

**THE EFFECTS OF CARBON DIOXIDE AND OXYGEN ON
RESPIRATORY DORSO-VENTRAL MUSCLE ACTIVITY DURING
NORMAL VENTILATION IN *ANAX IMPERATOR* LEACH
(ANISOPTERA: *AESHNIDAE*)**

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Sudden changes in tension of dissolved respiratory gases have been utilised to disrupt normal ventilation (V_n). Chronic records of respiratory dorso-ventral muscle activity obtained during such disruptions provide information on the nature of ventilatory control.

INTRODUCTION

Considerable attention has been paid to dragonfly larval ventilation in recent years (MILL & PICKARD, 1972a; PICKARD & MILL, 1974a). Recording of muscular activity in restrained and free-swimming larvae has provided information on the synchronisation of abdominal muscles during ventilatory behaviour (PICKARD & MILL, 1972, 1974b; MILL & PICKARD, 1974) and this has been correlated with mechanical parameters (HUGHES & MILL, 1966; MILL & PICKARD, 1972b, 1974).

Larvae have been subjected to continuous changes in temperature (WALLENLÖF, 1913; SAYLE, 1928; PATTEE, 1955) and gas tensions (MATULA, 1911; WALLENLÖF, 1914; STAHN, 1928). However, since water temperature is often inversely proportional to the water's gas retaining capacity, results tend to be inter-related. BEREZINA (1959) has considered the energy balance of anisopteran larvae with respect to metabolic rate, and oxygen consumption

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has also been assessed in the resting larva (PENNAK & McCOLL, 1944; PETIT-PREN & KNIGHT, 1970). In the current study, larvae were exposed to sudden changes in tension of dissolved respiratory gases in an attempt to disrupt the characteristically regular ventilatory rhythm (V_n). It was hoped that any resultant disruption or regulation would reflect upon the nature of ventilatory control in this animal.

MATERIAL AND METHODS

Larvae of *Anax imperator* Leach were used.

Electrodes were inserted into the respiratory dorso-ventral muscles to record their activity in free-swimming larvae (PICKARD & MILL, 1972, 1974b) and the electrode outputs were displayed on a Tektronix 561 oscilloscope and filmed with a Nihon Kohden PC-2A camera. The larvae were placed, individually, into a glass chamber (18 x 4 x 6 cm) filled with filtered pond water at a temperature of 18°C. Air, carbon dioxide, or oxygen was bubbled into this chamber through four air-stones to promote rapid saturation of the pond water as required.

RESULTS

During CO₂ saturation the larva initially becomes very agitated and tries to move away from the CO₂ source. Bubbling air or oxygen into the chamber at the same rate does not evoke such activity. The ventilatory rhythm tends to be maintained at first, sometimes at slightly increased frequencies, but the bursts themselves become irregular (Figs. 1b, cf. a and 3b & c, cf. a). Within two to three minutes the V_n rhythm gives way to maintained contractions with reduced pulse amplitude. The larva begins to float. Within a further minute, after a brief period of vigorous jetting, ventilation ceases completely (Figs. 1c & 3d). In this state the abdomen is distended and the anal appendages are splayed out and not closed (fully or partially) as in voluntary cessation of ventilation or "maintained contraction" (PICKARD & MILL, 1974a).

If at this stage, the CO₂ is turned off and air is bubbled into the chamber the larva can recover (15 minutes to 1 hour) and the restoration of V_n can be observed. Spontaneous activity appears in the muscles and this is associated with prolonged sternal lifting and violent abdominal movements (Figs. 1d & 3e, f & g). When the ventilatory rhythm first re-appears (Fig. 1e) it is weak and of much lower frequency than before the application of CO₂ but burst duration tends to be fairly regular. During the subsequent ten to fifteen minutes both ventilatory rate and burst activity gradually increase to produce a steady rhythm of deep ventilation (Fig. 1f). This takes several hours to return to normal.

During O₂ saturation the larval behaviour is entirely different, there being no signs of agitation and the anal appendages remain partially closed during periods

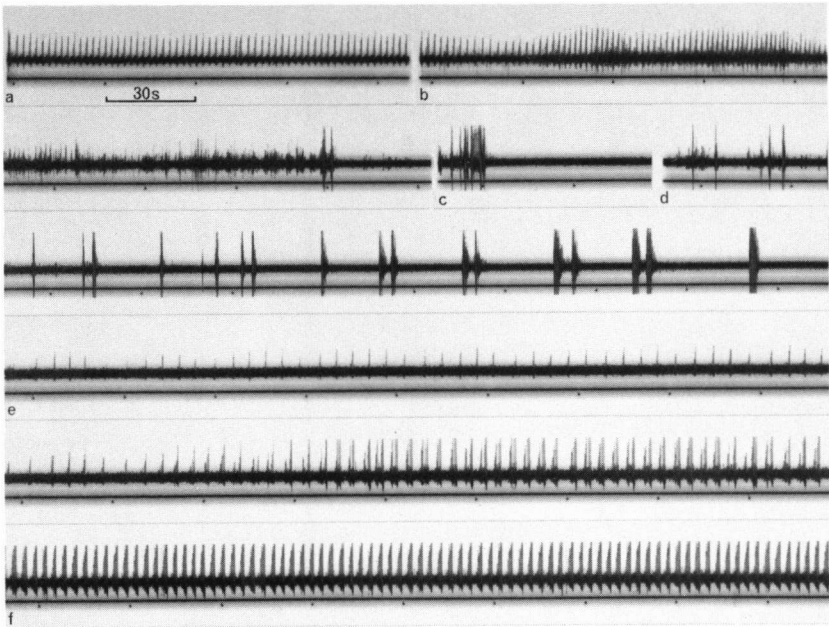


Fig. 1. Some effects of carbon dioxide on activity in the right respiratory dorso-ventral muscle (*RDV*) of segment five during a period of V_n in a free-swimming larva. Pieces of record have been removed at selected points where no changes in activity were evident. Sequence of events: (a) V_n before experimentation; CO_2 on; 40.5 s cut; – (b) V_n activity beginning to change; CO_2 off; 8.5 s cut; – (c) ventilation stops; 6 min 43 s cut; air supply on; 1 min 39 s cut; – (d) Spontaneous activity appears; 22 min 7 s cut; – (e) Ventilatory rhythm re-appears with short bursts at low frequency, then burst length and ventilatory rate increase; 16 min. 49 s cut; – (f) V_n re-established with vigorous expiratory bursts.

of no ventilation. Initially, muscle activity continues during oxygenation with little change from that shown before experimentation (Fig. 2b, cf. a). After two or three minutes ventilation becomes spasmodic, either stopping altogether or continuing with isolated periods of regular expiratory bursts (Fig. 2b, c). The gradation of response seen in CO_2 saturation experiments is absent.

If the O_2 is turned off at this point, spasmodic ventilation (occasionally with isolated potentials occurring, cf. Fig. 2d) continues for some time (1-3 hours) before V_n is resumed, again without the gradual introduction observed in recovery from CO_2 saturation. The larva also maintains normal postures throughout oxygenation. Addition of CO_2 to the preparation will stimulate renewed ventilatory activity for a few minutes, but this is soon followed by cessation of all normal ventilatory bursts (Fig. 2e).

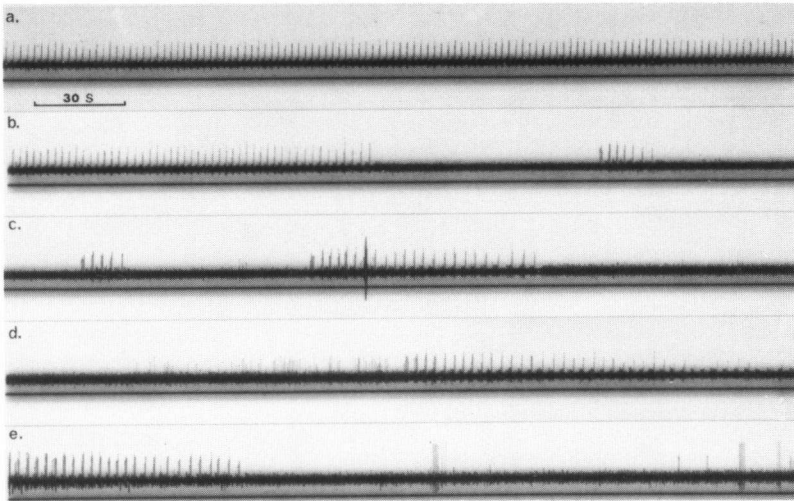


Fig. 2. Some effects of oxygen and carbon dioxide on activity in the right *RDV* of segment five during a period of V_n in a free-swimming larva (same preparation as in Figure 1 for comparison; 24 hours between the two experiments). Pieces of record have been removed at selected points where no changes in activity were evident. Sequence of events: (a) V_n before experimentation, cf. Figure 1 (a); O_2 on; 35 s cut; – (b & c) Ventilation becomes spasmodic; 1 min 5 s cut; O_2 off; 1 min 9 s cut; – (d) Ventilation still spasmodic; 35 s cut; CO_2 on; 20 s cut; – (e) Stronger ventilation returns for a short while and then stops altogether apart from some variable bursts of activity which occur at irregular intervals.

DISCUSSION

Larval responses to changing CO_2/O_2 tensions and temperature provide evidence for assessing the degree of adaptability inherent in ventilatory control. Both increasing temperature and decreasing oxygen content tend initially to cause a slight increase in ventilatory rate in dragonfly larvae. WALLENGREN (1913) suggested that adjustments such as this were under the control of the prothoracic ganglion. However, PLATEAU (1884) states that he was able to show that temperature, at least, will accelerate ventilation in both decapitated dragonfly adults and isolated abdominal segments, although it is difficult to know whether this was a temperature-gas effect or a general metabolic one. The situation is further complicated by the larva's inclination to stop ventilating altogether if its environment is changed quickly or dramatically. In the present study dramatic changes in oxygen tension in the inspired water tended to produce all or nothing responses, perhaps suggesting centralised command con-

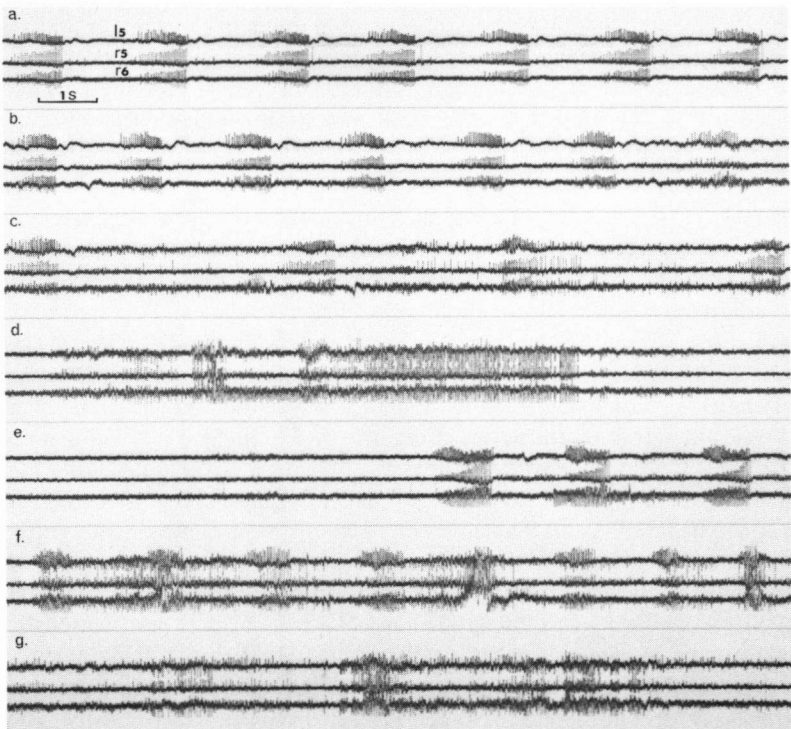


Fig. 3. Some effects of carbon dioxide on activity in three *RDVs* (left and right in segment five, and right in segment six) during a period of V_n in a free-swimming larva. Pieces of record have been removed at selected points where no changes in activity were evident. Sequence of events: (a) V_n before experimentation; CO_2 on; 46 s cut; - (b & c) Ventilatory rhythm begins to break down; 1 min 33 s cut; - (d) V_n has ceased and only isolated periods of maintained sternal lifting remain; CO_2 off; 1 min 3 s cut; no activity during this time apart from one isolated burst of less than 0.25 s duration; - (e & f) Regular bursts of activity re-appear; 1 min 15 s cut; - (g) Maintained variable activity between regular bursts of the type shown in (e & f).

trols in operation. At 17-18°C and low oxygen tension the larvae will actually move to the surface of the water and continue V_n with the tips of the anal appendages protruding through the water surface (WALLENGREN, 1914). It would be interesting to discover whether or not oxygen has a direct effect on the intrinsic musculature of the branchial apparatus, since LUTZ (1930) has shown that isolated strips of circular muscle from the cloacal wall of *Stichopus* (Holothuroidea) respond to oxygen tensions with varying amplitudes of contrac-

tion. An initial reduction in oxygen results in increased amplitude, strong periodicity in the pumping rhythm, and periods of inhibition (2-3 minutes). Further depletion of oxygen results in a total cessation of contractions (Dissolved oxygen below 60% of its air saturation value).

It was obvious in the present study that larvae resisted attempts to induce changes in V_n and preferred to move from areas of discomfort rather than adapt. The first effect of CO₂ saturation on ventilatory control is probably a peripheral one of the type found by HOYLE (1960, 1961) on the post-synaptic membrane of spiracle closer muscle in locust, since individual muscle burst characteristics alter before the centrally produced rhythm of burst initiation is completely upset. The first signs of recovery are non-rhythmic spasms in the muscles and only minutes later does the ventilatory rhythm re-appear. If a specific CO₂ receptor were present in the central nervous system one might expect to find a more controlled response to CO₂ changes, for example rhythmical changes in burst character rather than spasmodic ones. Once ventilation has re-started after CO₂ anaesthesia the return to V_n appears to be extremely controlled with both frequency and amplitude of cycles progressing with a positive regularity to V_n . With the close proximity of tracheolar cells and synapsing neural elements, it is difficult to believe that the whole central nervous system is not directly influenced by high CO₂ tensions in the trachea, and KROGH (1920) has shown tracheal partial pressures in *Aeshna* larvae to be proportionately related to gas tensions in inspired water. Both ROEDER (1953) and MILLER (1960) have demonstrated the sensitivity of cerebral ganglia in locust to CO₂ but it is unlikely that this sensitivity alone should account for cerebral influence over ventilation, since pacemaker centres in thoracic or abdominal ganglia appear to be equally exposed to CO₂ penetration directly, or indirectly as a pH effect (CASE, 1961).

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