VENTILATORY MUSCLE ACTIVITY IN INTACT PREPARATIONS OF AESHNID DRAGONFLY LARVAE

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INTRODUCTION

Various aspects of rhythmic activity in arthropods have recently been studied in some detail. Interest has centred in particular on: heart beat in decapods (Maynard, 1955; Bullock & Terzuolo, 1957); swimmeret movements in the Astacura (e.g. Hughes & Wiersma, 1960; Wiersma & Ikeda, 1964; Davis, 1969); locomotion (e.g. Wilson & Weis-Fogh, 1962; Wilson, 1964; Evoy, Barnes & Spirito, 1971); and insect ventilation (e.g. Miller, 1960, 1962, 1971 a, b; Hughes & Mill, 1966; Farley & Case, 1968; Mill, 1970).

The pacemaker varies in its position in different systems and preparations. However, in the case of swimmeret movements (Hughes & Wiersma, 1960; Ikeda & Wiersma, 1964), ventilation in the dragonfly larva (Mill & Hughes, 1966; Mill, 1970), and *Limulus* gill-plate movements (Fourtner, Drewes & Pax, 1971), it has been shown to lie in one of the posterior ganglia. In these cases activity spreads anteriorly to successive segments.

In this study, intact preparations have been used because they have the obvious advantage that the animal is not subjected to any dissection which may alter or disrupt the activity to be studied. Previous papers on the coordination of ventilation in dragonfly larvae using dissected preparations (Mill & Hughes, 1966; Mill, 1970) have shown that rhythmic bursts of activity emanate from the central nervous system. Expiratory bursts have been recorded from the second segmental nerves from the fourth to the eighth abdominal ganglion and in the corresponding nerve innervating the ninth abdominal segment. These bursts initiate contraction of the respiratory (expiratory) dorso-ventral muscles. Alternating with this activity, inspiratory bursts occur in the median nerves which innervate the two main inspiratory muscles; the subintestinal muscle and the diaphragm. Certain other nerves associated with the intrinsic musculature of the branchial chamber and vestibule show expiratory and inspiratory activity.

The mechanical movements associated with ventilation have also been described (Hughes & Mill, 1966; Mill & Pickard, 1972). The principle external feature is the raising and lowering of the posterior abdominal sterna. The point at which the sterna are maximally raised denotes the end of expiration and the beginning of inspiration.

The present paper presents a vindication of the use of dissected preparations in the study of many aspects of the control of dragonfly ventilation by demonstrating

similar patterns in intact animals. Furthermore, the technique of using intact prepara-
tions has made it possible to record simultaneously from several expiratory muscles
while obtaining mechanical records of the ventilatory behaviour itself.

MATERIALS AND METHODS

In all experiments late instar larvae of the Emperor dragonfly *Anax imperator* were
used.

The Perspex bath used for these experiments was divided into two compartments
by a thin wax partition (Text-fig. 1). In the centre of this partition an inverted
triangular hole was cut. The larger compartment was filled with wax to the level of
this hole and a depression was made to accommodate a late instar larva. The animal
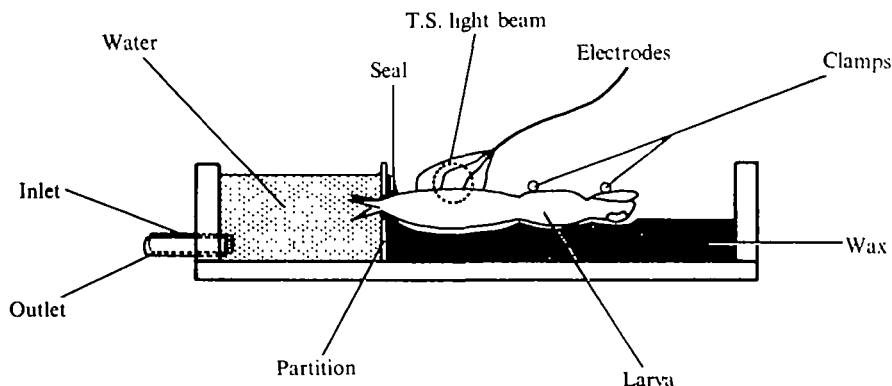
Text-fig. 1. Side elevation of experimental chamber with larva in recording position.

was positioned ventral surface uppermost, with its anal appendages protruding through
the partition hole into the smaller compartment. The body and legs were restrained
with clamps. The walls of the hole helped to stop lateral bending of the abdomen.
A steady flow of aerated water was passed through the small compartment. Leakage
from the smaller compartment to the larger could be almost entirely prevented by
using a small amount of vacuum grease to seal the joint between the animal and the
hole in the partition.

Muscle recordings. Electrical recordings were made by implanting electrodes
(48 s.w.g. Lewcosol-covered copper wire) into the ventral attachments of the respir-
atory dorso-ventral muscles and into one of the attachments of the sub-intestinal
muscle. Holes were made in the cuticle overlying or adjacent to the muscle attach-
ments, using Swiss watch drills of 0·07 mm diameter. The electrodes were inserted
through the holes, and within 1–4 h were sealed in position by the larva's natural
wound-repairing mechanisms and by the rapid drying of a tissue matrix around the
Lewcosol-cuticle joint. This sealing was then reinforced with a non-toxic medical
preparation of Eastman 910 adhesive. In most cases a single recording electrode was
used, with an indifferent electrode in the body cavity. However, a pair of electrodes
were sometimes implanted into a muscle. The muscle potentials were amplified with
Grass P4 pre-amplifiers and displayed on a Tektronix 565 or Telequipment D43
oscilloscope.

Mechanical recordings. Sternal movement was monitored by passing a narrow light beam across the fifth abdominal sternum to an OCP 71 photo-transistor.

In some experiments an isotonic SRI strain gauge was used to measure forces being exerted on the pleura by contractions of respiratory dorso-ventral muscles. The following procedure was used to determine the force exerted by an individual muscle. Two holes of about 0·07 mm diameter were drilled in the pleural cuticle of one segment. They were 1 mm apart and on the posterior margin of the respiratory dorso-ventral attachment. 48 S.W.G. wire was threaded into the animal through one hole and out again through the other. The ends were twisted and attached vertically to the strain gauge.

Records of pleural strain on one side of segment six and activity in the corresponding respiratory dorso-ventral muscle were taken simultaneously. The area of cutical containing the respiratory dorso-ventral insertion and the strain gauge attachment was then isolated from the entire animal by drilling round it. Simultaneous records at this point were then solely concerned with one respiratory dorso-ventral muscle and the strain it was exerting on the cuticle to which it was attached.

In experiments involving muscle ablation, or muscle isolation, 50 % of the specimens completely recovered after experimentation. In other experiments almost all specimens recovered fully, some even completing their metamorphosis months later.

RESULTS

Normal ventilation (V_n). Of the three pairs of dorso-ventral muscles in each of the posterior abdominal segments, only the respiratory dorso-ventrals were found to be active during V_n. Muscle potentials from the respiratory dorso-ventral muscle on the right side of abdominal segment five are shown in Text-fig. 2. It can be seen that these expiratory bursts show exactly the same characteristics observed in dissected preparations by Mill & Hughes (1966) and Mill (1970). There is an increase in frequency during the first part of the burst and this is associated with facilitation of the potentials. The upper trace in this figure shows movements of the hind abdominal sterna, upwards indicating lifting of the sterna. It was not surprising to observe that the initial low frequency in the expiratory bursts is associated with a rather gentle lifting of the sterna. As the frequency increases so the strength of the movement increases. However, what was not expected was the cessation of firing some 100 msec before the sterna had reached their fully raised position. This was so for all segments from five to eight at least.

Activity on opposite sides of the same segment is very closely synchronized (Text-fig. 3) and there is often a 1:1 relationship between the potentials on the two sides. The closeness to a 1:1 relationship varies somewhat between preparations and within a given preparation. Records (*c*) and (*d*) in Text-fig. 3 are single expiratory bursts from the same preparation. In the former there are the same number of potentials (40) on each side; whereas in (*d*) there are two more on the left side (43) than on the right (41). One of the extra ones on the left side can be accounted for by the single potential at the end of the burst, the other appears in the middle of the burst. It is of interest to note that, when there is a 1:1 relationship, one side does not always lead consistently throughout the burst; nor is there a constant delay between one side and

the other. Thus it is almost certain that two expiratory motor neurones are present (one for each side), in spite of the fact that there is some structural evidence that some motor cells send branches into both second roots (Mill, 1964). Perhaps these latter cells are associated with the jet-propulsion mechanism.
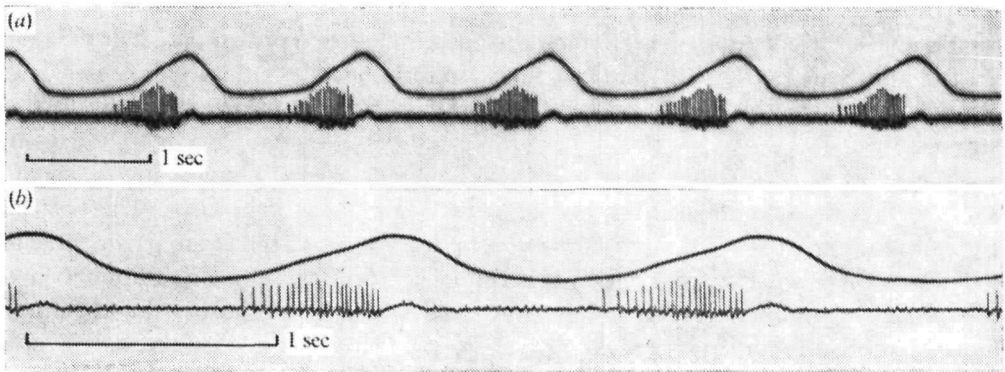

Text-fig. 2. Expiratory bursts (lower trace) in the right respiratory dorso-ventral muscle (*rdv.*) of segment 5 during normal ventilation (V_n). The upper trace monitors sternal movement of segments 4–6. (Upward deflexion indicates lifting of the sterna.) (*a*) and (*b*) Same preparation.
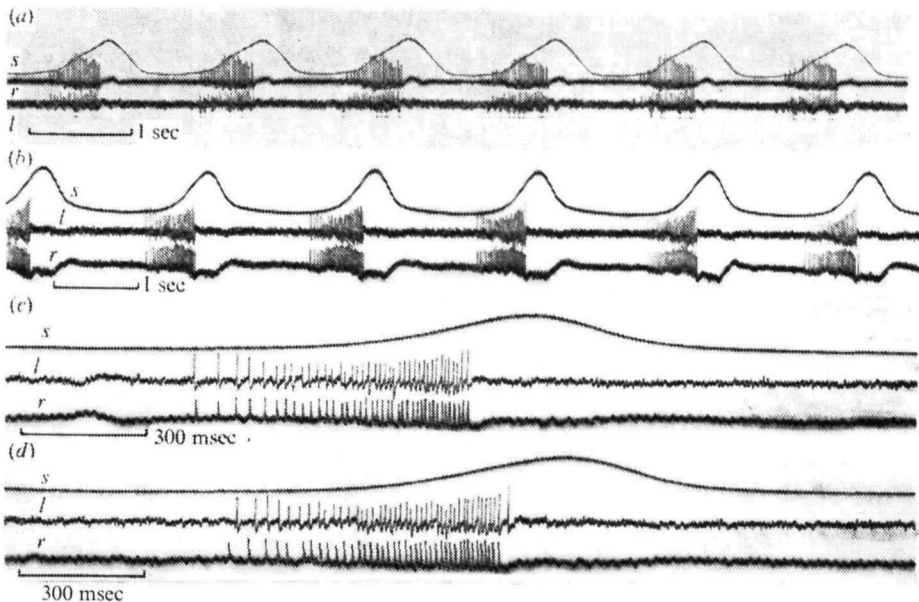
Text-fig. 3. Expiratory bursts in right (*r*) and left (*l*) *rdv.* muscles. (*a*) Segment 5; (*b*)–(*d*) segment 6 from a different preparation, cf. pulses in (*c*) 1:1, with those in (*d*), not 1:1. Top trace (*s*): movement of sterna of segments 4–6. (Upward deflexion indicates lifting of the sterna.)

Simultaneous recordings from the respiratory dorso-ventral muscles of several segments have confirmed that expiratory bursts first appear from the eighth (last) abdominal ganglion (Text-fig. 4). Successively more anterior ganglia produce expiratory bursts with a delay of about 100–150 msec per segment. Occasionally one

ganglion will start to fire out of sequence. This has been noticed particularly in the case of the sixth ganglion, which in some preparations produces bursts before the seventh, but always after the eighth (Pl. 1, fig. 2). However, when this happens the initial frequency of the potentials in the bursts from the sixth ganglion is particularly low. The expiratory bursts all end simultaneously about 100 msec before the sterna are fully raised.
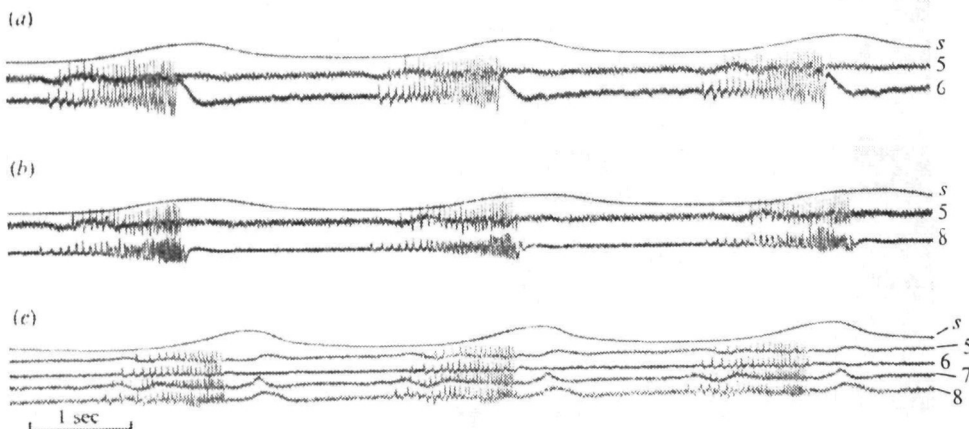

Text-fig. 4. Expiratory bursts in the right *rdv.* muscles of different abdominal segments, showing serial staggering of burst initiation in adjacent segments (5–8). (a) and (b) are from the same preparation. (*s*) *sternal movement of segments* 4–6. (Upward deflexion indicates lifting of the sterna.)

In order to determine the relationship between the premature termination of the expiratory bursts and the lifting of the sterna, preparations were made in which the respiratory dorso-ventral muscles were ablated from segments on either side of the segment from which recordings were being made. Ablations were performed by drilling out the small muscle-attachment points and sealing the holes with Eastman 910. Records from such preparations had reduced amplitude of sternal movement but still showed a delay of about 100 msec between cessation of electrical activity and peak sternal movement (Text-fig. 5). This helped to demonstrate that continued peak sternal movement was not due simply to adjacent segments pulling up the recording segment.
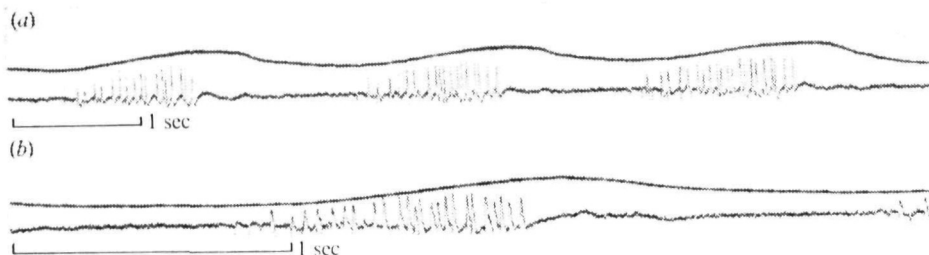
Text-fig. 5. Sternal movement in segments 5 and 6 (upper trace; upward deflexion indicates lifting of sterna) and expiratory bursts in the right *rdv.* muscle of segment 6. Left and right *rdvs* of segments 5 and 7 have been ablated. Note reduced amplitude of sternal movement, and presence of delay between the end of the expiratory burst and peak sternal movement.

Records of the strain developed on a single pleuron in intact preparations (Pl. 1, fig. 1 *a, b*) showed that a delay also existed between the end of electrical activity in the muscle and the development of maximum tension; although in these preparations the delay was less than 100 msec. The tension recorded was in the order of 10^{-4} Newtons (about 1 g wt.). However, the tension exerted by an individual respiratory muscle reached maximum at approximately the same time as the expiratory burst ended (Pl. 1, fig. 1 *c*). Recordings of activity in the subintestinal muscle confirm that this is an inspiratory muscle, and muscle potentials appear in it at or just before peak sternal movement (Text-fig. 6). The subintestinal muscle is innervated by two branches of the same axon from the unpaired median nerve (Zawarzin, 1924; Mill, 1965). The branches enter the muscle on opposite sides of the segment, and this is likely to be the reason for two different impulse sizes appearing in a 1:1 relationship within a single burst recorded from one muscle-attachment point.
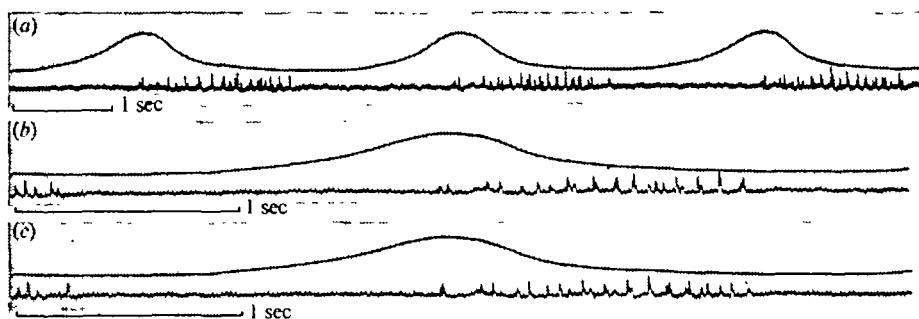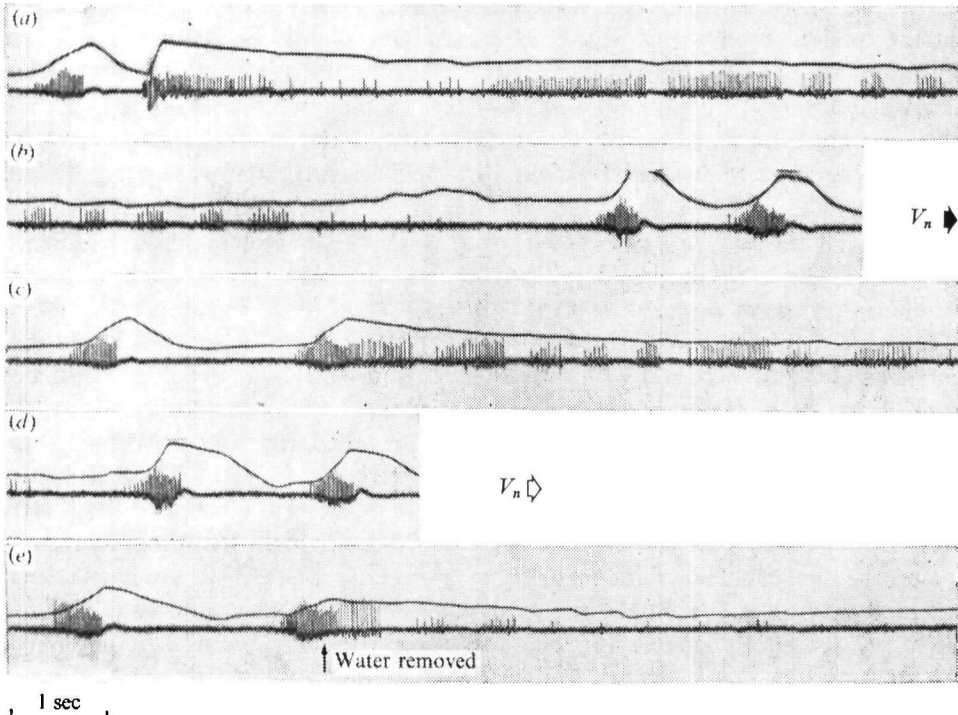

Text-fig. 6. Inspiratory bursts (lower trace) in the subintestinal muscle, and sternal movement (upper trace; upward deflexion indicates lifting of sterna) of segment 5 during V_n.

Other forms of ventilation. Other forms of ventilation have been noted by Tonner (1936) and by Hughes & Mill (1966) and have generally been described as 'gulping' and 'chewing' ventilation. 'Gulping' has been observed in the current series of experiments and the maintained lift of the sterna, characteristic of this form of ventilation, is associated with intermittent bursts of activity in the respiratory dorso-ventral muscles. Another type of ventilation frequently observed is shown in Text-fig. 7 and Pl. 1, fig. 2, and this will now be described.

The sterna are raised as in normal ventilation, but the expiratory burst may be maintained for longer than usual. The sterna then start to fall extremely slowly, their descent being interrupted by brief lifting movements. These movements are brought about by a series of rapid bursts of activity in the respiratory dorso-ventral muscles. The frequency in these bursts is not always as high as the maximum attained in a normal expiratory burst, but the muscle potentials are almost invariably of high amplitude. Occasionally this form of ventilation starts with a burst of high frequency before an expiratory burst is due. This ventilation is terminated either by the sterna eventually falling to their rest position (Text-fig. 7 *b, d, e*) or by the recurrence of normal ventilation while the sterna are still partially raised (Pl. 1, fig. 2 *a*). This behaviour can be induced by touching the paraprocts or by removing water from the experimental bath.

Records from the respiratory dorso-ventral muscles of several segments show that, although those in abdominal segments five to eight at least are active during this form of ventilation, the amount of activity decreases from posterior to anterior. In other words the respiratory dorso-ventral muscles in segment eight are more active than those in segment seven and so on (Pl. 1, fig. 2b, c).

Text-fig. 7. Expiratory bursts (lower trace) in the right *rdv.* muscle of segment 5 during ventilatory activity other than V_n. Sternal movement is shown in the upper trace (upward deflexion indicates lifting of sterna). (a) and (b) continuous records of spontaneously occurring behaviour; (c) and (d) continuous records of behaviour induced by touching paraprocts with fine needle; (e) behaviour induced by sudden drainage of water from the experimental chamber. V_n continued between (b) and (c) and between (d) and (e).

DISCUSSION

The results obtained using intact preparations confirm the earlier work of Mill & Hughes (1966) and Mill (1970) in showing that ventilation is initiated by segmental expiratory motor neurones which produce bursts of muscle potentials in the respiratory dorso-ventral muscles. Furthermore, the respiratory dorso-ventral muscles at the posterior end of the abdomen are activated first, with successively anterior pairs of muscles becoming involved with a delay of about 100 msec per segment. The bursts in each segment cease simultaneously. There is then a short delay, after which activity can be recorded from the subintestinal muscle. Thus the inspiratory role of the latter is confirmed. Since the diaphragm is active at exactly the same time as the sub-intestinal muscle (Mill & Hughes, 1966) presumably it too has an inspiratory role.

The delay between the end of the expiratory burst and the peak of sternal movement may occur because the role of the respiratory dorso-ventral muscles is to pull the

sterna past some critical level, after which they 'click' into a stable raised position. Alternatively, it could be due to the inertia of the expiratory flow of water. This, together with the importance of the timing of activity in the inspiratory subintestinal muscle, is discussed elsewhere (Mill & Pickard, 1972).

The fact that the respiratory dorso-ventral muscles of one segment may fire out of turn in some instances, i.e. not sequentially from posterior to anterior, indicates that the model proposed by Mill & Hughes (1966) should be modified to include connexions from the command interneurone(s) to each expiratory centre. It is suggested that successively more anterior expiratory motor neurones have higher thresholds, so that they are normally only activated when the input from the command system is augmented by activity from the next most posterior segment. Future experiments should reveal that more anterior ganglia are capable of acting as pacemakers, as in the crayfish swimmeret system (Ikeda & Wiersma, 1964). There is presumably also a rhythmically firing interneurone between each pair of ganglia, carrying information anteriorly.

Hughes & Mill (1966) described two forms of ventilation in aeshnid larvae other than normal ventilation and jet-propulsion. In one of these, 'gulping' ventilation (Tonner, 1936), the abdominal sterna were raised and held in this position for several seconds. During this time the pressure in the branchial chamber oscillated around 10 cm H_2O. In the other type, which was observed less frequently, the abdominal sterna were extended downwards below their rest position in a series of small steps. The overall downward movement was matched by a corresponding increase in pressure in the branchial chamber to about 4 cm H_2O and the steps coincided with brief increases in pressure of about 2 cm H_2O. The latter were reminiscent of normal respiratory pressure changes, but with the negative phase obscured by the overall pressure increase. The frequency of their occurrence was about twice that of normal ventilation. After reaching a maximum the sterna remained extended, while the pressure started to fall slowly. This form of ventilation was terminated by the occurrence of a normal ventilatory cycle.

The current study has confirmed the existence of 'gulping' as described by Hughes & Mill (1966). The other type of ventilation described in this paper corresponds to 'gulping' in the initial raising of the sterna; and to the step-like form of ventilation described by Hughes & Mill (1966) in the periodic downward movements of the sterna. It is felt that this other ventilation is more closely allied to the 'stepping' behaviour than to 'gulping'.

Hughes & Mill attributed the pumping up of the branchial chamber pressure during this 'stepping' behaviour to the possibility of intrinsic muscular activity. However, results of the present study suggest that this pumping is due in part to contractions of the respiratory dorso-ventral muscles. The anal valve is tightly closed during the procedure. Such modification of V_n may well serve to clear residual pockets of water or debris from the branchial chamber, and rearrange the contained tracheal gills. The behaviour is always followed with an abnormally strong expiratory movement before V_n is resumed. It is hoped that further studies, using free swimming-animals, will throw more light on the various types of ventilation.

SUMMARY

1. The expiratory role of the segmental, respiratory dorso-ventral muscles, and the inspiratory role of the subintestinal muscle, have been confirmed using intact preparations of aeshnid dragonfly larvae.

2. The strain developed by individual respiratory dorso-ventral muscles has been measured.

3. The respiratory dorso-ventral muscles all cease firing simultaneously, about 100 msec before the sterna are fully raised, and do not have any mechanical effect on the sterna after this time. It is suggested that the delay is caused either because the role of these muscles is to lift the sterna past some critical position, and/or because of the inertia of the expiratory current.

4. Periodically the sterna are raised and then lowered slowly in a series of steps, each pause in the lowering coinciding with activity in the respiratory dorso-ventral muscles. This form of ventilation is compared with others previously described.

5. In normal ventilation, and in other types of ventilation, activity in the respiratory dorso-ventral muscles shows a pronounced tendency to begin in the most posterior segments and to continue for longer periods in those segments.

6. Some aspects of the central neural connexions involved in normal ventilation are discussed.

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EXPLANATION OF PLATE

Fig. 1. (a) and (b) Right pleural strain in segment 6 (upper trace) and expiratory bursts (lower trace) in the *rdv*. muscle attached to the pleuron. (c) Same preparation with cuticle containing *rdv*. attachment now isolated from the pleuron and upper trace thus monitoring the strain developed by the muscle alone. (1 g wt. = 9.81×10^{-3} N.)

Fig. 2. Ventilatory activity other than V_n. (a) Activity in the left (l) and right (r) *rdvs* of segment 6. Upper trace; sternal movement (s) in segments 4–6 (upward deflexion indicates lifting of sterna). (b) and (c) Continuous records of four *rdvs* on the right side of segments 5–8 showing decreasing activity from posterior to anterior during pumping type of ventilation.
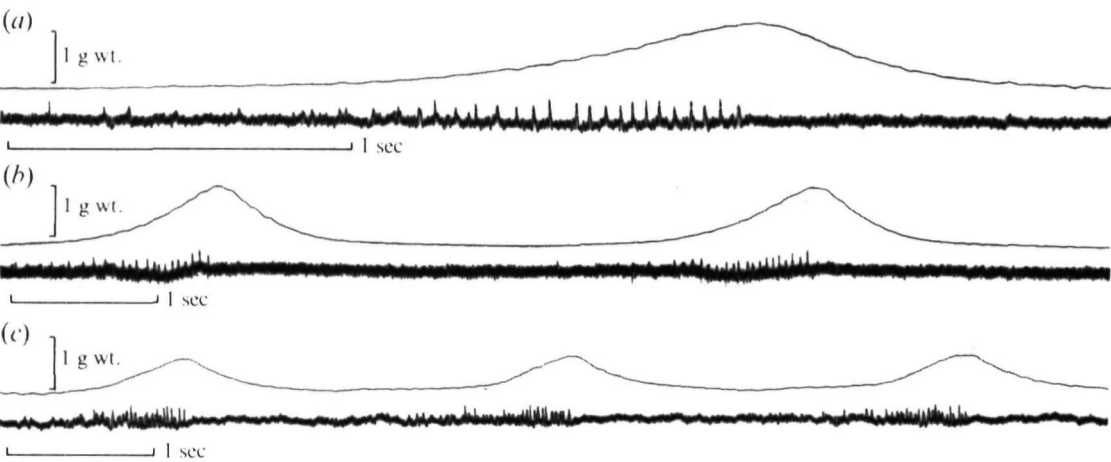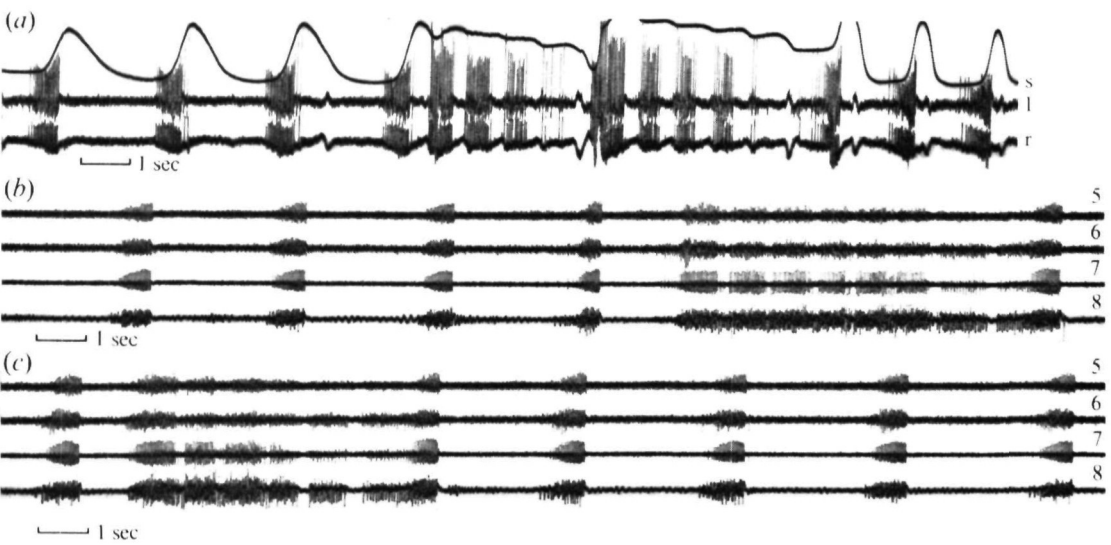
Fig. 1

Fig. 2