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SOME *IN VIVO* AND *IN VITRO* CHARACTERISTICS OF *APLYSIA CALIFORNICA* HAEMOLYMPH*

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The nervous system of the opisthobranch mollusc, *Aplysia californica* Cooper has been extensively studied and a great deal is known about the electrophysiological properties of a number of identifiable neurones (see Kandel, 1976, 1979). A variety of synaptic and electrical connections have been mapped, permitting identification of the mode in which nervous control is exerted over certain motor functions. Substantially less is known regarding other physiological systems of *Aplysia* (Kandel, 1979), particularly the respiratory system, and this limits the description of how nervous control is affected by internal physiological state. Chalazonitis & Nahas (1965) reported data for haemolymph pH, CO_2 tension (P_{CO_2}) and O_2 tension (P_{O_2}) in the related species, *Aplysia fasciata*, and Bevelaqua *et al.* (1975) identified the respiratory pigment, haemocyanin, in the haemolymph of *A. californica*. However, there has been no detailed study of *in vivo* oxygen levels or acid-base status of *A. californica*. Neither are there any data for the *in vitro* properties of *A. californica* haemolymph, such as oxygen carrying capacity, oxygen affinity or buffering properties. Such data are of particular importance in *A. californica*, for two reasons. Firstly, motor function, such as the often-studied gill withdrawal reflex, may be directly influenced by factors such as pH, P_{CO_2} or P_{O_2}, as are the respiratory performances of other marine invertebrates (Mangum & Burnett, 1975; McMahon & Wilkens, 1975; Bayne, 1976; Batterton & Cameron, 1978). Secondly, Chalazonitis (1974) has shown clearly that membrane potential, spike frequency and membrane resistance of many single cells in *A. fasciata* are directly affected by pH, P_{O_2} and P_{CO_2} of the bathing fluid. Therefore, the present study was undertaken to describe some of the *in vivo* and *in vitro* properties of the haemolymph of *A. californica* with regards to acid-base status and oxygenation characteristics.

A. californica (37–370 g) were obtained commercially from Pacific Biomarine Supply Co. (Venice, California) during September–December, 1980 or were freshly collected by hand in the vicinity of San Diego, California, January 3, 1981. Animals were transported to the University of Calgary and maintained in a recirculating sea

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Table 1. In vivo *haemolymph acid-base status and oxygen levels in the pedal sinus of* A. californica, 15 °C; 32–35‰. *Data are* $\bar{x} \pm 1$ S.E.

	pH	P_{CO_2} (mmHg)	C_{CO_2} (mM)	P_{O_2} (mmHg)	C_{O_2} (mM)	$C_{O_2}^{max}$ (mM)	*n*
Freshly collected San Diego	7·522 ±0·017	3·04 ±0·35	4·48 ±0·45	26·3 ±5·2	0·038 ±0·012	0·32 ±0·017	5
Commercially obtained Venice	7·560 ±0·032	3·7 ±0·17	5·30 ±0·42	39·7 ±4·3	0·060 ±0·011	0·21 ±0·007	6

water system at 15 °C, 32–35‰, pH 7·9–8·1 (elevation *ca.* 1050 m). Animals from Venice, were fed once per week (Lukowiak, 1980), those from San Diego, fed *ad libitum* and all experiments were conducted within 4 weeks of animal arrival.

Haemolymph samples of 0·5–1·0 ml were withdrawn anaerobically from the pedal sinus into 1·0 ml glass syringes and immediately analysed for pH, CO_2 tension (P_{CO_2}), and O_2 tension (P_{O_2}), using Radiometer electrodes thermostatted to 15 °C and connected to an acid-base analyser (Radiometer) (deFur, Wilkes & McMahon, 1980). Additionally, total CO_2 (C_{CO_2}) was determined via the method of Cameron (1971) and total O_2 was measured with a Lex-O_2-Con O_2 analyser.

The *in vivo* acid-base status of the two groups of animals (freshly collected from San Diego, and commercially obtained from Venice) was similar (Table 1) with no significant differences ($P > 0{\cdot}05$) between mean pH, C_{CO_2} or P_{CO_2}. The non-bicarbonate buffer capacity of *A. californica* haemolymph determined *in vitro* on individual haemolymph samples from both groups of animals, according to the method of Truchot (1976), was moderate: $\bar{x} \pm$ S.E. $= -3{\cdot}01 \pm 0{\cdot}24$ mM-C_{CO_2}/pH; $n = 10$. These pH and P_{CO_2} data agree well with those reported for the related species, *A. fasciata* (Chalazonitis & Nahas, 1965), but differ from those for the prosobranch molluscs, *Busycon canaliculatum* (Mangum & Polites, 1980) and *Pleuroploca gigantea* (P. L. deFur & P. R. H. Wilkes, unpublished). In *A. californica*, pedal haemolymph pH is lower and P_{CO_2} higher than in either species of prosobranch mollusc.

The important question is not why haemolymph pH is lower in *A. californica*, than in the two species of conch, at similar temperatures, but rather, why is P_{CO_2}, one determinant of pH, higher. In *A. californica*, the large surface area directly in contact with the sea water – much greater than in the two conch species – and the diffusion gradient across the body wall (sea water $P_{CO_2} < 1{\cdot}0$) should favour a loss of CO_2 into the sea water. Several factors may be responsible for the higher P_{CO_2} in *A. californica*: lower cardiac output, poor vascularization of the foot and body wall, higher rate of oxidative metabolism and, therefore, CO_2 production, or a greater diffusion barrier presented by the muscular body wall and foot.

Haemolymph in the pedal sinus is venous (see Kandel, 1979), yet mean pedal O_2 tension (P_{O_2}) was high in both groups of animals (Table 1). O_2 content (C_{O_2}), however, was very low (Table 1) and not significantly different ($P > 0{\cdot}05$) from sea water C_{O_2} at similar P_{O_2}. These low C_{O_2} values suggest that either there is no enhancement of O_2 carrying capacity by a respiratory pigment, or the respiratory pigment is not saturated with O_2 to any significant extent at *in vivo* P_{O_2}.

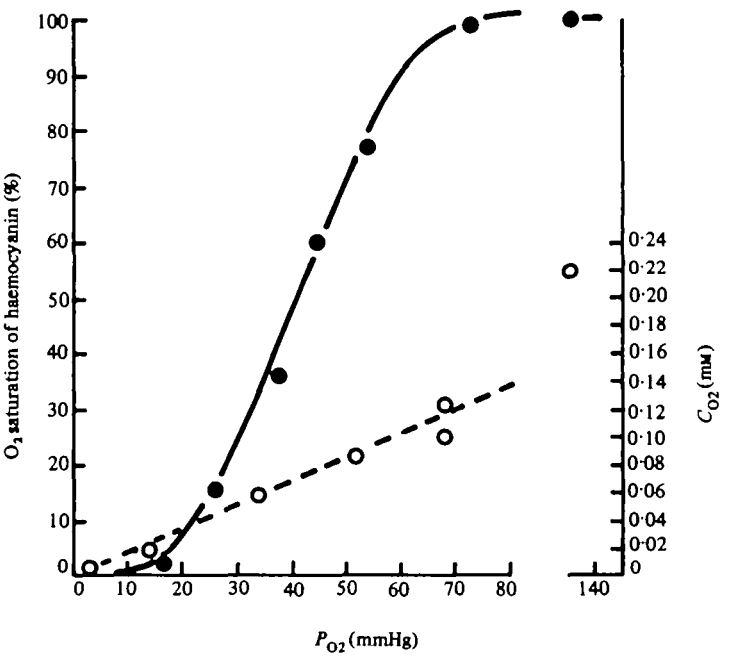

Fig. 1. Oxygen binding curve (●) for a pooled sample of *A. californica* haemolymph at 15 °C, pH 7·52, $C_{O_2}^{max}$ = 0·308 mM and the relationship between C_{O_2} and P_{O_2} (– – –) for a pooled sample of *A. californica* haemolymph at 15 °C, pH 7·54, $C_{O_2}^{max}$ = 0·22 mM.

Absorption spectra and O_2 carrying capacities (Table 1) for *A. californica* haemolymph indicate that haemocyanin is present, but there is a wide range of haemocyanin levels in this species. Absorption spectra did show a peak in the region of 340 nm, the absorbance band for other haemocyanins (Bonaventura & Bonaventura, 1980), and the peak could be abolished by chemically deoxygenating the haemolymph. The magnitude of this peak varied considerably, however, and consistently higher absorbance values were obtained with haemolymph from animals which had been freshly collected in San Diego. Haemolymph O_2 carrying capacity ($C_{O_2}^{max}$) was also greater in this group of animals (Table 1), further suggesting higher haemocyanin levels.

Copper concentrations were also measured on aliquots of haemolymph that were frozen and later analysed with an atomic absorption spectrophotometer (Jarrel-Ash, Model 850), using a hollow cathode lamp (Corning). Haemolymph Cu^{2+} was 4·5 ± 4·3 μM (n = 6) in *A. californica* freshly collected from San Diego, and the Cu/haemocyanin ratio (w/w) was calculated using estimates of haemocyanin concentration from the present absorption spectra and extinction coefficients for other molluscan haemocyanins (Nickerson & van Holde, 1971; van Holde & van Bruggen, 1971). The Cu/haemocyanin ratio of 0·23 ± 0·02% (n = 6) compares well with the values for purified haemocyanin (0·24–0·26%) for other molluscs (Ghiretti-Magaldi, Nuzzolo & Ghiretti, 1966).

The oxygen combining properties of *A. californica* haemolymph were determined on two pooled samples consisting of 1·5 ml obtained from each of five animals from each group (San Diego and Venice). O_2 binding curves (Fig. 1) were obtained by

equilibrating the pooled samples at a range of P_{O_2} from 0 to 132 mmHg and P_{CO_2} = 3·4 mmHg and measuring C_{O_2}. The oxygen affinity of *A. californica* haemocyanin with a $C_{O_2}^{max}$ of 0·088 mM was 42·0 mmHg at 15 °C, pH 7·52 (Fig. 1). Haemolymph with a $C_{O_2}^{max}$ similar to that of sea water (0·22 mM) was found to have a P_{O_2} *vs.* C_{O_2} curve almost identical to that of sea water (Fig. 1). These data suggest that haemolymph C_{O_2} in the pedal sinus may be lower for two reasons. Firstly, haemocyanin levels are so low in some animals that there is no significant enhancement of $C_{O_2}^{max}$. Secondly, when haemocyanin levels are higher, the pigment is nearly completely deoxygenated at *in vivo* P_{O_2} (Table 1).

An interesting aspect of the present data is the intrinsic variability in haemocyanin observable in the absorption spectra, haemolymph O_2-carrying capacity and P_{O_2} *vs.* C_{O_2} curves. van Holde & van Bruggen (1971) observe a similar variability in their review of the literature on a wide range of molluscs. *A. californica* examined in the present study seem to differ from other molluscs, however, in having a lower range of concentrations than in other species. Higher haemocyanin levels were observed in freshly collected animals and may also occur in other populations, at specific seasons, or under other environmental conditions. Neither the cause of this variability nor its effect on tissue oxygen supply can be assessed at this time.

Neurophysiologists utilizing the gill–mantle–siphon–abdominal ganglion preparation of *Aplysia* choose seawater as the most physiological saline because the two fluids are nearly isosmotic and isoionic (Hayes & Pelluet, 1947). The present data demonstrate that sea water and *Aplysia* haemolymph do, however, differ considerably with respect to acid-base characteristics and, in some animals, oxygen content. These differences in pH, C_{CO_2}, P_{CO_2} and C_{O_2} may affect specific neuronal activities, as demonstrated by Chalazonitis & Nahas, (1965) Brown (1972, 1974), Chalazonitis (1974) and Carpenter *et al.* (1974). These authors have clearly shown that pH, P_{CO_2} and P_{O_2} may affect membrane potential, spike frequency, membrane resistance, specific ion conductances and whether or not a neurone is silent in identifiable cells of the abdominal ganglion in *Aplysia*. Although for the most part these studies have utilized changes of pH, P_{CO_2} and P_{O_2} outside the physiological range, Brown (1974) points out that electrophysiological alterations do occur when more physiologically relevant levels of P_{CO_2} and pH are utilized. Furthermore, the effects of pH and P_{CO_2} changes are not the same for all cells: some cells are hyperpolarized, resulting in a reduced spike frequency upon administralion of low pH or high P_{CO_2} medium (Brown, 1974).

Chalazonitis & Arvanitaki (1970) have found that there is an inverse relationship between oxygen tension of the bathing medium and membrane potential and spike frequency of spontaneously active cells in the abdominal ganglion of *Aplysia fasciata*. Although postbranchial P_{O_2} was not measured in the present study, haemolymph bathing the abdominal ganglion is certainly not air-saturated (P_{O_2} 133–159 torr), because of the diffusion barrier at the gill and because postbranchial (high P_{O_2}) and renal haemolymph (low P_{O_2}) are mixed in the heart before flowing to the abdominal ganglion. Use of air-saturated sea water as the bathing medium may elevate intracellular P_{O_2} enough to alter both membrane potential and possibly baseline spike frequencies. Certain changes in spike frequency or membrane potential observed upon changing the bathing medium may be due to alteration of oxygen levels or acid-base status in the solution rather than due to administration of an exogenous substance

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