that buffering by nonhaemocyanin proteins and other substances is minimal. This relationship between β and $C^{max}_{Hcy\ O_2}$ in *Cardisoma* at 25°C was almost identical to that in the marine crab *Carcinas maenas* at 15°C (Truchot, '76) and similar to that for *Cancer magister* at 8°C (McDonald et al., '79). The ratio of proton-accepting to O_2-binding sites on the haemolymph molecule therefore appears independent of species, temperature, or haemocyanin concentration. The higher haemocyanin levels in terrestrial crabs mean that they will have higher nonbicarbonate buffer capacities than aquatic crabs. For example, β averaged 12.1 slykes in the present *Cardisoma carnifex*, 14.4 slykes in *Gecarcinus lateralis* (Smatresk et al., '79), ≃16.0 slykes in *Birgus latro* (Cameron and Mecklenburg, '73), and 16.0 slykes in *Coenobita clypeatus* (McMahon and Burggren, '79) *versus* ≃5 slykes in *Cancer magister* (McDonald et al., '79), ≃6 slykes in *Callinectes sapidus* (Cameron, '78), and ≃7 slykes in *Carcinus maenas* (Truchot, '76). Arguments can be made for benefits derived from increasing both $C^{max}_{Hcy\ O_2}$ and β with terrestrial invasion (cf. McMahon and Burggren, '79), but it is unclear whether one, the other, both, or something entirely different such as elevated oncotic pressure constitutes the primary advantage of high haemocyanin levels.

Oxygen transport in the haemolymph

Perhaps the most surprising feature of O_2 transport in *Cardisoma carnifex* was its similarity to that in aquatic crabs (Johansen et al., '70; Mangum and Weiland, '75; McMahon et al., '79). Table 2 compares the present data for the severely exercised group with the data of McMahon et al. ('79) on *Cancer magister* similarly exercised to exhaustion. The overall agreement is remarkable. The only pronounced difference, that in the percentage of O_2 delivery by physical solution, simply reflects the greater $C^{max}_{Hcy\ O_2}$ in the land crab. There seems to have been little modification of the internal O_2 transport system with the invasion of land. *A priori*, there is no obvious reason why an O_2 transport system which works well in water should not work equally well in air.

W.W. Burggren and B.R. McMahon (personal communication) have recorded values of P_{aO_2} in *Cardisoma* approximately 50% of our figures, but virtually identical P_{vO_2} values. The

TABLE 2. A comparison of O_2 transport at rest and immediately after exhausting exercise in the land crab Cardisoma carnifex *and the marine crab* Cancer magister

	Cardisoma carnifex		Cancer magister[a]	
	Rest	Exercise	Rest	Exercise
$\dot{M}_{O_2}$ (μM $O_2 \cdot kg^{-1} \cdot min^{-1}$)[b]	39.6	102.5	24.6	60.9
Pa_{O_2} (torr)	53	38	75	45
Pv_{O_2} (torr)	14	8	15	10
ΔP_{O_2} (torr)[b,c]	98	126	74	95
$\frac{\dot{M}_{O_2}}{\Delta P_{O_2}}$ (μM $O_2 \cdot kg^{-1} \cdot min^{-1} \cdot torr^{-1}$)[b,d]	0.406	0.812	0.332	0.641
$C_{Hcy}^{max}{}_{O_2}$ (mM $O_2 \cdot L^{-1}$)	0.67	—	0.33	—
pH_a	7.514	7.339	7.902	7.512
pH_v	7.487	7.240	7.895	7.493
S_{aO_2} (%)	91	71	99	94
S_{vO_2} (%)	50	9	55	15
$C_{aO_2} - C_{vO_2}$ (mM $O_2 \cdot L^{-1}$)	0.33	0.45	0.27	0.36
O_2 transport in physical solution (% of total)	18	10	58	21
U_{b,O_2} (%)[e]	57	82	56	86
$\dot{V}$(ml.kg^{-1}.min^{-1})[b]	122	230	72	185
$\frac{\dot{V}_b}{\dot{M}_{O_2}}$ (L. , O_2^{-1})[b,f]	3.08	2.24	3.74	2.79

[a] McMahon et al. ('79).
[b] Data from Wood and Randall ('81).
[c] Mean O_2 diffusion gradient across respiratory epithelium.
[d] O_2 diffusing capacity.
[e] % Utilization of O_2 by the tissues from the haemolymph $\left(\frac{C_{aO_2} - C_{vO_2}}{C_{aO_2}} \times 100\%\right)$
[f] Haemolymph convection requirement for O_2.

reason for this discrepancy is unknown. Nevertheless, both data sets, as well as Burggren and McMahon's O_2 dissociation curves, are in total disagreement with those of Redmond ('62, '68a) on *Cardisoma guanhami*. Redmond reported extremely low in vivo blood P_{O_2}'s, arterial saturations, and in vitro P_{50}'s on resting animals. However the P_{O_2} and saturation values were calculated from spectrophotometric dissociation curves rather than measured directly. In his 1968a paper, Redmond expressed some reservations about the data, and noted that direct measurements of P_{O_2} in the haemolymph of other species were greater than the similarly calculated values. Redmond's ('68b) direct measurements of P_{O_2} in *Gecarcinus lateralis* are very similar to our data on *Cardisoma*.

The basic strategy of both *Cardisoma* and *Cancer* for elevating O_2 transport during exercise is to allow haemolymph O_2 tensions to fall (Table 2) while $\dot{V}_b$ rises. Increased O_2 extraction by the tissues lowers P_{vO_2}. This effect, combined with the decreased residence time for haemolymph at the respiratory surface caused by the rise in $\dot{V}_b$, in turn, reduces P_{aO_2}. The net effect is to move O_2 transport onto the steep part of the dissociation curve. By so doing, the animal is able to raise the mean diffusion gradient (ΔP_{O_2}) at the respiratory membrane, tap the haemolymph O_2 store, and at the same time maintain or slightly increase $C_{aO_2} - C_{vO_2}$. The latter two effects are augmented by the Bohr shift due to postexercise acidosis (Fig. 4), which accounts for about 30% of $C_{aO_2} - C_{vO_2}$ at this time in *Cardisoma*, and about 60% in *Cancer*, where the pH change is much larger (Table 2). Assuming a haemolymph volume of 306 ml/kg in *Cardisoma* (J.N. Cameron, personal communication), the O_2 released from the venous reserve is $\simeq$91 μM O_2/kg, or enough to fuel the extra aerobic metabolic demands of running at 0.5 BL/second for about 1.5 minutes (cf. $\dot{M}_{O_2}$ data in Table 2). Considering that the mean fatigue time at this speed was only 3.73 $\pm$ 0.40 (8) minutes (Wood and Randall, '81), this is a highly significant contribution.

Carbon dioxide transport and acid-base regulation in the haemolymph

CO_2 will not be dealt with in detail here, apart from its influence on acid-base regulation, as the subject is considered at length by

Randall and Wood ('81). Suffice it to say that the mechanism for CO_2 excretion appears extremely effective. Like all air breathers relative to water breathers, *Cardisoma* is in a state of "compensated respiratory acidosis" (Dejours, '75), but the extent of this CO_2 retention is much less than in comparable vertebrate air breathers. For example, turtles, which breathe in a similar intermittent fashion to land crabs, maintain P_{aCO_2} levels approximately twice those of *Cardisoma* (Burggren and Shelton, '79). Furthermore, a greater than 5-fold increase in CO_2 flux (cf. Fig. 10B of Wood and Randall, '81) after severe exercise in *Cardisoma* raised P_{aCO_2} by only 2.7 torr (Fig. 3B).

Haemolymph pH levels were only slightly and nonsignificantly depressed after mild exercise (Fig. 4A). With severe exercise, the acidosis (≈0.2 pH units) became significant, but was followed by an alkalosis (+ 0.05 to 0.15 pH units) from 2 to 6 hours during the recovery period (Fig. 4B). In contrast, the aquatic *Cancer magister* exhibited a 2.5-fold greater pH depression after exhausting activity which persisted for 4–8 hours with no ensuing alkalosis.

The relative contribution of metabolic (i.e., noncarbonic) acid and respiratory acid (i.e., elevated P_{CO_2}) to these changes in pH can be calculated from the known buffer characteristics (β) of the haemolymph for each animal and the measured changes in C_{CO_2} (Fig. 3C, D) and P_{CO_2} (Fig. 3A, B) using a Davenport diagram analysis (Davenport, '74). The metabolic acid load is defined as the elevation, relative to the control condition, in the amount of protons of noncarbonic origin only, which are buffered in the haemolymph. The details have been explained by Wood et al. ('77) and McDonald et al. ('80). This analysis showed that the pH drop after mild exercise (Fig. 4A) was totally metabolic in origin, there being no rise in P_{CO_2} (Fig. 3A). Immediately after severe exercise, elevated metabolic acid loads accounted for 74.9 ± 7.1 (7)% of the acidosis on the arterial side and 54.7 ± 5.1 (7)% on the venous side. Elevated P_{CO_2} was therefore responsible for 25.1% and 45.9%, respectively. The much greater pH depression on the venous side (Fig. 4B) was due solely to the greater P_{vCO_2} increase (Fig. 3B); metabolic proton loads were identical (arterial = 5.94 ± 2.00 (7) mM/L; venous = 6.18 ± 1.49 (7) mM/L). By 30 minutes after severe exercise, P_{CO_2} levels had returned to normal or below (Fig. 3B) so the remaining acidosis was totally metabolic. The ensuing alkalosis at 4 and 6 hours (Fig. 4B) was again mainly metabolic in origin (i.e., negative metabolic acid or metabolic base load) with only a minor contribution (18–38%) from the slightly reduced P_{CO_2} levels (Fig. 3B).

There was considerable variability in β values between animals, and thus we initially expected to see greater pH depressions in those with smaller buffer capacities. In fact, exactly the opposite was true, as illustrated in Table 3 based on venous data immediately after mild and severe exercise. In both groups, there was a direct and highly significant correlation between β and pH depression. This relationship resulted from the fact that metabolic acid loads increased directly with β. This correlation was again highly significant (Table 3). One probable explanation is that metabolic acid efflux is a function of the buffer capacity of the haemolymph as described for amphibian muscle by Mainwood and Worsley-Brown ('75). Another is the fact that β is a linear function of $C^{max}_{Hcy\ O_2}$ (Fig. 1), so the correlation in Table 3 would be just as high if $C^{max}_{Hcy\ O_2}$ were substituted for β. Per unit O_2 delivery, haemolymph of high $C^{max}_{Hcy\ O_2}$ would not have to circulate as rapidly and therefore its residence time in the tissues would be longer, allowing greater loading of both metabolic acid and CO_2 per unit volume.

The rapid correction of postexercise metabolic acidosis and the subsequent metabolic alkalosis in *Cardisoma* (Fig. 4) are intriguing. Figure 8 shows the changes in Δ metabolic acid and Δ lactate in arterial haemolymph over time after mild and severe exercise; a virtually identical relationship was seen for venous haemolymph. Immediately after exercise, lactate loads and metabolic acid loads were identical. This was not a spurious result of averaging, for the regression relationship of Δ metabolic acid on Δ lactate in all individual crabs at time 0 hour was

$$\Delta \text{ metabolic acid} = 1.07\ (\Delta \text{ lactate}) - 0.37 \quad (r = 0.78,\ P < 0.001)$$

In other words, with slope ≈1 and intercept ≈0, proton and lactate anions were entering the blood from the tissues in equivalent amounts. However, by 1 hour, a consistent discrepancy of 2–4 mEq/L developed with Δ lactate significantly exceeding Δ metabolic acid; after severe exercise, this caused the significant metabolic alkalosis at 4 and 6 hours (Figs. 4B, 8B). Therefore metabolic proton removal from the blood (or metabolic base addition, an identical event in terms of acid-base equilibrium) occurred at a faster rate than lactate anion removal. Mangum and Towle ('77) have

TABLE 3. The relationships between the nonbicarbonate buffer capacity (β) and Δ pH, Δ metabolic acid load, and Δ P_{CO_2} in venous haemolymph immediately after mild and severe exercise

Crab	β (slykes)	Δ pH_v	Δ Metabolic Acid (mM/L)	Δ P_{vCO_2} (torr)
Mild exercise				
1	7.35	+ .015	+ 0.30	− 7.4
2	9.80	− .074	+ 2.54	− 2.2
3	10.61	− .071	+ 2.15	− 3.0
4	11.86	.000	+ 2.29	− 1.7
5	13.03	− .192	+ 4.79	+ 2.7
6	15.36	− .325	+10.60	+ 8.1
r[a]		−0.86	+ 0.90	+ 0.97
p[b]		<0.01	< 0.01	< 0.001
Severe exercise				
7	6.00	− .012	+ 0.89	+ 1.7
8	10.00	− .205	+ 4.14	+ 0.9
9	10.43	− .344	+ 6.00	+10.7
10	10.91	− .059	+ 1.82	+ 1.3
11	12.16	−0.295	+ 5.03	+13.1
12	16.97	−0.383	+10.21	+ 5.7
13	17.01	−0.258	+11.06	− 3.0
14	17.32	−0.420	+10.25	+14.7
r[a]		−0.75	+ 0.94	+ 0.39
p[b]		<0.01	< 0.001	n.s.

[a] Correlation coefficient between β and parameter tabulated.
[b] Significance of correlation coefficient.

argued that NH_3 mobilization as metabolic base is an important mechanism for raising haemolymph pH in aquatic crabs. Figure 6 shows that while C_{NH_3}'s increased significantly after exercise, probably as a result of increased protein and adenylate catabolism, the rise was only ≈0.2 mM/L, or about 1/15th of the measured discrepancy. In any event, this would have been cancelled out by an almost identical rise in pyruvic acid levels (Fig. 5C, D).

DeFur, Wilkes, and McMahon ('81) have recently proposed and provided evidence for the concept that carbonate can be mobilized from calcium carbonate reserves into the haemolymph of *Cancer productus* to help compensate the acidosis associated with air exposure in this aquatic species. We strongly suspect that a similar situation applies in the land crab after exercise so that the metabolic proton removal at this time was due to the mobilization of calcium carbonate from the carapace or other stores. The significant rise in haemolymph Ca^{++} levels against a background of constant Na^+ and Cl^- concentrations (Fig. 7) supports this interpretation (cf. Randall and Wood, '81). The $[Ca^{++}]$ peak occurred at 1 hour postexercise (Fig. 7), which was the time at which the significant Δ lactate versus Δ metabolic acid discrepancy developed (Fig. 8). The magnitude of the $[Ca^{++}]$ rise (+3.7 mEq/L) was very similar to the discrepancy (2–4mEq/L); assuming $CaCO_3$ as the base source, 1 mEq/L of Ca^{++} would correspond to −1mEq/L of Δ metabolic acid. The overshoot in pH (Fig. 4B) and net negative Δ metabolic acid load late in recovery (Fig. 8; metabolic alkalosis) would result from metabolism of lactate and protons in equivalent amounts while the base load persisted. Note however that $[Ca^{++}]$ tended to fall during this period (Fig. 7), suggesting its separate removal by unknown mechanisms.

We believe that the carapace (and perhaps other sites such as the gut) represents a readily mobilizable source of buffer base ($CaCO_3$) of particular importance in land crabs. This store might compensate to some extent for the lack of external seawater as a HCO_3^- source (cf. Truchot, '79). The store would be gradually built up at rest by retention of respiratory CO_2 thereby contributing to shell growth. This would explain the abnormally low resting R value (cf. Fig. 11 of Wood and Randall, '81). Haemolymph acidosis would cause leaching of the $CaCO_3$, thereby tending automatically towards self-correction. If this theory is correct, the animal would have to excrete two H^+ ions for every CO_2 stored as $CO_3^=$. We suggest that the branchial chamber water would be the most likely site to explore for this proton secretion.

Qualitatively, the effects of exhausting exercise on acid-base status in *Cardisoma* were fairly similar to those in *Cancer magister* (McDonald et al., '79). Like *Cardisoma*, *Cancer*

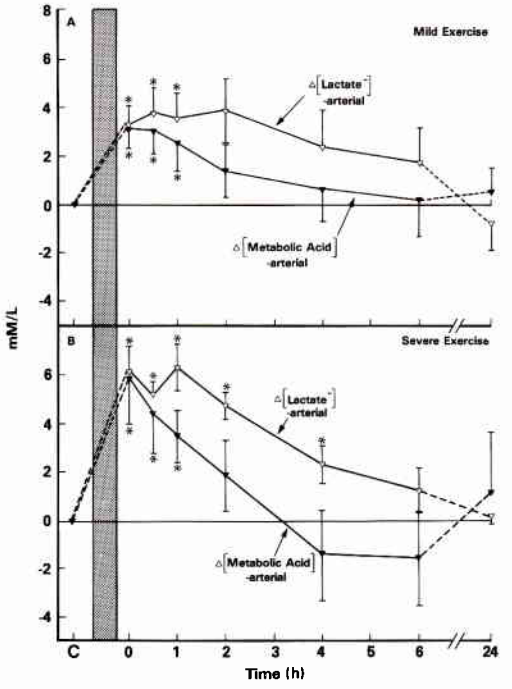

Fig. 8. Changes in the measured lactate anion load (Δ lactate) and calculated metabolic proton load (Δ metabolic acid) in arterial haemolymph of *Cardisoma* at 25°C after 10 minutes of either (A) mild or (B) severe exercise. Note the agreement of Δ lactate and Δ metabolic acid immediately after exercise and the discrepancy in these parameters which develops by 1h and persists through 6h postexercise. Other details as in Figure 2.

showed a small respiratory acidosis, large metabolic acidosis, relatively high lactate levels, and an excess of Δ lactate over Δ metabolic acid throughout most of the recovery period. However the latter discrepancy was much larger, was fully developed as soon as exercise finished, and was not followed by an alkalosis. McDonald et al.('79) suggested that the phenomenon reflected preferential retention of protons in the tissues. Our results with *Cardisoma* were very different from those of Smatresk et al. ('79) on the land crab *Gecarcinus lateralis*. These workers found a large rise in P_{CO_2}, only a small rise in lactate, and a large increase in metabolic acid of unknown origin in excess of the lactate (i.e., the opposite discrepancy to the present study). This may reflect a real species difference or a difference in methodology.

Anaerobic metabolism and oxygen debt

Exercise metabolism in *Cardisoma* appears to rely heavily on essentially anaerobic mechanisms. This was shown by the depletion of haemolymph O_2 reserves (see above), the accumulation of high lactate and pyruvate levels (Fig. 5), and the repayment of a substantial O_2 debt during recovery (cf. Fig. 10 of Wood and Randall, '81). This was true of mild (0.2 BL/second) as well as severe exercise (0.5 BL/second), despite the fact that the former could be maintained for 2+ hours. As our exercise period was only 10 minutes, it is possible that these effects reflected a failure of the animal to come into a steady state with respect to O_2 delivery over the short time period, and therefore mainly O_2 "deficit" rather than true debt. However, the sheer size of the effects makes this difficult to accept. For example the O_2 debt (calculated from Fig. 10 of Wood and Randall, '81) after 10 minutes of mild exercise was ≃3500 μM O_2/kg, or enough to fuel resting aerobic metabolism for ≃1.5 hours. This was about 8-fold greater than the actual extra O_2 uptake during the 10-minute exercise period. (The data of Herreid et al. ('79) on *Cardisoma guanhami* after similar mild exercise (≃0.2 BL/second) indicate almost identical figures.) The O_2 debt after severe exercise was almost twice as large, ≃7300 μM O_2/kg, or 11-fold the actual increase during exercise.

In vertebrates, the whole subject of O_2 debt is controversial and unsettled (Hagberg et al., '80). Traditionally, the debt has been divided into a rapidly repaid "alactic portion" and a slowly repaid "lactic portion." The former was thought to represent O_2 used to restore depleted O_2 reserves and the intracellular phosphate energy change, and the latter to represent O_2 used to metabolize lactic acid back to glycogen (Astrand and Rodahl '70; Bennet '78). However, an alternative view has arisen that elevated postexercise $\dot{M}_{O_2}$ is not necessarily repayment of an energetic debt incurred during exercise but rather simply a reflection of elevated tissue metabolism during the postexercise period. Recent work has stressed the importance of elevated tissue temperatures after exercise as a cause of this elevated metabolism (Brooks et al., '71; Hagberg et al., '80) during the slow phase.

In *Oxygen transport in the haemolymph* above, we have calculated that restoration of haemolymph O_2 reserves would account for ≃91 μM O_2/kg. As haemolymph, containing haemocyanin, is about 31% of body weight (J.N. Cameron, personal communication) and there is apparently no intracellular O_2 pigment (Prosser, '73), then the figure for the whole body should be no more than 200 μM O_2/kg. By analogy to the mammalian situa-

tion, the phosphate debt at most might be 3-fold higher (≈600 μM O_2/kg) (Astrand and Rodahl, '70). Measured O_2 debts in *Cardisoma* were 3,500–7,300 μM O_2/kg. Clearly, in terms of traditional theory, the major portions of the debts were of the slow "lactic" type, persisting 3–5 hours postexercise (cf. Fig. 10 of Wood and Randall, '81). In a poikilotherm such as *Cardisoma* it is most unlikely that body temperature would be increased to a sufficient extent and duration after exercise to explain this long-term elevation of $\dot{M}_{O_2}$. The question is therefore whether the traditional explanation of lactate metabolism is applicable.

There was no relationship between haemolymph lactate levels (Fig. 5A, B) and the extent of the O_2 debt (cf. Fig. 10 of Wood and Randall, '81). This is illustrated in Figure 9, which compares the measured O_2 debt with the standard Δ lactate estimate of this parameter used in mammals. If it is assumed that lactate is freely distributed throughout the body water, this estimate is calculated as the measured Δ lactate concentration × the O_2 equivalence factor (≈0.5 mM O_2/mM lactate) × the body water content (643 ml/kg, J.N. Cameron, personal communication) (Astrand and Rodahl, '70). The Δ lactate index underestimated the O_2 debt early in recovery and overestimated it late in recovery because lactate levels remained elevated well after the O_2 debt had been paid off.

A similar situation has been reported in some lower vertebrates (Bennet, '78). This lack of relation is perhaps not surprising in view of the different time courses of lactate and pyruvate variation (Figs. 5, 8). The lactate: pyruvate ratio (Table 3) paralleled the O_2 debt quite closely. Huckabee ('58a, b) has pointed out that lactate may be elevated simply because pyruvate is elevated, and not because of deficiency of O_2, and that pyruvate levels may vary for a variety of reasons not directly associated with anaerobiosis. To correct for this, he introduced the "excess lactate" concept which compensates for pyruvate elevation:

$$\text{"excess lactate"} = [\text{Le} - \text{Lc}] - [\text{Pe} - \text{Pc}]\frac{\text{Lc}}{\text{Pc}}$$

where L = lactate, P = pyruvate, c = control, and e = experimental. In the mammalian literature, "excess lactate" is as controversal as the concept of O_2 debt itself. While the expression has been validly criticized on theoretical grounds (e.g., Harris, '69), in practice it appears a more reliable index of O_2 debt than

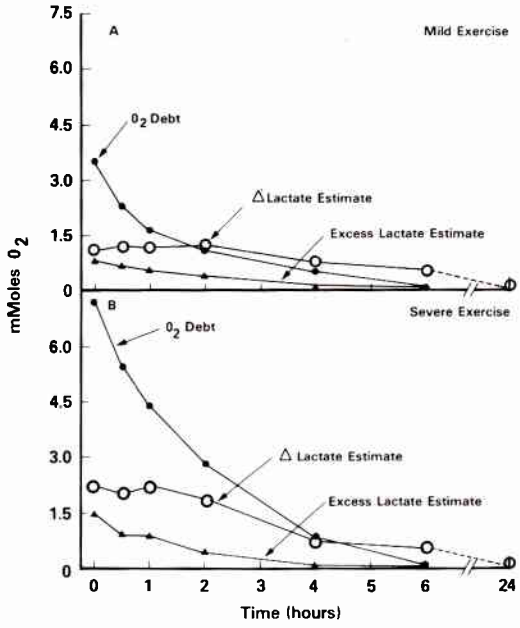

Fig. 9. Changes in the measured oxygen debt and the "Δ lactate" and "excess lactate" estimates of this debt in *Cardisoma* after (A) mild or (B) severe exercise. The oxygen debt is derived from the data in Figure 10 of Wood and Randall ('81). The "Δ lactate" and "excess lactate" estimates of the debt are derived from the data in Figure 5A, B, and Figures 5A, B, C, D, respectively, by methods outlined in the text. Note the proportionality between the measured O_2 debt and the "excess lactate" estimate, but the complete lack of correlation with the "Δ lactate" estimate.

lactate alone (Huckabee, '58a, b; Cain, '77; Vanuxem et al., '80). Substituting "excess lactate" for Δ lactate in the standard formula provides a much more satisfactory index of the O_2 debt in *Cardisoma* (Fig. 9). The "excess lactate" estimate is directly proportional to the measured O_2 debt throughout recovery, but quantitatively accounts for only about 30% after mild exercise (Fig. 9A) and 20% after severe exercise (Fig. 9B). The most likely explanation for this discrepancy is that in *Cardisoma* lactate is not freely distributed throughout the body water as assumed for the mammalian situation, but rather that there are high tissue to blood lactate gradients, as for example, in fish (Wardle, '78), and thus haemolymph levels underestimate whole body levels. Phillips et al. ('77) have provided some evidence that this is indeed the case in the lobster *Homarus gammarus*.

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