THE FUNCTION OF HAEMOGLOBIN IN RELATION TO FILTER FEEDING IN LEAF-MINING CHIRONOMID LARVAE

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(With One Text-figure)

Tendipes (=Chironomus) plumosus larvae are unique among species of Tendipes in employing a filter-feeding mechanism for obtaining their food (Walshe, 1947a). The larva spins a sheet of salivary secretion across the lumen of its tube, after which, by rapid anteroposterior undulations of the body, it irrigates the tube. Plankton or other organic particles in the incoming water current are thereby caught in the salivary net and subsequently eaten. This filter feeding is an energetic process involving glandular activity in spinning the salivary sheet and muscular activity in maintaining a rapid current of water during the filtration of food particles. A study (Walshe, 1950) of the amount of filter feeding at different oxygen concentrations has shown that this is essentially an aerobic process, declining at low oxygen concentrations and absent under anaerobic conditions. Normal larvae are incapable of feeding below about 10% air saturation of the water, while larvae without functional haemoglobin (i.e. after carbon monoxide treatment) cease to feed at 26% air saturation. Even above this value larvae with carboxyhaemoglobin feed less than normal animals. Thus the haemoglobin, in addition to its established significance as an oxygen carrier at low oxygen pressures (Harnisch, 1936; Ewer, 1942) and during recovery from oxygen lack (Harnisch, 1936; Walshe, 1947b), also plays a part in enabling the larvae to continue an active aerobic feeding process even when little oxygen is available.

Other chironomid larvae, namely, the leaf-mining genera, employ a filter mechanism for obtaining their phytoplanktonic food and, although the mechanism is slightly different from that of T. plumosus in that the larvae turn round in their tubes after spinning the net (Burtt, 1940), the principle is the same and also demands considerable activity on the part of the animal. Some of the species of these genera contain abundant haemoglobin, so a number of experiments was made to see whether in these larvae also the blood pigment is of functional value whilst filter feeding.

Larvae of two filter-feeding genera, namely, Glyptotendipes and Endochironomus, were removed from the leaves of their host plants and put into straight glass tubes of the same dimensions as their natural burrows in or on the plants. The larvae lived well in these glass tubes, lining them with salivary secretion, filter-feeding normally and remaining in them until the end of pupation. Their feeding behaviour could be
clearly seen within these tubes, which were stuck vertically through holes in a hori-
zontal celluloid sheet suspended in an aquarium in which the respiratory conditions
of the water could be controlled. In this way several larvae could be observed
simultaneously. The amount of filter feeding of normal larvae was compared with
that of carbon monoxide-treated larvae at a range of different oxygen concentrations.
At each oxygen concentration between five and ten larvae were studied; after an
initial 2 hr. under the desired respiratory conditions the numbers of larvae filter
feeding were determined at half-hourly intervals for the next 3 hr. Larvae filter
feeding could be distinguished from those irrigating the tube for respiratory purposes
or carrying on other activities, by the very characteristic net-spinning move-
ments and by the nets, made visible by phytoplankton added to the experimental water.
Experiments were carried out in a dilute phytoplankton suspension, since larvae
failing to catch any suspended matter in their nets soon abandon filter feeding.
The larvae were observed in a dim light in order to lessen any change in oxygen
concentration due to photosynthesis by the plankton. The experiments were made
at room temperature (14.5-18° C).

The first leaf-mining larva studied was *Glyptotendipes pallens* Meig., a red or dark
red larva containing much haemoglobin (according to Scheer (1934) the concentra-
tion is twice that in the *Tendipes plumosus* group), and collected from *Stratiotes* and
*Potomogeton* in a pond. The amount of filter feeding at different oxygen concentra-
tions, with and without functional haemoglobin, is given in Table 1 and Fig. 1 A.
In well-aerated water the carbon monoxide-treated larvae fed as much as normal
animals, but at lower oxygen concentrations they always showed less filter feeding
than those with functional haemoglobin. The same result was obtained with
*Endochironomus dispar* Meig., another red chironomid found mining in dead *Phrag-
mites* stalks (Fig. 1 B). In these two species therefore the haemoglobin plays the
same role in filter feeding as it does in *Tendipes plumosus*.

Other species of *Endochironomus*, however, have considerably less haemoglobin
in their blood, *E. tendens* Fabr. being pink rather than red in colour and *E. albipennis*
Meig. being very pale pink or colourless. These two species, incidentally, were found
in habitats where the average oxygen content of the water was probably considerably
higher than that in the stagnant peaty pond from which *E. dispar* was collected.
From the previous experiments it was expected that these larvae would be less
efficient filter feeders at low oxygen pressures than the red larvae, since they had less
pigment to pick up oxygen with which to supply their demands. Such, however, was
not the case (Table 1, Fig. 1 C, D). They were capable of maintaining their filter
feeding at reduced oxygen concentrations as effectively as their haemoglobin-
containing relatives. Carbon monoxide treatment caused a slight diminution in
amount of feeding in the pink species (*E. tendens*) and had no effect in the very pale
species (*E. albipennis*). These carbon monoxide experiments incidentally serve as
a useful check on the validity of drawing conclusions about the function of haemo-
globin from carbon monoxide treatment. Such conclusions are always based on the
assumption that the carbon monoxide only eliminates the oxygen-carrying capacity
of the haemoglobin and has no adverse effect on other physiological processes. Here
Function of haemoglobin in relation to filter feeding

its action of diminishing the filter feeding of chironomids containing haemoglobin must be due solely to its effect on the pigment, since it has no effect on larvae without haemoglobin.

Presumably therefore, these two pale species have a capacity for maintaining an output of energy under partly anaerobic conditions of a different nature from that of the red species. To see whether the resistance to total anaerobiosis threw any light on their behaviour at low oxygen concentrations, ten larvae of each of the four species were kept in nitrogenated water in large bottles, and their rate of death during the course of a number of days was recorded. Under such conditions none of the larvae filter fed, but for a number of hours they maintained a continuous irrigation current through their tubes, after which they left their tubes, became increasingly immobile and finally died. Of the four species, *Glyptotendipes* and *Endochironomus dispar* remained in their tubes and were active longest, a few individuals surviving for 89 hr., whereas the pale *Endochironomus* species were all out of their tubes and immobile or dead considerably before this. Thus the haemoglobin-containing species have the greater capacity for complete anaerobiosis, although in surroundings totally

Table 1. The amount of filter feeding of four species of leaf-mining chironomid larvae at different oxygen concentrations, with and without carboxyhaemoglobin

<table>
<thead>
<tr>
<th>Species</th>
<th>Normal animals</th>
<th>Animals with carboxyhaemoglobin</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>% air saturation</td>
<td>No. of animals</td>
</tr>
<tr>
<td><em>Glyptotendipes pallens</em> Meig.</td>
<td>100–80</td>
<td>8</td>
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<td></td>
<td>56</td>
<td>8</td>
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<td>&lt;3</td>
<td>6</td>
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<td><em>Endochironomus dispar</em> Meig.</td>
<td>100–80</td>
<td>5</td>
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<td>50</td>
<td>8</td>
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<td>39</td>
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<td></td>
<td>7</td>
<td>5</td>
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<tr>
<td><em>Endochironomus tendens</em> Fabr.</td>
<td>100–80</td>
<td>11</td>
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<td>15</td>
<td>6</td>
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<td>&lt;3</td>
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<td><em>Endochironomus albipennis</em> Meig.</td>
<td>100–80</td>
<td>10</td>
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devoid of oxygen this can have no direct connexion with the possession of the blood pigment.

Because of their inhibitory action on certain oxygen-utilizing cellular enzymes, respiratory poisons may also be used to explore animals' powers of anaerobic activity. The resistance of *Glyptotendipes pallens* and *Endochironomus tendens* to potassium cyanide was therefore tested. Larvae in glass tubes were put into M/5000- and M/10,000-KCN solutions in aerated pond water. At both concentrations the larvae abandoned filter feeding and behaved as if under anaerobic conditions, ultimately coming out of their tubes. After 24 hr. all larvae of both species recovered when removed from the cyanide. There was thus no striking difference in total susceptibility of the two species at these concentrations. The effects of greater concentrations were not explored, since even in the stronger of these solutions the unnatural twitchings of the larvae indicated that the cyanide was having a physiological effect other than the desired one of blocking part of the respiratory process. At both concentrations, however, there was a difference between the species in the rate at which the signs of respiratory poisoning appeared (as indicated by their abandoning respiratory irrigation and leaving the tubes), the pale *Endochironomus* larvae remaining in their tubes longer than the red *Glyptotendipes*. This would seem to
Function of haemoglobin in relation to filter feeding

indicate that the pale species is less immediately inconvenienced by an inability to utilize oxygen through the oxidase-dehydrogenase system. The capacity of the pale larvae to filter feed in the presence of little oxygen may thus be related to the possession of an alternative system of respiratory enzymes efficient at low oxygen concentrations, at which the red species, on the other hand, need haemoglobin as an oxygen source. This suggestion must be a tentative one, however, since it is based only on a difference in speed of susceptibility of the two species as judged by relatively few data.

However, the fact remains that the haemoglobin of red leaf-mining chironomids is of functional value to them in carrying on filter feeding at low oxygen pressures, as it is in Tendipes plumosus. Nevertheless, the possession of haemoglobin is not the only respiratory modification in the chironomids for activity under partly anaerobic conditions, since other larvae can maintain active feeding without the intervention of the blood pigment.

SUMMARY

1. The filter feeding of four species of leaf-mining chironomid larvae with and without functional haemoglobin was studied at different oxygen concentrations.

2. Two red species, Glyptotendipes pallens and Endochironomus dispar, showed at low oxygen concentrations a reduced amount of filter feeding after treatment with carbon monoxide (to render the haemoglobin functionless). The blood pigment is therefore of significance to these larvae in increasing the amount of feeding possible at low oxygen concentrations.

3. Two pale species, Endochironomus tendens and E. albipennis, however, despite their having little haemoglobin, were nevertheless capable of filter feeding in water poor in oxygen.

4. The capacity to live anaerobically was greatest in the red species.

5. In potassium cyanide solutions, on the other hand, the red Glyptotendipes pallens larvae were more rapidly affected than the pale Endochironomus tendens larvae. It is tentatively suggested, therefore, that the ability of the larvae poor in haemoglobin to filter feed at low oxygen concentrations may be due to their possession of respiratory enzyme systems, alternative to the cyanide-sensitive type, which are efficient at low oxygen pressures.

My grateful thanks are due to Prof. Kaj Berg in whose laboratory in Hillerød, Denmark, this work was done.

REFERENCES


