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FACTORS ALTERING SPIRACLE CONTROL IN ADULT DRAGONFLIES: HYPOXIA AND TEMPERATURE

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INTRODUCTION

Oxygen lack causes a reduction in the frequency of motor impulses to the closer muscles of the spiracles of certain African dragonflies (Miller, 1962). This results in fluttering and opening of the valves. The decreased frequency also probably makes the spiracles more sensitive to carbon dioxide. The spiracles of flies (Case, 1956) and of silkmoth pupae (Schneiderman, 1960) show an increased sensitivity to carbon dioxide under hypoxic conditions and this might be explained by a similar effect. Experiments in which the action of oxygen shortage on the motor discharge from the mesothoracic ganglion to spiracle 2 is studied in more detail are reported here, together with observations on the interaction of water balance and hypoxia on spiracle control. The effect of desiccation on the motor frequency is shown to be separable from its action on the central mechanism responsible for sensitivity to hypoxia. The influence of temperature on this system is also considered with particular reference to a large dragonfly species whose method of hunting necessitates accurate temperature control in the pterothorax (Corbet, 1962).

MATERIAL AND METHODS

Material

Ictinogomphus ferox Rambur (Gomphidae, Odonata) was used in most of the experiments to be described. Teneral adults were obtained from emerging larvae and mature individuals were caught in the wild in Uganda as already described (Miller, 1962). Other species employed are mentioned in the appropriate sections.

Methods

Gas mixtures were supplied through flowmeters as previously indicated (Miller, 1960). They were bubbled through water at a rate of 2–3 l./min. and passed into a gassing chamber of 1·2 l. capacity. Electrodes for extracellular recording from the motor nerve to spiracle 2 entered the chamber through an oil seal which permitted their control from outside (Fig. 1). Impulses were recorded close to the mesothoracic ganglion through the coxal cavity. The nerve was lifted into air and the cavity was roofed over with Vaseline so that drying out was prevented. This method has permitted continuous recording from the nerve for up to 6 hr. without touching the preparation.

For measurements of the effect of temperature the dragonfly was placed inside a vessel fitted with double walls through which steam could be passed or into which ice

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was placed. Localized heating of the thorax or head was carried out by means of a small heating coil implanted through the cuticle into the appropriate region. Temperature measurements were made with a bead thermistor (0·3–0·4 mm. in diameter) embedded in Araldite and inserted into the thorax so as to lie just dorsal to the mesothoracic ganglion, or into the frons close to the supra-oesophageal ganglion.

Impulse frequencies are given as the sum of the values in the two motor axons to spiracle 2, determined from counts made on oscilloscope film. Methods for counting and other techniques have already been described (Miller, 1962, 1963*a*).

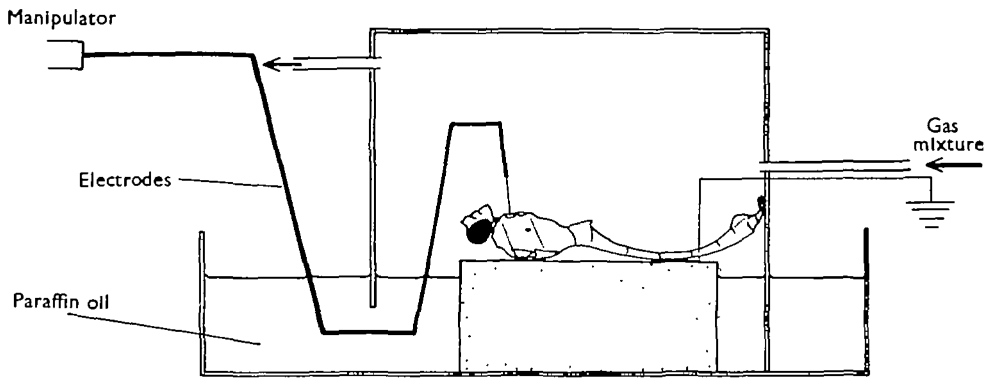

Fig. 1. Diagram of the chamber used for treating dragonflies with known gas mixtures. The electrodes are shown entering the box through an oil seal.

RESULTS

Observations on the spiracular response to hypoxia

When a mature *Ictinogomphus ferox* is placed in the gassing chamber and 2% oxygen in nitrogen is passed through, spiracle 2 starts to flutter within a few seconds and is usually wide open and motionless within 1 min. A similar reaction is seen with 0·5% oxygen or less, but after 5–10 min. gassing irregular slow movements start independently in both spiracles 2, and these may lead to closure (Fig. 2A). Spiracles 1 usually show similar renewed activity. This behaviour may be maintained for a further 10 min. after which the spiracles again open. By this time ventilation has become extremely irregular and asphyxiation may follow shortly. If air is re-admitted at any stage short of asphyxiation, neurogenic spiracle fluttering is resumed within a few seconds and closure follows in less than 1 min. After asphyxiation, however, recovery takes much longer. The slow irregular movements of the spiracles in 0·5% oxygen are considered to be myogenic in origin for the following reasons: they are unsynchronized on the two sides, they are not accompanied by motor impulses in the spiracle nerves (see below) and they can still be seen after extirpation of the ganglion. Earlier reports of myogenic or independent activity by the spiracles of various insects were made after spiracular denervation (Case, 1957; Beckel & Schneiderman, 1957), but the more recent results of van der Kloot (1963) show that the myogenic mechanism may be functional in the intact preparation, as appears to be the case here.

These movements in the dragonfly may arise from an increase in the potassium level of the haemolymph, brought about by water loss through the open spiracle, as appears to happen with similar behaviour of spiracle 2 in the locust (Hoyle, 1961). An alternative explanation, however, is that the myogenic mechanism becomes more excitable with hypoxia which is known to have a depolarizing effect on some excitable tissues (Chalazonitis, 1963). When denervated in air the dragonfly spiracle 2 may show no activity for several hours after which closure sometimes occurs; when placed in 0·5 % oxygen, however, it may show movements of the type described above and these disappear on return to air. Thus it seems that hypoxia can excite the myogenic pacemaker if it is already near the threshold for activity, and oxygen lack therefore has the opposite effect to carbon dioxide on the spiracle muscle.

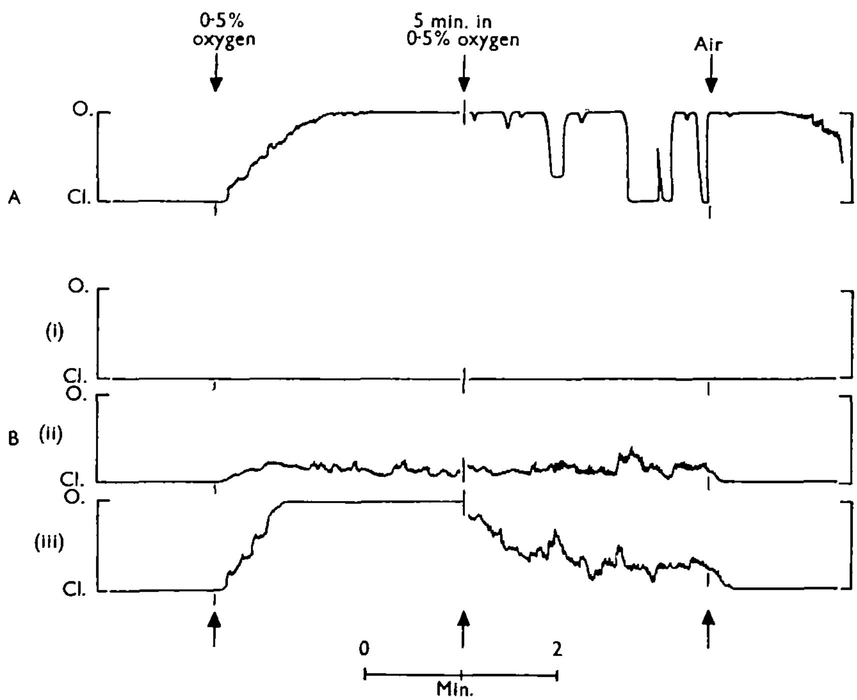

Fig. 2. Diagram illustrating the activity of spiracle 2 of dragonflies in 0·5 % oxygen, based on kymograph records. A, Mature *Ictinogomphus ferox*. The spiracle initially opens wide but after about 5 min. myogenic movements commence. B, Teneral *Ictinogomphus ferox* (i and ii), in which the spiracle either remains closed or makes small fluttering movements, and *Trithemis annulata* (iii), in which the spiracle initially opens fully but after about 5 min. commences fluttering.

In tenerals of *I. ferox* the spiracles are much less sensitive to hypoxia than in mature insects; this stands in contrast to their greater responsiveness to changes in water balance (Miller, 1963*a*). In 0·5 % oxygen spiracle 2 of a teneral *I. ferox* may remain closed indefinitely or it may commence fluttering movements (Fig. 2B), but it does not normally open fully. Similar teneral insensitivity has been found in several other species which breed in Lake Victoria (e.g. *Trithemis annulata* (Palisot de Beauvois), *Phyllogomphus orientalis* Fraser). It is associated with a tolerance of oxygen shortage greater than that found in the mature insect (Miller, 1962). For example after 26 hr. in 0·5 % oxygen a teneral *P. orientalis* was found to be still active and to perform weak abdominal pumping movements while spiracle 2 continued to flutter. The last larval instar is likewise able to remain unasphyxiated under the same conditions provided desiccation is prevented. On the other hand, mature insects, tested together with larvae and tenerals, are usually asphyxiated after 15–20 min. gassing.

The tenerals of certain species (e.g. *Pantala flavescens* Fabricius, *Philomonon luminans* (Karsch)) may show an initial spiracular response to oxygen lack by opening fully. After a variable interval, usually between 3 and 10 min., fluttering commences and then continues for as long as gassing is maintained (Fig. 2 B (*iii*)). Subsequent tests with hypoxia produce only fluttering or no change at all. The initial response of full spiracle opening may have allowed slight desiccation to take place which effectively abolished the full response subsequently. The possible significance of teneral insensitivity to hypoxia will be discussed later.

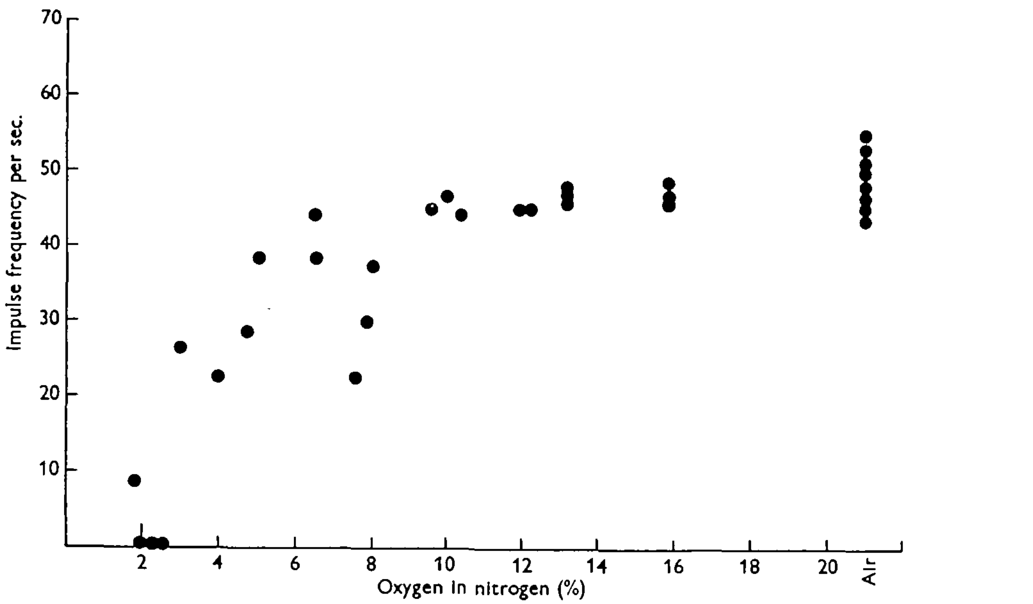

Fig. 3 *Ictinogomphus ferox*. The frequency of motor impulses to spiracle 2 plotted against concentrations of oxygen in nitrogen. Data from three mature insects. In 2 % oxygen the impulses are extinguished.

The effect of hypoxia on the frequency of motor impulses to spiracle 2

When *I. ferox* is perfused with low oxygen concentrations and the motor impulses to spiracle 2 are simultaneously recorded, the results are in agreement with those obtained by direct observation of the spiracle. Each reading was made after a steady value had been reached, usually after about 5 min. gassing, and this was maintained for a further 20 min. at least. Between administering different gas mixtures air was passed through the chamber at the same rate and the frequency was checked. Oxygen mixtures were not given in any particular order of increasing or decreasing concentration. About 0·05 ml. saline was added to the preparation during dissection but no more subsequently.

With oxygen concentrations of less than 10% in nitrogen there is a marked decline in the frequency in mature insects (Fig. 3), and in about 2% oxygen the impulses are extinguished. There is no return of the spikes when gassing is maintained for 16 min., although myogenic movements may start after 5 min. as mentioned earlier. After extinction of the impulses in 2% or less, ventilation by the abdomen continues and the spiracle touch reflex can still be evoked as previously described (Miller, 1962). On re-admission of air impulses are resumed within 10–15 sec. and the original frequency is achieved in about 1 min. Experiments similar to those already undertaken (Miller, 1963*a*) have shown that the meso- and metathoracic ganglia are still able to respond to hypoxia after complete de-afferentation and there is no need to postulate extra-ganglionic receptors.

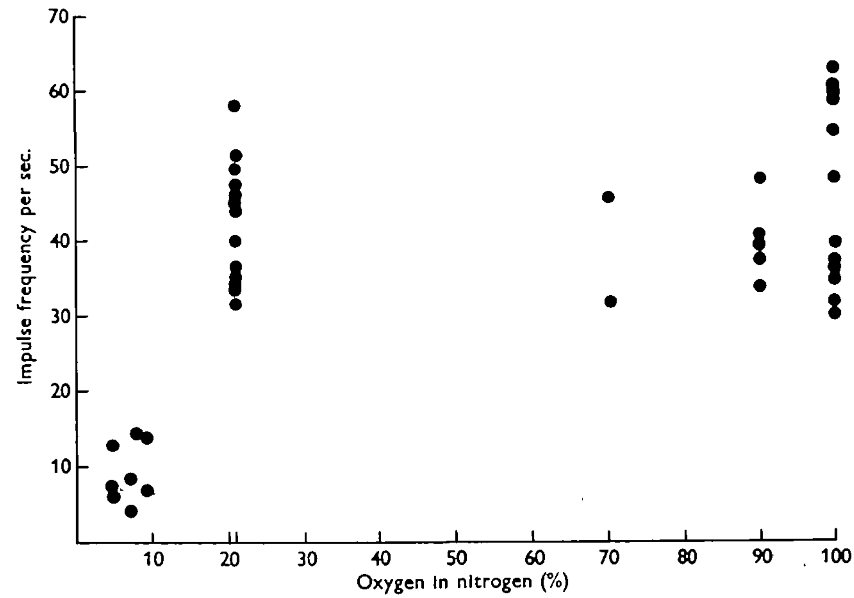

Fig. 4. *Aeshna cyanea* (Müll.). The frequency of motor impulses to spiracle 2 plotted against concentration of oxygen in nitrogen.

When high oxygen concentrations are used no change in frequency has been observed, and even in 100% oxygen maintained for 30 min. the value remains unaltered (Fig. 4). In the silkworm pupa Schneiderman (1960) has shown that the threshold of the spiracular response to carbon dioxide was further raised by high oxygen pressures and this suggested that the motor frequency might also reach values higher than in air, but this is not so in the dragonfly.

The interaction of salines of different strengths and hypoxia on the frequency of motor impulses to spiracle 2

After perfusion of the mature dragonfly with various salines, as described elsewhere (Miller, 1963*a*), the reaction was measured. The results, illustrated in Fig. 5, show that when normal saline is perfused through the insect the response is the same as in the unperfused insect. Following perfusion with double strength saline, however, the threshold is raised and impulses are not extinguished even after 25 min. gassing with 0·5% oxygen, whereas perfusion with half strength saline lowers the threshold and the discharge may cease in 7% oxygen. These results amplify those reported earlier (Miller, 1963*a*) when direct measurements of spiracle activity were made.

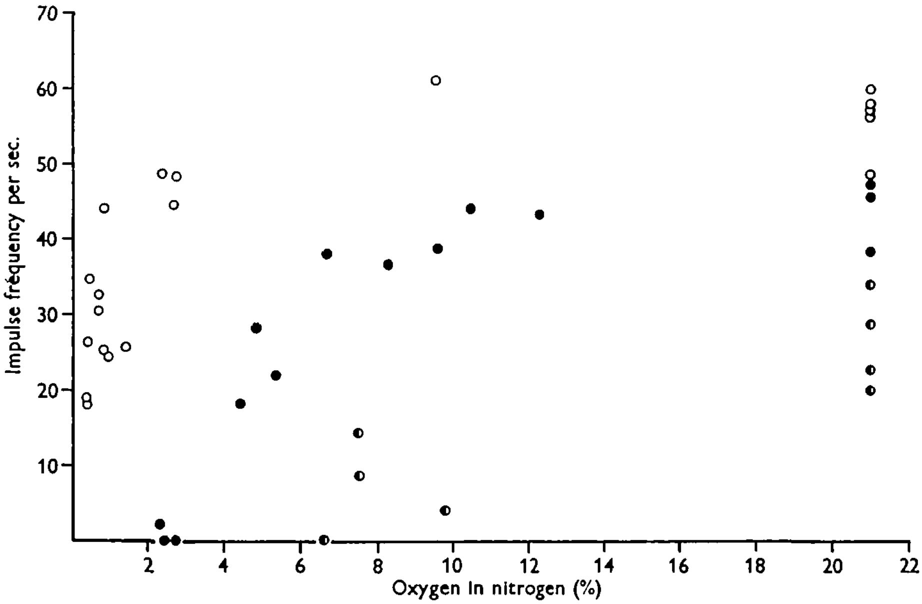

Fig. 5. *Ictinogomphus ferox* (mature). The frequency of motor impulses to spiracle 2 plotted against oxygen concentration after perfusion of the insect with salines of various strengths. ○, Double strength saline; ●, normal strength saline; ◐, half strength saline.

The response of the teneral dragonfly to hypoxia

As already described the spiracles of teneral *I. ferox* give only a weak response to severe hypoxia. Likewise, recordings from the motor nerve to spiracle 2 show only a small change with oxygen lack—similar in fact to that of the mature insect perfused with double strength saline. This led to the hypothesis that a factor, responsible for raising the threshold of the response to hypoxia, was present in the teneral in greater concentration than in the mature insect. Moreover, the failure to respond to hypoxia is associated with a high frequency in the spiracle nerve: for example in 24 tenerals the mean frequency in air was 58 ± 2·01/sec., whereas for 23 matures it was 48 ± 3·10/sec. This would be expected if tenerals were slightly desiccated, but it would also occur if they failed to respond to a mild hypoxia which was normally present. In fact desiccation alone cannot account for the observations since the volume of haemolymph in tenerals is greater than in matures, as is also found in *Drosophila* (Wigglesworth, 1963). After perfusion of tenerals with normal saline a small drop in frequency and a lowering of the threshold to hypoxia were measured, but the latter did not normally achieve the sensitivity found in mature insects, even after intermittent perfusion for several hours. It seems possible therefore that since the postulated factor cannot be completely removed by perfusion it is not confined to the haemolymph. The results, however, are at present open to other interpretations.

The action of carbon dioxide on the motor frequency to spiracle 2

Carbon dioxide mixtures in air were passed through the gassing chamber and the frequency was measured as above (Fig. 6). The frequency is unaffected by mixtures containing less than 20–24% carbon dioxide; at higher concentrations a fall occurs but this is usually accompanied by struggling and wing flapping. Such concentrations are probably well above the normal physiological range and it seems likely, therefore, that carbon dioxide plays little part in controlling the spiracles through the central nervous system, in marked contrast to its effect on centres controlling ventilation and to its direct action on the spiracle muscles. This situation is different from that found by van der Kloot (1963) in silkmoth pupae where 10% carbon dioxide in oxygen silenced the motor output to the spiracle in one axon completely. Moreover, it suggests that hypoxia may have its effect not through a lowering of the pH but through a more specific mechanism.

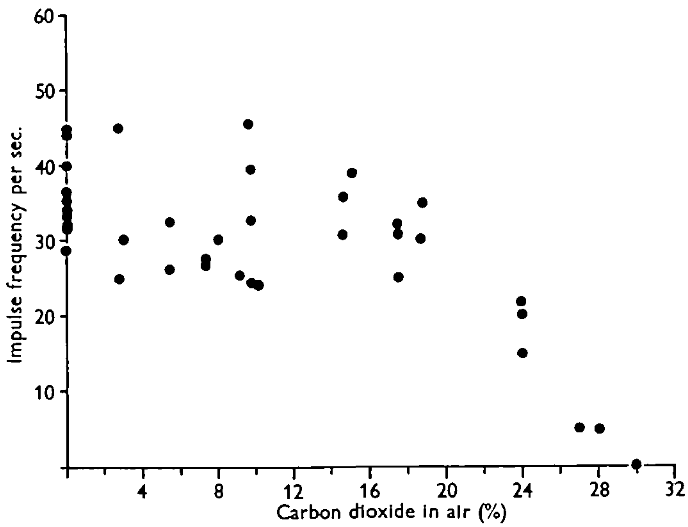

Fig. 6. *Ictinogomphus ferox* (mature). The frequency of motor impulses to spiracle 2 plotted against concentration of carbon dioxide in air.

The effect of temperature on the motor frequency to spiracle 2

Ictinogomphus ferox adopts the perching method of hunting (Corbet, 1962), as will be discussed below, and for this method accurate temperature regulation of the thorax is essential. Most of the experimental work on the effects of hypoxia and changes in water balance was carried out at room temperature (*c.* 25° C.), but when the insect is perching and on the look-out for prey a thoracic temperature of 30–35° C. is probably maintained (see Discussion). It was therefore of interest to measure the frequency of motor impulses to spiracle 2 after raising or lowering the temperature. Readings were made only when a steady temperature had been maintained for 5–10 min. after either heating or cooling. In Fig. 7 the results from 3 tenerals are shown; there is a logarithmic increase in frequency up to 35–40° C. but at higher temperatures the frequency declines. The Q_{10} for the reaction is 2·7 (15–25° C.). In mature insects the frequency falls off rapidly above 30–35° C. (Fig. 8), and at about 40° C. struggling often starts and a further reduction in frequency may be caused by the increased oxygen consumption. The decline at lower temperatures in matures, as compared with tenerals, may reflect their greater sensitivity to hypoxia.

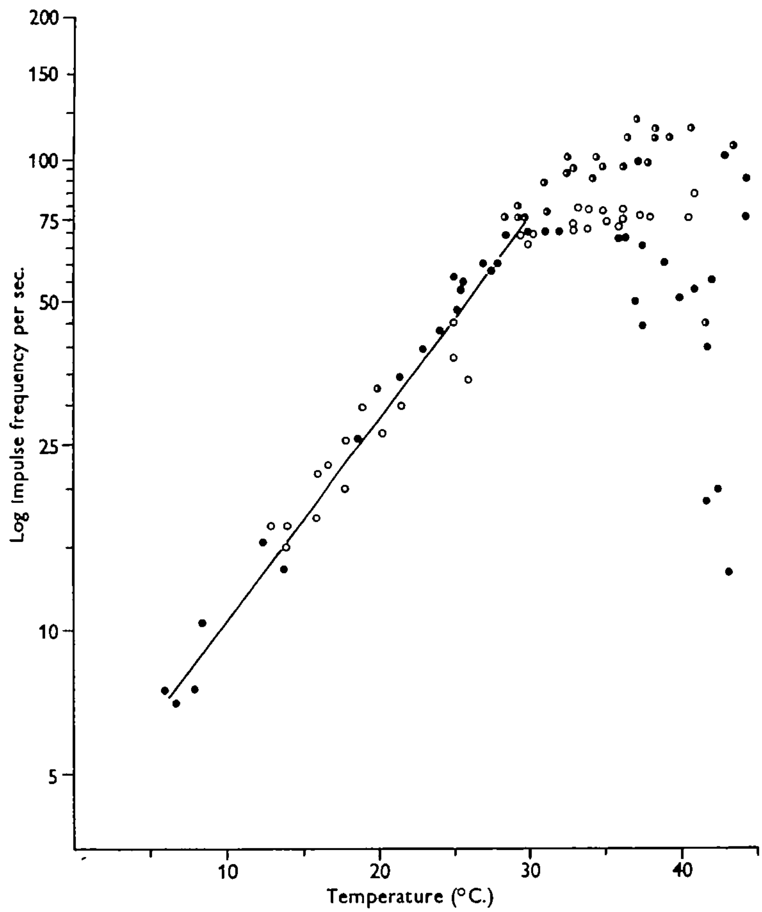

Fig. 7. *Ictinogomphus ferox*. The frequency of motor impulses to spiracle 2 plotted against temperature. Data for three teneral insects.

When the head alone is heated by means of a small coil implanted in the frons a similar increase in frequency is obtained. A thermistor inserted near the mesothoracic ganglia was used to check that the thorax remained close to room temperature during this experiment (Fig. 9). Similarly when the thorax alone is heated and the head kept at room temperature (checked with a thermistor) a comparable rise in frequency is seen. The results suggest that temperature may affect the motor neurones of the spiracle muscles either directly or via a controller neurone in the head, but that the effect on both is not summed. After decapitation a rise in temperature still produces an increase in frequency, but this is smaller than in the intact insect.

The motor frequency to spiracle 2 can therefore be increased either by raising the strength of the perfusing saline or by warming the insect. A rise in the threshold of the response to hypoxia accompanies the former, but this rise is not necessarily caused by the increase in frequency; it may merely be a concomitant of it in desiccated insects. By measuring the hypoxic response after raising the temperature we may determine whether the two can be varied independently. When this is done the results show that with a frequency of about 55/sec. at 30° C. the sensitivity to hypoxia is as great as when it is about 40/sec. at 25° C. (Fig. 10). In 1·0–2·0% oxygen the impulses are extinguished regardless of whether the frequency in air is 40, 55 or 75/sec. It seems therefore that desiccation (or perfusion with double strength saline) produces two separable effects: first, an increase in frequency with a consequent rise of the threshold of the *peripheral* response to carbon dioxide and, secondly, a rise in the threshold of the *central* response to oxygen lack. A rise in temperature, on the other hand, produces the former alone.

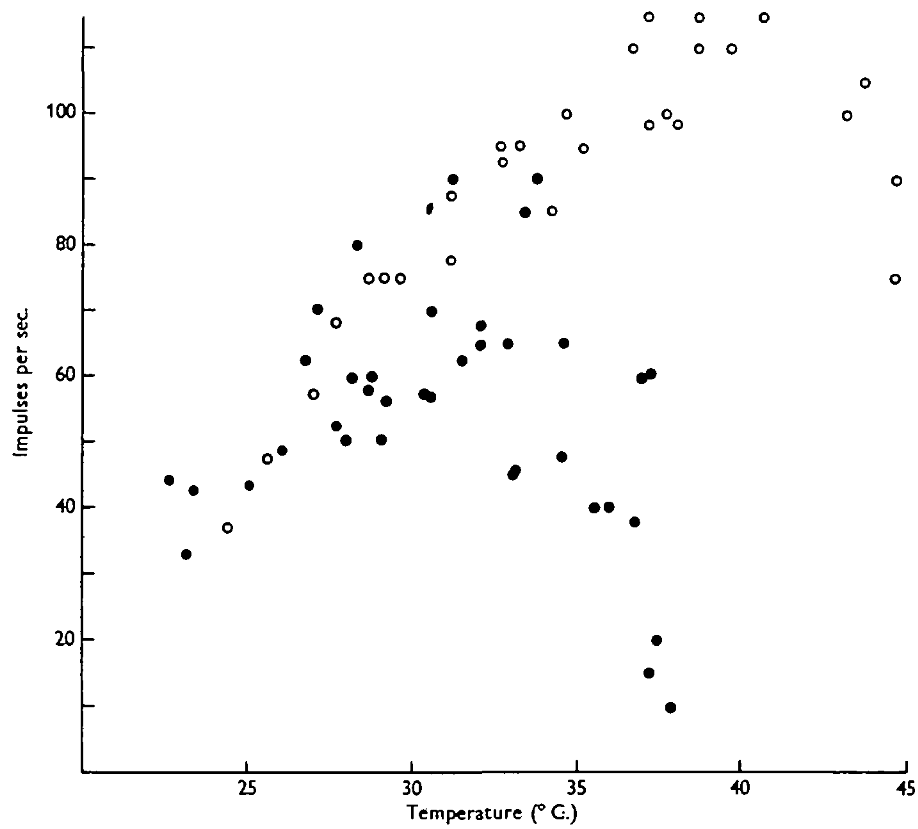

Fig. 8. *Ictinogomphus ferox*. The frequency of motor impulses to spiracle 2 of a teneral (○) and a mature (●) insect at various temperatures.

DISCUSSION

Various attempts may be made to account for the remarkable insensitivity of the spiracles of the teneral *I. ferox* to hypoxia as compared with the mature insect. As already suggested, there may be present in the teneral a factor in high concentration which suppresses the reaction. Since perfusion for long periods usually increases the sensitivity by a limited amount only, the factor may not be confined to the haemolymph.

Alternatively the mechanism may not be fully developed until after the first few days of adult life.

The adaptive value of spiracular insensitivity to hypoxia in tenerals may be in reducing water loss. Some species of dragonflies perform long maiden flights away from water immediately after emergence (Corbet, 1962). Little is known of the habits of *I. ferox* and the length of its maiden flight has not been ascertained. Nor is it known how soon the adult commences feeding. However, there is probably a period of starvation, lasting a day or longer, immediately following emergence, during which the flight system is insufficiently developed to perform the rapid manoeuvres necessary for prey capture, but at which time the maiden flight is undertaken. Considerable water loss might take place during such a period of activity when there was no replacement through feeding, and strict spiracle control in tenerals may reduce this loss.

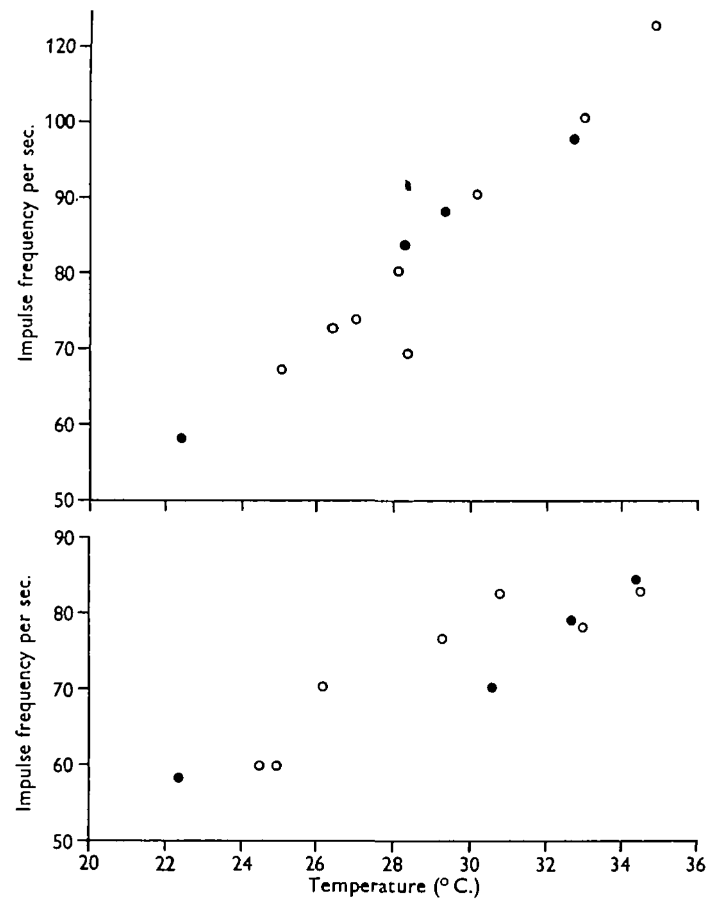

Fig. 9. *Ictinogomphus ferox*. The frequency of motor impulses to spiracle 2 in two teneral insects when the head alone (●) or the thorax alone (○) is heated by means of an implanted coil.

The larvae of *I. ferox* inhabit an environment which is likely to contain only small amounts of dissolved oxygen; larvae have been found up to about 0·8 km. from the shore of Lake Victoria in 10 m. of water and they probably rest partly buried in soft bottom deposits (Corbet, 1962). The absence of emergent aquatic vegetation means that they are unable to approach the water surface when the oxygen supply is lower than normal—the means used by aeshnids to overcome temporary oxygen lack. Moreover spiracle 1, adapted for aerial respiration in the last larval instar of most species, is unusually small in gomphids (Miller, 1963*b*).

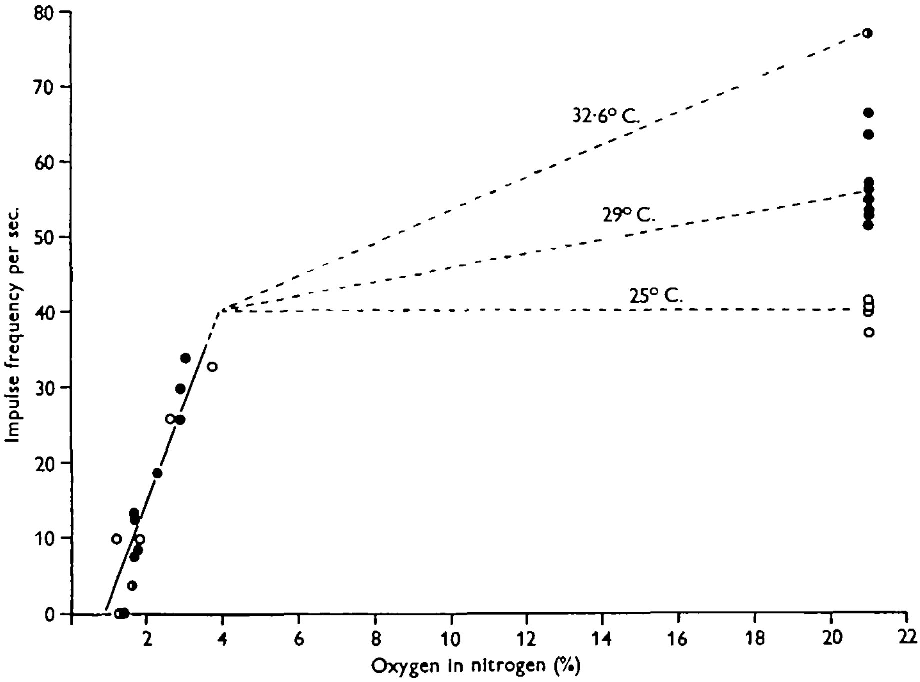

Fig. 10. *Ictinogomphus ferox*. The frequency of motor impulses to spiracle 2 in a mature insect at 32·5° C. (◑), 29° C. (●) and 25° C. (○), plotted against oxygen concentrations.

In consequence the larvae probably tolerate conditions of low oxygen supply, and their inactivity may help in this. They may also be able to accumulate oxygen debts and repay them slowly. Survival by the teneral for several hours in low oxygen concentrations may also reflect their low oxygen consumption when immature and inactive. Since ventilation continues under these conditions, the diffusion pathway to the respiring tissues probably remains short.

Ictinogomphus ferox hunts mostly from perches. It chooses a prominent site from which it makes short rapid flights in pursuit of prey often returning to the same perch between flights (Miller, 1963*b*). Experiments carried out in a heated room have indicated that spontaneous flight is not possible below an air temperature of 32–35° C., unless it is preceded by wing whirring to raise the thoracic temperature. Air temperatures near the northern shores of Lake Victoria are seldom higher than 25–27° C. so that a thoracic temperature several degrees above the air temperature must be maintained to make possible instant flight. This is accomplished by careful adjustment of the body angle with respect to the incident radiation (Corbet, 1962). When the sun is obscured by cloud, *I. ferox* either ceases to hunt or attempts to maintain a high thoracic temperature by intermittent bouts of wing whirring. The thorax of perching *I. ferox* is kept, therefore, around 30–35° C. at which temperature the frequency of motor impulses to spiracle 2 is high and spiracle control, with respect to carbon dioxide, is tight. In flight, when higher thoracic temperatures occur, the spiracle neurones are probably switched off (Miller, 1962). Immediately after flight the thorax, which is well insulated by an envelope of airsacs, cools slowly and a low impulse frequency allows the spiracle to flutter or remain open so that rapid gas exchange can occur until the temperature has returned to 30–35° C.

In conclusion some of the factors which are thought to alter spiracle control have been summarized in Fig. 11. Most of them are shown as having their effect through the central nervous system, but carbon dioxide and water balance are indicated as having a direct action on the closer muscle.

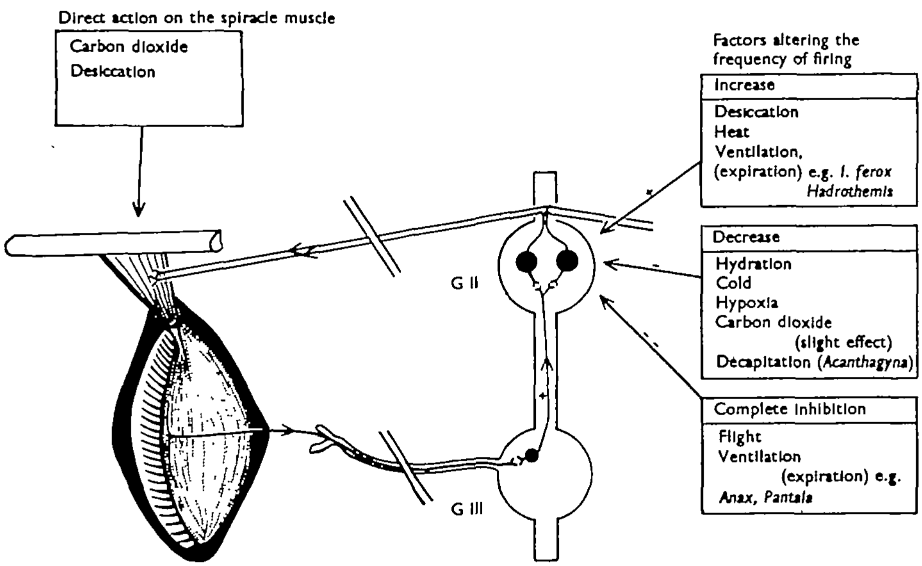

Fig. 11. Diagram summarizing some of the factors which are thought to alter spiracle control in dragonflies either by direct action on the spiracle or else through the central nervous system. A sensory nerve from mechano-receptors in the valve and peritreme to the metathoracic ganglion (G III) and a connecting interneurone to the mesothoracic ganglion (G II) are also shown. +, excitatory effect; −, inhibitory effect.

SUMMARY

1. The steady output of impulses in the two motor axons to spiracle 2 of mature individuals of the large African gomphid dragonfly, *Ictinogomphus ferox* Rambur, is reduced in frequency with hypoxia and abolished in about 2% oxygen. After perfusion of the insect with hypertonic saline the threshold of this response is raised, whereas, following perfusion with hypotonic solutions, it is lowered.

2. Teneral dragonflies give only a weak response to hypoxia—comparable to that of the mature insect perfused with hypertonic saline. This may help to conserve water before feeding commences.

3. Little change in the frequency of motor impulses follows gassing with carbon dioxide mixtures in air up to 20%; this is in contrast to the reactions reported in some other insects.

4. A raised temperature causes an increased frequency and the Q_{10} for the reaction is 2·7 (15–25° C.). Above 30–35° C. the frequency declines rapidly. A rise in the threshold of the hypoxic response does not accompany the increased frequency brought about by a raised temperature. A similar increase in frequency occurs when either the head or the thorax alone is heated.

5. Pterothoracic temperatures of about 30–35° C. are probably maintained in this species while perching in the sun. In this temperature range it is able to take off instantly when potential prey is sighted. High pterothoracic temperatures are considered in relation to spiracle control.

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