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SHORT COMMUNICATION

CONTINUOUS RECORDING OF ARTERIAL BLOOD P_{O_2} IN *OCTOPUS VULGARIS* DURING PROGRESSIVE HYPOXIA AND MOVEMENT

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The respiratory and acid-base status of the blood of animals continually changes in response to variations in internal and external conditions. Attempts to monitor transitory changes by repetitive sampling techniques can, however, lead to an unacceptable level of blood loss. An external circulatory shunt in which blood is continuously removed from one vessel, passed across monitoring electrodes and reintroduced into an appropriate second vessel would obviate this problem. Such a system has been developed to continuously monitor blood gas parameters and pH in fish (Belaud, Trotter & Peyraud, 1979; Thomas & Le Ruz, 1982). The present study attempts to apply a similar technique to an invertebrate, *Octopus vulgaris*.

Octopus vulgaris (Cuvier) ($N = 5$, weight range 800–1200 g) were caught at sea and maintained for 5 days in the aquarium facilities at Laboratoire Arago prior to experimentation. Individuals were anaesthetized in 2% ethanol in sea water and polyethylene cannulae (internal diameter 1 mm) were inserted into the auricles *via* the efferent branchial vessels. The cannulae were inserted through the blind endings of these vessels and tied in place. This resulted in the loss of the last few gill lamellae from the circuit. To prevent leakage the portions of the afferent branchial vessels feeding these lamellae were also ligatured. The cannulae were passed out through the back of the mantle sac and, providing the skin was not sewn, the animals showed no interest in the tubes. Each cannula was fitted with a luer ending for ease of subsequent connection. All tubing had an i.d. of 1 mm with the exception of the 20 cm length through the pump (i.d. 1·5 mm). The total volume of blood in the external circuit was 1 ml.

Blood clotting in the vertebrate sense, does not occur in the cephalopods. Haemocyte aggregation at the cannulae tips, particularly during the recovery period is, however, a complication. Pre-rinsing the cannulae overnight in Radiometer enzyme detergent reagent prevented any haemocytes from lodging in the tubes and minimized the problem.

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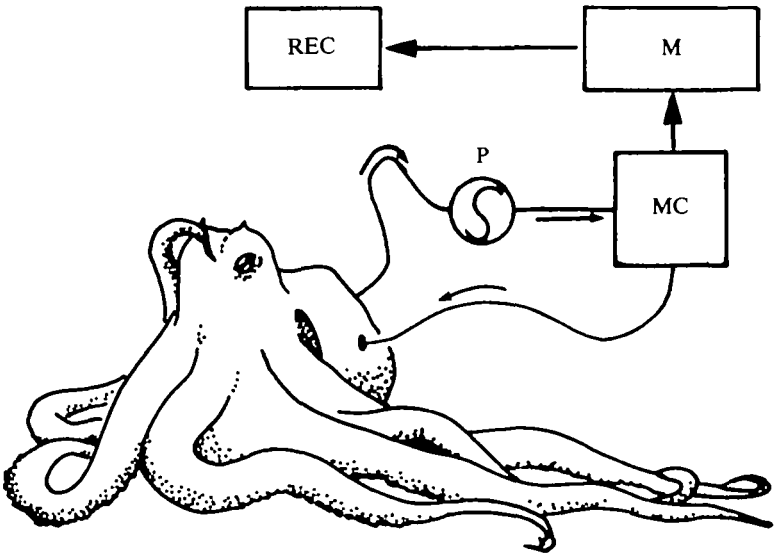

Fig. 1. Diagram of experimental set-up. P, peristaltic pump; MC, measuring cell (P_{O_2}); M, P_{O_2} meter; REC, pen recorder. Arrows indicate direction of blood flow.

After implantation of the cannulae, the octopus was transferred to a 20-litre tank, through which aerated sea water was passed at 25 °C, and left for 2 h. The cannulae were then connected to a LKB 2120 peristaltic pump assembly (Fig. 1), care being taken to ensure that no air bubbles remained in the tubing. Blood was pumped at 0·5 ml min^{-1} from one efferent branchial vessel across a Radiometer P_{O_2} electrode (E5047: Radiometer, Copenhagen, Denmark) thermostatted at 25 °C in a glass cuvette (D616: Radiometer, Copenhagen, Denmark) and into the contralateral efferent branchial vessel. Such a flow represented 2 % of the minute volume pumped by each branchial heart (Wells, 1983). It took 30 s for the blood to reach the electrode from the animal. Prior to attachment of the cannulae, the oxygen electrode was calibrated by continuously flushing the pump assembly and tubing with a zero P_{O_2} solution (0·1 mol l^{-1} disodium tetraborate + sodium sulphide crystals in excess) for 10 min at the above flow rate, followed by flushing with air-saturated sea water for 20–30 min. The volume of blood in the external shunt was approximately 2 % of total blood volume (Martin, Harrison, Huston & Stewart, 1958). Arterial P_{O_2} (Pa_{O_2}) was displayed on a Strathkelvin 781 oxygen meter (Strathkelvin Instruments, Glasgow, U.K.) and Washington pen recorder (Bioscience, Shearness, U.K.).

Progressive hypoxia was induced by either N_2 bubbling or closing the tank inflow. Water samples (0·2 ml) were removed at intervals for Pw_{O_2} determination with a BMS 3 MK2 blood microsystem (Radiometer, Copenhagen, Denmark). Activity was induced by manually chasing the animal around the tank. As a calibration check, 0·2 ml of blood was anaerobically removed before and after each trial and Pa_{O_2} independently determined with the BMS 3 MK2. In no case did these values differ by more than 5 mmHg from those of the shunt.

With the possible exception of one individual (Fig. 4A), under normoxic conditions individuals had a constant Pa_{O_2} at rest. However, Pa_{O_2} varied considerably between

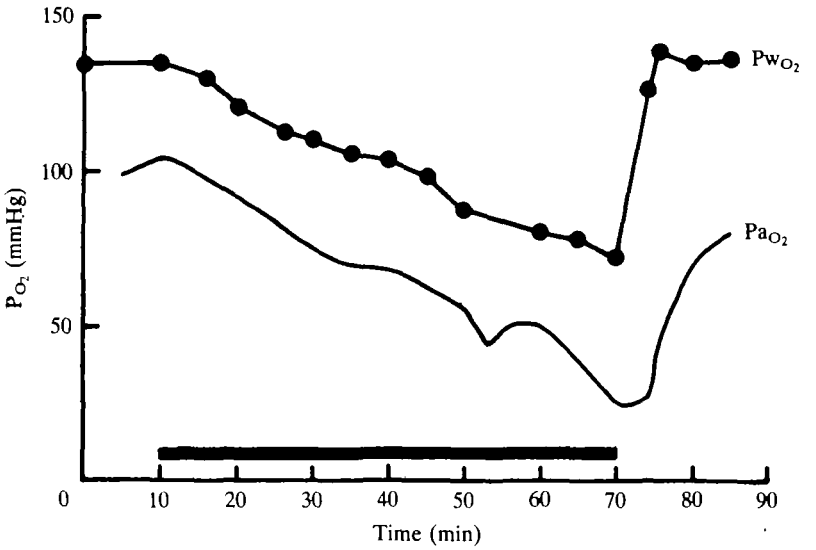

Fig. 2. Hypoxia induced by an individual respiring in a closed system. A relatively constant $Pw_{O_2} - Pa_{O_2}$ difference is maintained except on return to normoxic water. In this and all subsequent figures the delay in Pa_{O_2} readings due to transit of blood from animal to electrode has been corrected for and plotted to be simultaneous with Pw_{O_2}. The black bar is the period when the water flow is off.

the five individuals ranging from 60–100 mmHg. A mean Pa_{O_2} of 78·1 mmHg has been previously reported for this species (Houlihan, Innes, Wells & Wells, 1982). During either method of induced hypoxia a relatively constant $Pw_{O_2} - Pa_{O_2}$ difference was maintained (Figs 2, 3)., Using intermittent sampling techniques, similar observations have been made on *Octopus vulgaris* (Houlihan *et al.* 1982) and *Sepia officinalis* (Johansen, Brix & Lykkeboe, 1982). On return to normoxia a delay of 1–2 min was apparent before Pa_{O_2} began to rise towards prehypoxic levels, possibly indicating a delay in blood clearance time from the efferent branchial vessel/auricular reservoir and/or the presence of residual mantle water of low P_{O_2}. In one animal, prehypoxic levels were not reattained post-hypoxia for the duration of the experiment (Fig. 3B), a phenomenon also apparent in *Sepia* (Johansen *et al.* 1982).

The onset of movement initiated a decrease in Pa_{O_2} of 15–20 mmHg, an effect which was reversed when movement ceased (Fig. 4A, B). Unlike in hypoxia, no delay in Pa_{O_2} recovery was apparent. The initial decrease may reflect the recruitment of blood of low P_{O_2} from the circulatory sinuses or altered blood oxygenation at the gills to changes in ventilatory activity. For example, the pressure differential across the gills changes markedly from normal to strong ventilation (Wells & Smith, 1985).

The continuous recording of Pa_{O_2} during progressive hypoxia confirmed that the external shunt gave results similar to independent studies using intermittent sampling techniques. The response to movement has not been previously reported and illustrates the advantage of a continuous recording technique in the detection of changes of a transient nature. The inclusion of P_{O_2} and pH electrodes into the external shunt would greatly enhance the interpretive value of the data and could, for

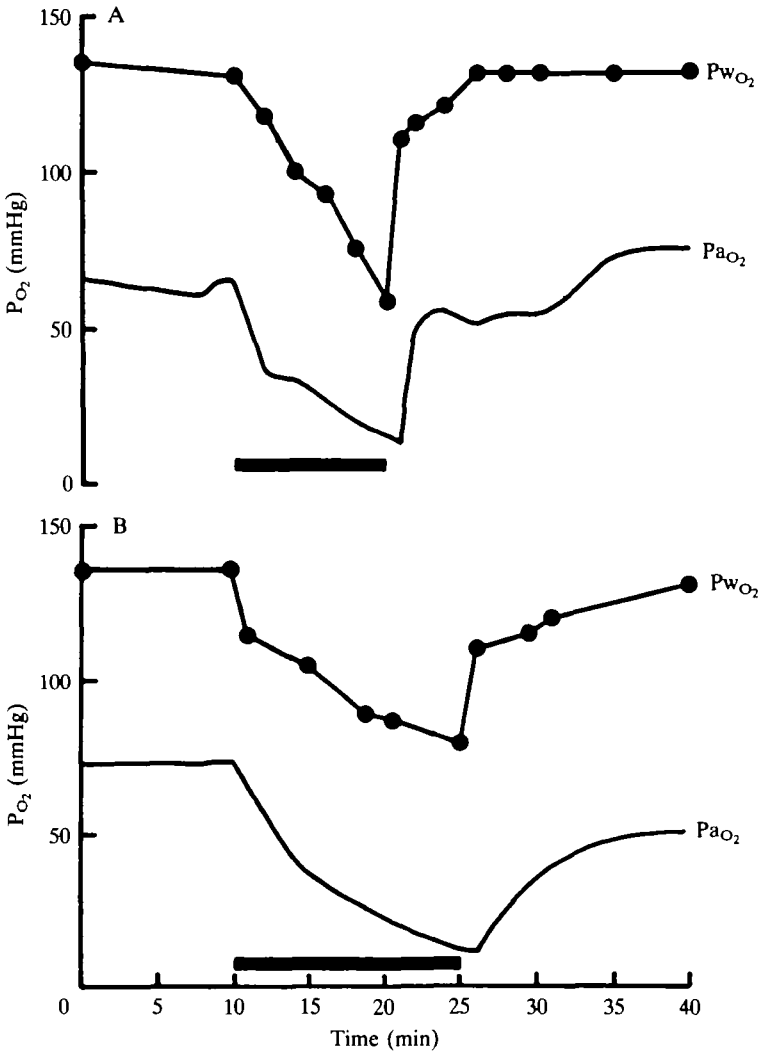

Fig. 3. Examples of the continuous recording of Pa_{O2} from two individuals (A, B) during N_2-induced hypoxia. The black bars denote the periods during which N_2 was bubbled into the tank.

example, resolve whether the pronounced Bohr and Haldane effects evident in cephalopod blood are primarily concerned with the facilitation of O_2 unloading to the tissues or, as is suggested by Lykkeboe & Johansen (1982), have their major effect on O_2 loading at the gills. The technique offers the possibility of making long-term observations on the respiratory physiology and acid-base status of cephalopods. In one experiment the shunt was maintained for over 6 h and there is little reason to suppose that continuous recording over longer periods is not feasible.

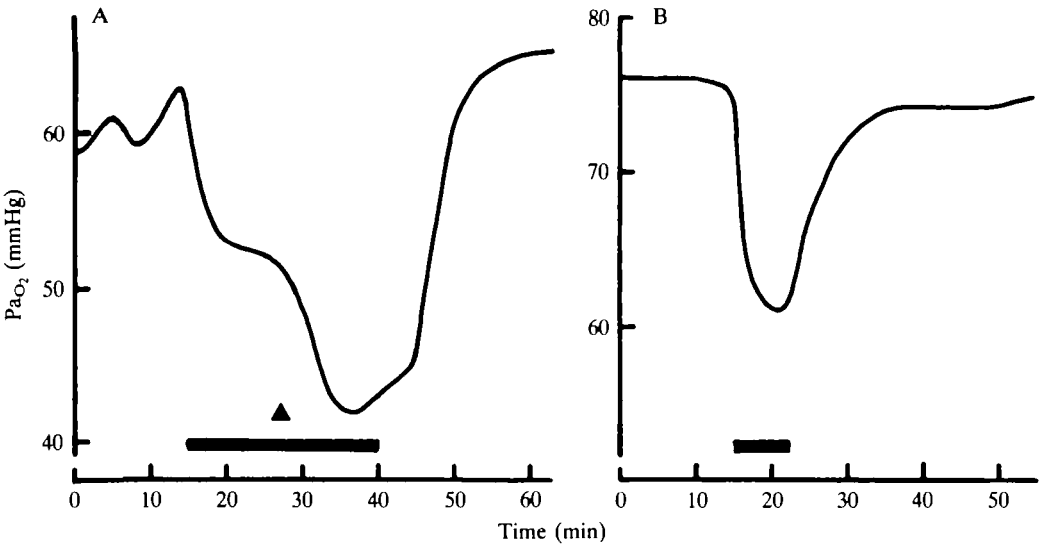

Fig. 4. Effects of short periods of activity on Pa_{O_2} of two individuals. Black bars denote periods of activity. Fig. 4A shows a decrease in Pa_{O_2} of 20 mmHg as a result of enforced movement plus an additional fall during a subsequent period of spontaneous activity. The onset of spontaneous activity is denoted by the black triangle. Fig. 4B shows the effect of spontaneous activity alone. In both cases Pa_{O_2} rapidly increases on the cessation of activity.

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