

Acid-base regulation in exercising squid (*Illex illecebrosus*, *Loligo pealei*)

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PÖRTNER, H. O., D. M. WEBBER, R. G. BOUTILIER, AND R. K. O'DOR. Acid-base regulation in exercising squid (*Illex illecebrosus*, *Loligo pealei*). *Am. J. Physiol.* 261 (Regulatory Integrative Comp. Physiol. 30): R239–R246, 1991.—Squid (*Illex illecebrosus*, *Loligo pealei*) were cannulated in the vena cava and swum in a Beamish-type respirometer. Gas tensions and acid-base variables as well as octopine levels were estimated in samples of the mantle and of venous blood collected from quiescent, exercised, and recovered animals. When exhausted, both species exhibited a decrease in vena cava oxygen tensions and a slight alkalosis. With high swimming speeds prior to exhaustion in *Illex* a slight acidosis developed in the blood, which was linked to a severe intracellular acidosis. Generally, the drop in intracellular pH was linearly correlated with octopine accumulation in this species. Metabolic proton (and end-product) release from the mantle, however, was minimal, thus protecting arterial oxygen binding. High PCO₂ values in the mantle of both species lead to the conclusion that the vena cava values analyzed in this and all literature studies on unrestrained cephalopods may not reflect the scope of respiratory acid-base changes in venous blood. Although metabolic changes in blood acid-base status are negligible, the respiratory acidification of venous mantle blood may allow for a classical function of Bohr and Haldane effects in these animals.

anaerobic glycolysis; cephalopod muscle; extracellular pH; intracellular pH; intracellular partial pressure of carbon dioxide; hemocyanin; muscular fatigue; octopine; oxygen binding; vena cava

PELAGIC SQUID are the most powerful invertebrate swimmers of the sea. Specializing in jet propulsion for speed generation, these animals come close to the performance of their piscine competitors. The major constraint on cephalopods, caused by the use of jet propulsion, is a maximization of metabolic rates, with high resting rates of oxygen consumption and limited factorial aerobic scopes (for review see Ref. 20). Squid are highly aerobic animals (2, 18) with a very limited capacity to tolerate hypoxia (32). The relationship between swimming speed and the rate of oxygen consumption is exponential (19, 36), as in fish (16), suggesting that the major fraction of the energy required for swimming activity is produced by aerobic means.

Nonetheless, squid are able to produce energy by anaerobic means during swimming to exhaustion. High octopine levels were found in fatigued squid, *Loligo vulgaris* (8). No octopine accumulation, however, occurred

in another species of squid, *L. pealei* (33). In accordance with theoretical evidence (11, 23, 24) octopine has been demonstrated to cause acidosis during both functional and environmental hypoxia (5, 27).

High aerobic performance of the muscular system of squid is supported by an efficient oxygen transport system including an adequate circulatory system (22). The respiratory pigment hemocyanin is highly pH sensitive (29, 32). However, the function of high Bohr and Haldane effects in cephalopods is controversial (e.g., Refs. 3, 29), and it is unknown whether metabolic acidification during octopine formation interferes with pigment function. Accordingly, our knowledge of hemocyanin O₂ carriage in squid is rather poor, since changes in the acid-base status have never been studied in unrestrained animals. Early studies by Redfield and Goodkind (32) were carried out on restrained (nailed down) specimens of *L. pealei*.

This study was designed as a comparative study of the intra- and extracellular acid-base variables during exhaustive muscular activity and subsequent recovery in two species of squid (*Illex illecebrosus* and *L. pealei*). These two species differ in their mode of locomotion in that *Loligo* relies on swimming by undulatory fin movements to a much larger extent and may use jet propulsion less frequently than *Illex*. Similar to all other studies on unrestrained cephalopods (e.g., Refs. 12, 15), a procedure was developed to cannulate the anterior vena cava as an accessible vessel in these animals. Octopine was analyzed in blood and tissue samples as a key substance of anaerobic activity metabolism. All experiments were performed on cannulated, unrestrained squid swimming in a tunnel respirometer.

MATERIALS AND METHODS

Animals. Squid (*I. illecebrosus*, 300–500 g, *L. pealei*, 200–400 g) were caught by commercial fishermen in St. Margarets Bay (close to Mill Cove) or close to Herring Cove, Nova Scotia, Canada, from October to December 1986. The animals were placed in plastic bags filled with oxygenated seawater at 2–6°C and transported to Halifax. There they were held in running seawater at ambient temperatures of 8–15°C. At high ambient temperatures, they were used as soon as they recovered from transport and handling (after 2–4 h). When ambient temperatures fell below 12°C, the animals were brought close to the experimental temperature for 12–24 h before being used.

Anesthesia and cannulation. Hypoxia sensitivity of squid is far greater than in cuttlefish (15) or octopuses (14). Therefore, surgery on anesthetized squid had to be complete within 10–12 min. Thus only one catheter could be implanted into one readily accessible vessel, the anterior vena cava. Individual animals were prepared for the anesthesia by keeping them in seawater of 5–7°C preequilibrated with pure oxygen. After 10–15 min, the animal was placed in oxygenated anesthetic (prepared from 7.5% $MgCl_2 \cdot 6H_2O$ in distilled water mixed with an equal volume of seawater; Ref. 17) at the same temperature. Muscle relaxation occurred within a few minutes. The anesthetized animal was placed on a plastic bag filled with ice and covered with a leather cloth soaked with seawater to prevent skin damage. The mantle cavity was perfused by recirculating cooled oxygenated anesthetic across both gills.

Successful cannulations were performed on animals with fully oxygenated (blue) blood in the vena cava, which was exposed by “unsnapping” the funnel from the ventral mantle. The vessel was punctured by use of a bent needle (20 gauge), colored with black india ink to mark the site of the puncture. A PE-50 cannula was drawn to a reduced diameter, blockage being prevented by three added holes at the tip. The catheter was bent (by 180°) 2–5 cm from the end (depending on the size of the animal), filled with filtered seawater, and fed into the lumen of the vena cava immediately after puncturing. There it was tied to the wall of the vena cava with a fine surgical suture and sealed using Bostik no. 7432 cyanoacrylate glue (Bostik, Oberursel, FRG). To prevent the animal from grabbing and chewing on the cannula, it was led back and fed through the mantle wall close to the posterior end of the mantle cavity, where it was secured in place by suture and cyanoacrylate glue. Surgery was finished by “resnapping” the funnel to the mantle.

After surgery, the animals were returned to oxygen-enriched seawater of 10–15°C and watched until mantle and heart contraction resumed. Recovery occurred within 5–7 min during which time stimulation of the mantle and the heart was carried out by gently squeezing the mantle so as to simulate contractions in the resting animal. This manipulation led to early ventilation of the mantle cavity, oxygenated blood at the gills, and an early onset of cardiac activity.

Experimental procedure. After an initial recovery period of at least 30 min, the cannulated animals were placed in a Beamish-type respirometer (Ref. 6, modified according to Ref. 36) filled with 92 liters of normoxic seawater at $15 \pm 0.5^\circ C$. Water flowed through the animal chamber continuously at ~ 0.07 m/s, with partial replacement on each circuit to maintain high oxygen tensions. In accordance with a rapid recovery of squid from exercise (19, 35), experimentation started after ~ 1.5 h of recovery from handling. The analysis of tissue phosphagen, octopine, and pH levels suggested that the animals were stable and that they were in aerobic steady state after this time period.

A control blood sample was withdrawn via the indwelling catheter using 1-ml syringes. Squid were then exercised tail first by subjecting them to increasing current

speeds. Cannulated squid reached swimming speeds close to those recorded by Webber and O’Dor (36) and Freadman et al. (7). Typically, the velocity was increased by steps of 0.07 m/s every 5–10 min, until the animals showed the first signs of fatigue (unstable swimming, touching the downstream grid). They were maintained at this speed, which was assumed to be close to the critical swimming velocity, until they collapsed from exhaustion. At the end of the exercise period, the water circulation was reduced to 0.07 m/s, and a blood sample was withdrawn from the exhausted animals. Some of the exhausted squid were removed from the respirometer thereafter and were quickly decapitated for tissue sampling (see below). Others were allowed to recover from exercise, and the blood was sampled during the recovery period.

For tissue sampling from control and recovering animals, the respirometer was closed and the animal anesthetized by adding 2 liters of pure ethanol to the water circulation downstream of the animal, so that it was fully mixed as it returned. Animals progressed to full anesthesia (indicated by the cessation of ventilation) during 3–5 min with no agitation or startle response. Thereafter, the animals were removed from the respirometer and quickly decapitated. A piece of muscle (6–10 cm long) was immediately excised from the left or right ventral mantle using two scalpel blades that had been arranged in parallel at a distance of 11 mm. The excision was made against an aluminium ruler 2.5 cm wide inserted into the mantle cavity. The muscle sample was freeze-clamped immediately (39), wrapped in aluminium foil, and stored under liquid nitrogen until analyzed.

Analyses. Blood samples were analyzed for pH, PO_2 , and PCO_2 using Radiometer (Copenhagen) equipment (BMS 3, thermostated to the incubation temperature of the animals, $\pm 0.1^\circ C$). The electrodes were calibrated with precision phosphate buffers (pH, Radiometer) or calibration gases (O_2 , CO_2 electrodes). All gases were prepared from pure nitrogen, CO_2 , and O_2 by gas-mixing pumps (type 303/a-F, Wösthoff, Bochum, FRG) and saturated with water at $15 \pm 0.1^\circ C$. Blood bicarbonate values were obtained by calculation using values of CO_2 solubility α and pK'''' , as calculated according to Heisler (10).

With the use of tissue samples stored under liquid nitrogen, intracellular pH and tissue concentration of CO_2 (CCO_2) were evaluated applying the homogenate technique described by Pörtner et al. (31). In brief, tissue samples were ground under liquid nitrogen using a porcelain mortar and pestle. Care was taken so that, during the work with liquid nitrogen, contamination of the muscle powder with condensing CO_2 was minimized. In a closed Eppendorf tube, the tissue powder was mixed with ice-cold medium (pH ~ 7) containing potassium fluoride (160 mmol/l) and nitrilotriacetic acid (2.9 mmol/l; Sigma, St. Louis, MO). After brief centrifugation (5–15 s), pH and total CO_2 were analyzed in the supernatant as described by Pörtner et al. (31). Based on the analysis of mean intracellular pH in the homogenates and on the evaluation of appropriate values for pK'''' and CO_2 solubility (10), intracellular PCO_2 values were then calculated for the mantle muscle using a modified Henderson-Hasselbalch equation (31).

TABLE 1. Model calculations to evaluate influence of mitochondria (%fractions based on Refs. 2, 18) on calculation of intracellular PCO₂ values (31) for squid (*Illex illecebrosus*) mantle tissue under control conditions

Model Parameters	n
Vena cava	
PVCO ₂ 1.8 ± 0.3 Torr	12
Mantle tissue	
81% anaerobic fibers: 6.4% mitochondria	
19% aerobic fibers: 47% mitochondria	
Whole tissue: 14.1% mitochondria	
F _{matrix} = 0.08	
CiCO ₂ = 4.79 ± 1.07 mmol/l	5
pH _{i,hom} = 7.38 ± 0.02	5
PiCO ₂ = 5.8 ± 1.3 Torr = PiCO _{2,max}	5
ΔpH _{m-c} = 0.6; β _{CNB} /β _{MNB} ~2.7	
pH _c = 7.36	
pH _m = 7.96	
CcCO ₂ = 3.9 mmol/l	
CmCO ₂ = 14.8 mmol/l	
pH _{i,weakacid} = 7.45	
PcCO ₂ = PmCO ₂ = 4.9 ± 1.1 Torr = PiCO _{2,min}	5

Values are means ± SD; n, no. of animals. Calculation of weak acid intracellular pH (pH_i) is a high estimate valid with a mitochondrial-cytosolic ΔpH of 0.6, as cited for mammalian tissue, and with a ratio of cytosolic-to-mitochondrial buffer values of ~2.7 (Refs. 30, 31, and unpublished observations). Intracellular PCO₂ (PiCO₂) values derived from homogenate pH_i and from weak-acid pH_i are discussed to represent minimal and maximal estimates of mean PiCO₂ (see text). C, concentration; c, cytosolic; hom, homogenate; i, mean intracellular; m, mitochondrial; v, venous; F_{matrix}, fraction of mitochondrial matrix in tissue; β_{NB}, nonbicarbonate buffer value.

As discussed earlier (31), effective mean intracellular pH (pH_i) values obtained by use of the homogenate technique are lower than those derived from weak acid [5,5-dimethylloxazolidine-2,4-dione (DMO)] distribution in tissues containing high fractions of mitochondria. Because CO₂ as a weak acid requires a weak acid-derived value of pH_i for PCO₂ calculations, the use of homogenate-derived pH_i values may yield overestimates of mean intracellular PCO₂. Table 1 provides an estimate of the maximum error introduced. The difference between the calculated PCO₂ values is small. In addition, in the special case of squid mantle tissue, pH values obtained from weak-acid distribution may yield only a low estimate for mantle PCO₂, because gas exchange via the skin (22, 29) may cause an unequal CO₂ profile across the muscle. Thus PCO₂ in the more peripheral aerobic layers is probably lower than in the central, anaerobic part of the mantle. The maximum PCO₂ estimate obtained by use of the homogenate pH_i (Table 1) is, therefore, assumed to be closer to the central PCO₂ value of the mantle.

Respiratory and nonrespiratory changes in the acid-base status were analyzed with a pH/bicarbonate diagram. The nonbicarbonate buffer values of the blood and of the mantle muscle of these animals have previously been published (28-30).

Samples of the tissue powder (see above) were extracted in perchloric acid according to Beis and Newsholme (1). Blood samples were deproteinated by the addition of perchloric acid (3 mol/l) to a final concentration of 0.6 mol/l. After centrifugation, the extracts were neutralized with KOH (5 mol/l) and solid K₂CO₃/KHCO₃ (1:6, wt/wt; Ref. 30). The precipitate was removed by centrifugation. Octopine was assayed in the extracts

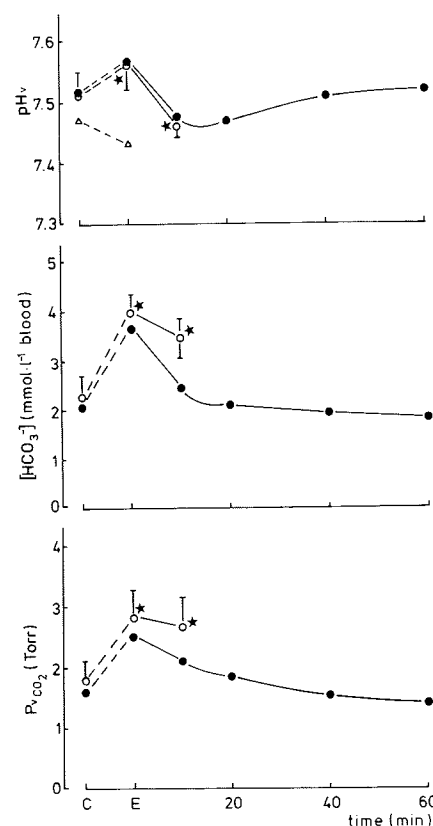


FIG. 1. Acid-base parameters in blood withdrawn from anterior vena cava of squid (*Illex illecebrosus*) during exercise (E) and subsequent recovery (E = 0 min; n = 12 for controls, C). Changes are depicted as they occurred in 8 animals swum to exhaustion (open circles, means ± SD; *significantly different from control values). Triangles, pH values for 1 additional animal that reached high swimming speeds and exhibited an extracellular acidosis (instead of an alkalosis) after exercise (see text). Five exhausted animals were allowed to recover for 10 min. One additional animal was used to monitor blood values during exercise and a subsequent 60-min period of recovery (closed circles) before sampling of tissue occurred (cf. Fig. 3). PVCO₂, venous partial pressure of CO₂; pH_v, venous pH.

according to Grieshaber et al. (9).

Differences were tested for significance at the 5% level by using Student's *t* test for unpaired samples. Extreme values that differ significantly from the norm (Nalimov's test) are discussed separately.

RESULTS

Figures 1-4 and Table 2 depict the changes in the intra- and extracellular gas tensions and acid-base status during and after activity in *I. illecebrosus* and *L. pealei*. Table 3 gives the lengths of the exercise periods and the maximum swimming speeds. Extracellular data given for control and exercised animals comprise all values measured under control and exercise conditions in animals killed after 10 min of recovery (cf. legend of Fig. 1).

In *I. illecebrosus*, pH changes in the venous blood were small during exercise compared with those occurring intracellularly (Figs. 1 and 3). In most animals, there was a significant 0.05 pH unit increase in venous (vena cava) pH starting at pH = 7.51 ± 0.04 (mean ± SD, n = 12). However, among the animals sampled directly after fatigue, the specimen with the highest swimming speed

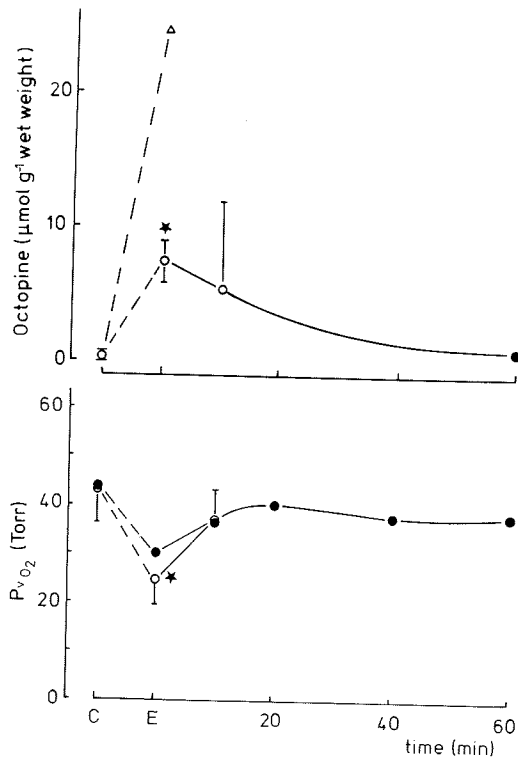


FIG. 2. Changes in mantle muscle octopine levels and in vena cava blood PO₂ (means \pm SD, n as in Fig. 1) during exercise and recovery in squid (*I. illecebrosus*). For octopine measurements animals were anesthetized and/or killed after blood samples had been withdrawn (C: open circles, $n = 5$; E: $n = 3$; 10 min, $n = 5$; *significantly different from controls). P_{vO₂}, venous partial pressure of O₂. For further explanations see Fig. 1.

(0.81 m/s instead of a mean of 0.68 ± 0.11 m/s; Table 3) showed a slight extracellular acidosis with pH falling from 7.47 to 7.43. Because extracellular pH and all intracellular parameters in this animal could be identified to differ significantly from the other values, they are given as examples showing extreme changes during fatigue (in Figs. 1–3, 5, and 6). This conclusion is based on Figs. 4 and 6 (see DISCUSSION). We have seen in previous studies that some animals are dominant in their schools and are more active and aggressive. Presumably, the single "athletic" animal represents such an extreme.

Exhausted animals exhibited a significant increase in venous bicarbonate levels by 1.7 mmol/l blood (starting from 2.3 ± 0.4 mmol/l blood) associated with a significant increase in PCO₂. The PO₂ in blood withdrawn from the vena cava decreased by 19 Torr (starting from 43.2 ± 7.0 Torr) during the exercise period (Fig. 2). Octopine accumulated in the mantle of *I. illecebrosus* starting from 0.5 ± 0.2 $\mu\text{mol/g}$ wet wt and reached 24.7 $\mu\text{mol/g}$ wet wt in the fastest animal (Fig. 2). Blood octopine levels remained below 15 $\mu\text{mol/l}$ and did not change significantly during activity or subsequent recovery.

Mean pH_i values found in squid mantle muscle by use of the homogenate technique (31) are close to pH_i values determined by use of DMO in the muscle tissue of the marine invertebrate *Sipunculus nudus* and by use of ³¹P-nuclear magnetic resonance (NMR) and DMO in white molluscan muscle at the same temperature (25, 28, 34, 40). The intracellular pH in the mantle fell significantly

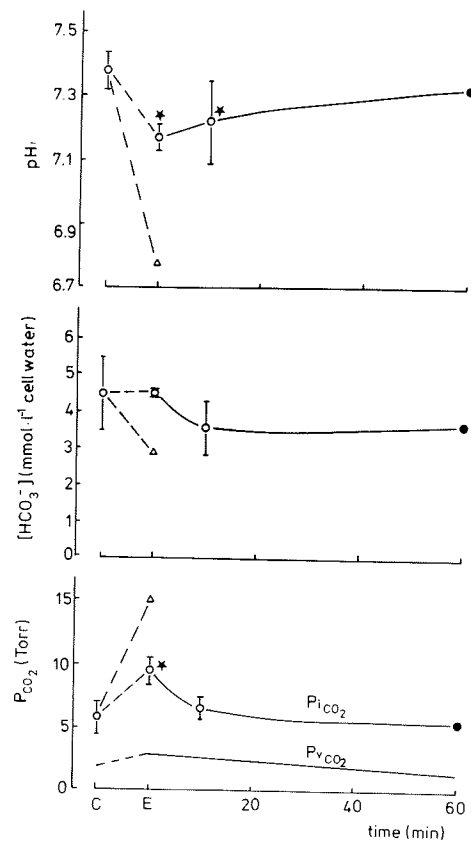


FIG. 3. Intracellular acid-base parameters in mantle tissue of squid (*I. illecebrosus*) sampled after exercise and subsequent recovery (cf. Figs. 1 and 2 for symbol definitions). P_{iCO₂}, intracellular PCO₂; pH_i, intracellular pH.

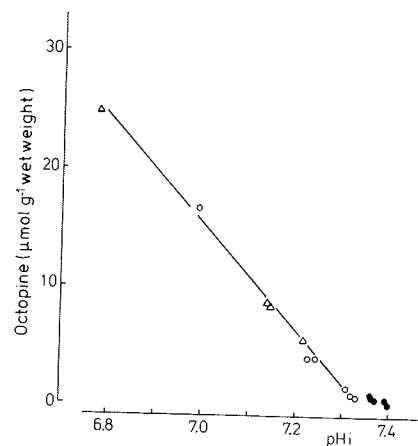


FIG. 4. Comparison of intracellular pH and octopine levels in mantle muscle of squid (*I. illecebrosus*) subjected to exhaustive swimming and subsequent recovery (closed circles, control; open circles, recovery; triangles, exhausted).

by an average of 0.21 pH units in three animals starting from 7.38 ± 0.02 ($n = 5$). In the fastest specimen, it reached a low value of 6.78 instead and was correlated with an extracellular acidosis and a high octopine content in the mantle (see above, Fig. 4). Intracellular bicarbonate levels remained more or less constant during exercise (only the fastest animal experienced a drop by 1.6 mmol/l cell water). Under control conditions, PCO₂ values in the intracellular space of the mantle (5.8 ± 1.3 Torr, $n = 5$) were more than three times higher than in the vena

TABLE 2. Changes in blood oxygen tensions, tissue octopine levels, and in the intra- and extracellular acid-base status of squid *Loligo pealei* during and 10 min after swimming to exhaustion

	Vena Cava				Mantle			
	pH _v	P _{vCO₂} , Torr	[HCO ₃ ⁻], mmol/l	PO ₂ , Torr	pH _i	P _{iCO₂} , Torr	[HCO ₃ ⁻], mmol/l	Octopine, μmol/g wet wt
Control	7.46±0.03	2.0±0.2	2.2±0.4	43.4±8.0	7.24	8.0	4.6	0.5
<i>n</i>	7	7	7	7	(7.21, 7.28) 2	(7.1, 8.9) 2	(3.8, 5.5) 2	1
Exhaustion	7.53±0.04*	2.9±0.4*	3.8±0.6*	24.5±7.2*	7.22±0.09	8.2±2.0	4.4±0.3	3.0±0.9
<i>n</i>	6	6	6	6	3	3	3	3
Recovery (10 min)	7.52±0.02*	2.7±0.5*	3.4±0.8*	38.9±11.9*	7.30±0.05	6.0±1.3	3.9±0.5	1.9±1.4
<i>n</i>	3	3	3	3	3	3	3	3

Values are means ± SD; *n*, no. of animals. For *n* = 2, table shows means and individual values (in parentheses). Octopine levels in blood remained below 11 μmol/l and did not change significantly under experimental conditions applied; T = 15°C. Brackets indicate concentration. * Significantly different from controls.

TABLE 3. Maximum swimming speeds, lengths of exercise periods, and lengths of time at highest swimming speeds for specimens of *Illex illecebrosus* and *Loligo pealei* swum to exhaustion

	<i>Illex</i>			<i>Loligo</i>		
	V _{max} , m/s	t _{Vmax} , min	t _{ex} , min	V _{max} , m/s	t _{Vmax} , min	t _{ex} , min
Exhaustion	0.68±0.11	11.5±5.0	42.8±10.8	0.60±0.04	7.0±4.6	52.3±8.4
<i>n</i>	4	4	4	3	3	3
Recovery (10 min)	0.67±0.15	5.9±2.6	45.2±13.7	0.54±0.13	8.7±4.2	52.0±14.4
<i>n</i>	5	5	5	3	3	3
Recovery (60 min)	0.55	8.0	32.0			

Values are means ± SD; *n*, no. of animals. V_{max}, maximum velocity; t_{ex}, length of exercise period; t_{Vmax}, length of time at highest swimming speed.

cava. Exercise caused intracellular PCO₂ to increase significantly by 3.8 Torr (up to 9.3 Torr in the fastest animal).

During 10 min of recovery, blood oxygen levels recovered to values that were close to those observed under control conditions (Fig. 2). Venous pH fell slightly but significantly below that seen under control conditions and reached an average of 7.46 ± 0.02 (*n* = 5, Fig. 1). This drop was associated with a decrease in bicarbonate levels (to 3.0 ± 0.4 mmol/l) and a drop in venous PCO₂ (to 2.7 ± 0.5 Torr). Both variables, however, remained significantly above control values. Blood acid-base changes during long-term recovery were investigated in one animal, which had exhibited changes during the exercise period similar to those observed in the other specimens (Figs. 1 and 2). The extracellular acidosis at 10-min recovery was reversed in this one animal during 40–60 min of recovery, associated with a drop in venous PCO₂ and bicarbonate levels (Fig. 1).

Intracellular PCO₂ had already fallen close to control values after 10 min of recovery (6.5 ± 0.8 Torr, *n* = 5). Intracellular pH was still below that seen under control conditions by an average of 0.16 units (Fig. 3), and octopine levels were still elevated (Fig. 2). Intracellular bicarbonate levels had fallen during the initial recovery period but were still not significantly different from those observed under control conditions. The tissue sample taken after 60 min of repeated blood sampling (see above) indicates that recovery of all metabolic, gas, and acid-

base parameters was complete by this time.

Table 2 shows the data available for *L. pealei*. As in *Illex*, extracellular pH increased significantly during activity (by 0.07 pH units). Vena cava PCO₂ also rose, whereas the PO₂ fell to the same extent as in *Illex*. As in *Illex*, intracellular PCO₂ values in the mantle were three to four times higher than in vena cava blood. At maximum swimming speeds (0.60 ± 0.04 m/s, Table 1) similar to *Illex*, pH_i and intracellular PCO₂ changes in *Loligo* as well as changes in intracellular bicarbonate levels were minor. Octopine levels remained below 4 μmol/g wet wt. During recovery, the PCO₂ in the vena cava decreased, whereas the PO₂ rose, coming close to control values. The extracellular acidosis observed after 10 min of recovery in *Illex* was not found in *Loligo*.

DISCUSSION

We report here for the first time measurements of blood and tissue acid-base variables of cannulated free-swimming squid. Swimming to fatigue caused intracellular pH to fall in *I. illecebrosus*. This drop is clearly related to the accumulation of octopine (Figs. 2 and 3), leading to the conclusion that anaerobic glycolysis predominates in determining the changes in the intracellular acid-base status (Fig. 4). When *L. pealei* were swum to exhaustion, changes in the intracellular acid-base status were not as extensive as in *I. illecebrosus*. This can be explained by the comparatively lower octopine formation in *Loligo* (Table 3 and Ref. 33), even though it is higher

in the present study than previously described (33). Lower octopine formation in *L. pealei* may mean that the ability to extend the swimming performance beyond the aerobic scope is lower in *Loligo* than in *Illex*. Indeed, *Loligo* may be better suited for low-speed aerobic cruising using its fin undulations; *Illex*, on the other hand, possesses much smaller fins and probably relies on jet propulsion more frequently and/or may operate at higher speeds more often (cf. Refs. 21, 37). However, the similarity of all other changes observed during exercise and recovery would suggest that both oegopsid (oceanic) and myopsid (inshore) squid have evolved similar mechanisms for maximizing performance.

Blood collected from the anterior vena cava of both species exhibited changes that indicate an increase in metabolic rate with exercise, i.e., venous PO_2 fell, whereas PCO_2 increased. Analysis of the extracellular acid-base changes in *Illex* (Fig. 5) and *Loligo* (based on Table 2) reveals a nonrespiratory alkalosis in most animals. At least part of the extracellular alkalosis seen in vena cava blood accords with proton removal during hemocyanin deoxygenation, as evidenced by the drop in venous PO_2 (Fig. 2). Bohr and Haldane coefficients around -1 would almost certainly contribute to an alkalosis if the oxygen carried by the pigment is replaced by CO_2 (based on a respiratory quotient of 0.85; Ref. 29). pH changes similar to those in exercising *Illex* and *Loligo* were also observed in the vena cava blood of other cephalopods during hypoxia or exercise (hypoxic *Sepia officinalis*, Ref. 15; hypoxic *Octopus vulgaris*, Ref. 12; *O. vulgaris* after 10 min of exercise, Ref. 13).

Only when a severe intracellular acidosis was observed in the mantle (E' in Fig. 6) was the proton uptake during hemocyanin deoxygenation compensated for by some minor nonrespiratory (metabolic) acidification (E' in Fig. 5) leading to a drop instead of an increase in venous pH (see below). These findings may be comparable with a decrease in vena cava blood pH found during 20 min of exercise in walking *O. vulgaris* (13).

In most animals, the base excess formed in the vena

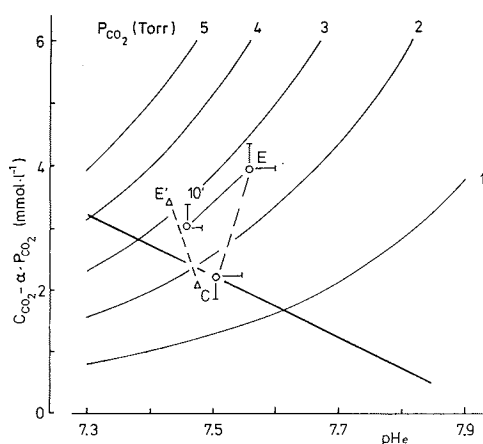


FIG. 5. Changes in acid-base status of blood withdrawn from vena cava of squid (*I. illecebrosus*) during exercise and 10 min of subsequent recovery. Value obtained after 60 min is virtually identical with control value. Buffer line represents nonbicarbonate buffer value of blood, as adopted from Ref. 29. Position of buffer line is valid for value of hemocyanin oxygenation under control conditions. For further explanations see Figs. 1 and 2 and text. CO_2 , concentration of CO_2 .

cava blood during exercise was removed during subsequent recovery, very likely with an increase in hemocyanin oxygenation being involved. When PO_2 returned to control during 10 min of recovery (Fig. 2), a slight respiratory acidification remained (Fig. 5), which was reversed during the subsequent 40 min of recovery (Fig. 1). Such a transient acidification was not observed after 10 min of recovery in *L. pealei* blood.

In the mantle (Fig. 6), a large respiratory component contributed to the intracellular acidosis, as indicated by the large increase in intracellular PCO_2 . The deviation of changes from the buffer line reflects the influence of metabolic acidification. The acidosis found in the athletic specimen of *Illex* (E') can be explained by an extrapolation from the values found in the other animals after exercise, with an increasing nonrespiratory component. This treatment is supported by the observation of a linear relationship between octopine levels and mantle pH_i (Fig. 4). A complete analysis of squid metabolism during exercise (unpublished observations) and of Fig. 6 reveals that the proton quantities formed in metabolism fully account for the changes in the intracellular mantle acid-base status and thus do not leave the cells. Based on blood and tissue measurements, a minor release of metabolic protons into the blood was only evident during severe acidification of the tissue (see above). The negligible changes in blood octopine levels and the observation that metabolic proton quantities remain intracellularly would suggest that both H^+ and octopine are metabolized during recovery in situ and do not disturb blood homeostasis.

The slight increase in pH_i during 10 min of recovery is associated with a rapid fall in intracellular PCO_2 and with decreasing octopine levels in most animals (Figs. 2-4). The large variability among the recovering animals is due to one animal, which reached a swimming velocity close to the maximum observed during the activity period and undoubtedly experienced high octopine levels (Fig. 4) and a severe intracellular acidosis during fatigue (i.e., close to point E' in Fig. 6). Consequently, the recovery time course for the athletic specimen (not shown in Figs.

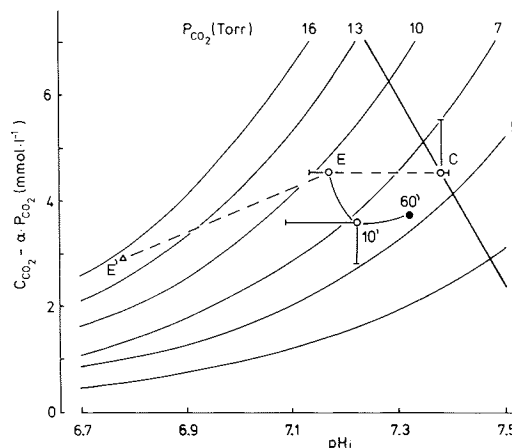


FIG. 6. Intracellular acid-base changes during exhaustive swimming and recovery in squid (*I. illecebrosus*) mantle tissue depicted in a pH/bicarbonate diagram. For explanation of symbols and n values see Figs. 1-3 and text. Slope of buffer line represents nonbicarbonate buffer value as adopted from Refs. 28 and 30.

1-6) would have been close to that depicted for the other animals. Generally, recovery from exhaustion is more rapid in squid than, e.g., in similar-sized fish (35), possibly related to the fact that the standard metabolic rate is close to ten times higher in these cephalopods.

Hemocyanin function. At a first sight, the finding of an alkalization in vena cava blood during exercise would support the generalized hypothesis brought forward by Brix et al. (3) that Bohr and Haldane effects in cephalopods would not serve to support venous oxygen unloading in the tissues but rather to enhance oxygen loading and CO_2 unloading at the gills. This conclusion is based on blood gas data from the vena cava of cephalopods (e.g., Refs. 13, 14, 16) and on the magnitude of the Bohr and Haldane coefficients (for a more complete discussion see Ref. 29). However, morphological data would already suggest that perfusion of the head and of the mantle occurs in two separate parts of the circulatory system (based on Refs. 2, 38). Mixed venous blood collected in the branchial hearts is oxygenated at the gills and divided between mantle and head via the ventricle. The venous blood traditionally analyzed in cephalopod studies would, therefore, be blood having come predominantly from the head and not from the mantle. The question arises whether the above explanation can really hold for the situation in all cephalopod tissues.

Changes observed in the vena cava during exercise may be due to an increase in metabolic rate in the head and arms. This change in cephalic metabolic rate may be related to an increase in brain activity or to the maintenance of posture in the head region, including the use of the arms as active "rudders." Alternatively, the changes in blood gas parameters could reflect an enhanced oxygen consumption by the mantle so great that arterial conditions are affected. If the gas exchange capacity of the gills is compromised during exercise, lower oxygen and higher CO_2 levels in arterial blood would result, thus affecting the gas values of the blood perfusing the head. However, for animals with such a high metabolic rate and small factorial aerobic scopes it seems unlikely that branchial gas exchange at the gills should become compromised, especially since branchial water flow necessarily increases with increased mantle contractions. High arterial oxygen tensions are maintained during exercise in another jet-propelled cephalopod, the *Octopus* (13). Therefore, increased oxygen consumption by the cephalic region seems more likely to cause the changes in vena cava parameters.

Based on the evidence discussed above, the morphological situation does not change the conclusion that metabolic acidification of all blood is negligible. If octopine is formed during functional anaerobiosis, all metabolic protons remain trapped in the tissue cells until very high octopine levels are reached. Therefore, metabolic acidification does not influence hemocyanin function. This is important in arterial blood where, despite a respiratory release of CO_2 , any metabolic acidification could decrease arterial oxygen binding. Maintenance of pH in arterial blood is essential to stabilize the oxygen-carrying function of the hemocyanin (29).

However, respiratory changes in blood collected from the anterior vena cava may be smaller than those in

venous blood returning from the most active mantle tissue, already under control conditions. Under all circumstances, the difference of P_{CO_2} values in the mantle and in the vena cava blood of both *Illex* and *Loligo* may be considered large. Changes in intracellular mantle P_{CO_2} during exercise in *Illex* were much larger than those observed in vena cava blood (Figs. 1 and 3), causing an intracellular and probably also an extracellular respiratory acidosis. As a corollary, P_{CO_2} values found in the vena cava under control and exercise conditions may not fully reflect the respiratory status of all tissues, and thus conclusions concerning blood pigment function in cephalopods should not be exclusively based on the alkalization prevailing in vena cava blood during exercise or hypoxia. Although metabolic influences on blood pH are minor, respiratory CO_2 may cause mantle blood to be more acidic than blood returning from the head, thus compensating for the alkalizing effect of hemocyanin deoxygenation. Bohr and Haldane effects could then function according to the classical understanding (cf. Ref. 29). Further analyses are required to investigate this hypothesis.

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