# THE METABOLISM OF CHIRONOMUS ( DIPTERA )

and other related genera.

by

Barbara M. Walshe M.Sc.(Birm.).

a candidate for the degree of Ph.D.

University of London.

Bedford College for Women.

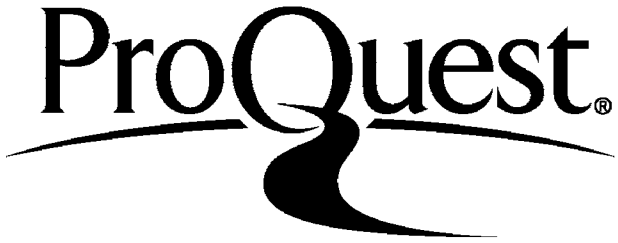
June 1948.

ProQuest Number: 10097946

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.



ProQuest 10097946

Published by ProQuest LLC(2016). Copyright of the Dissertation is held by the Author.

All rights reserved.
This work is protected against unauthorized copying under Title 17, United States Code.
Microform Edition © ProQuest LLC.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Introduction.

The pigment haemoglobin, universally present in the vertebrates, occurs spasmodically in the invertebrate phyla. The diversity of the modes of life of the haemoglobin-bearing invertebrates, and the variations in site and quantity of the pigment, make it untenable to assume that, as in vertebrates, its chief function is necessarily that of transporting oxygen from the external environment to the tissues of the body. Generalisations as to the function of invertebrate haemoglobins cannot be made.

A considerable amount of work has been done with a view to elucidating the functions of haemoglobin in different animal forms. The problem may be tackled in a number of ways : a knowledge of the biochemical and physical properties and quantity of the ~~of the~~ particular haemoglobin gives an indication of its potentialities within the organism, while an experimental study of the metabolism of living animals, making use especially of the carbon monoxide method for studying haemoglobin function provides information as to the role of the haemoglobin under known laboratory conditions. Finally, a knowledge of the organism's mode of life and behaviour under various contolled external environments, accompanied by ecological data of the conditions actually encountered in nature, allow the experimentally acquired laboratory data to be interpreted in terms of the significance of the haemoglobin in the life of the animal. Only by an integration of such biochemical,

physiological and ecological work can haemoglobin function be assessed.

The papers forming this thesis are concerned with experimental work on certain aspects of the function of haemoglobin in chironomid larvae.

Chironomus larvae live in mud often deficient in oxygen, their haemoglobin has a high affinity for oxygen, and enables them to maintain a normal metabolic rate at oxygen pressures too low to allow of this otherwise. It is therefore of functional value to the larvae in poorly-aerated water. Certain species of Chironomus, however, also possess the capacity of living for many hours under completely anaerobic conditions. During the subsequent recovery it has been claimed that their metabolic rate rose in the repayment of an oxygem debt, and that the haemoglobin was then functional at all external pressures of oxygen. This work is, however, open to serious critisism and it was considered necessary to reinvestigate the metabolism of Chironomus after oxygen deficiency under carefully controlled conditions. This forms the content of the first paper in this thesis. The previous results were not confirmed, for the oxygen debt repaid after a period of anaerobiosis was found to be very small, and the haemoglobin did not function during its repayment in fully aerated water. It is, however, of functional significance to the post-anaerobic larvae, for the initial stages of recovery are accompanied by a high degree of respiratory undulation, which activity, with the help of haemoglobin as an oxygen carrier, is largely aerobic and

therefore economical, for without haemoglobin this activity would be anaerobic and consequently produced at the expense of a greater quantity of metabolic substances. These undulations would, under natural conditions, serve to irrigate the larval burrows, making a large quantity of oxygen-containing water available, and so hastening the return of larvae to normal aerobic metabolism after periods of oxygen lack.

However, the possession of haemoglobin by a chironomid larva does not necessarily imply a capacity to live in surroundings deficientin oxygen, an apparent anomaly which has been commented upon in the past. Its role in stenoxybiotic species must differ from that in Chironomus. Consequently the stenoxybiotic, stream-living larvae of Tanytarsus were selected for an experimental study of the metabolism with and without functional haemoglobin. This work is described in the second paper. Although in fully aerated water the metabolism is high the haemoglobin plays no part in transfering oxygen to the tissues. It is in fact functionless in oxygen transport over a wide range of oxygen concentrations. Only below 25 % air saturation of the water has it any such function : at these low oxygen concentrations it lessens the rate of death of larvae from asphyxia. Even with the help of their haemoglobin, however, they die quickly : the significance of the haemoglobin in the life of Tanytarsus larvae is therefore doubtful.

The lack of correlation between haemoglobin possession and oxygen deficiency in the environment suggested that other

physiological attributes are more important in determining the ecological distribution of chironomid larvae. Certain aspects of the metabolism of a number of larvae from different habitats were therefore studied. The results are given in the third paper. Larvae from stagnant, oxygen-deficient environments were found to have a lower metabolic rate in fully aerated water than morphologically similar larvae from streams. Also their ability to withstand oxygen deficiency and high temperatures, and to maintain a normal metabolic rate at low oxygen pressures, was greater. These attributes were independent of the possession of haemoglobin, or the morphological structure of the larvae, but obviously correlated with the physical and chemical nature of the environments.

Considering chironomid larvae as a whole, therefore, one cannot generalise on the role played by the haemoglobin in their lives. In Chironomus it is useful at low oxygen pressures and helps a quick, economical recovery from oxygen lack. In Tanytarsus and in other stream-living species however, it does very little to improve the animal's resistance to low oxygen pressures. All these data, however, were gained from experimental work on larvae under highly artificial conditions. A study of the metabolism and behaviour under more natural conditions might be expected to throw additional light on the function of the haemoglobin. Such a study has been undertaken in the case of Chironomus plumosus. It is not reported in this thesis, except that during the course of watching these larvae in observation tubes similar to the mud tubes which they construct

in nature, a previously unknown filter-feeding mechanism was discovered which is described in the fourth paper in this thesis.

Reprinted from THE JOURNAL OF EXPERIMENTAL BIOLOGY,
Vol. 24, Nos. 3 & 4, pp. 329–342, December 1947
[*All rights reserved.*]
PRINTED IN GREAT BRITAIN

# ON THE FUNCTION OF HAEMOGLOBIN IN *CHIRONOMUS* AFTER OXYGEN LACK

By BARBARA M. WALSHE
*Bedford College, University of London*

(*Received* 17 *March* 1947)

(With Four Text-figures)

## INTRODUCTION

The function of haemoglobin in the large chironomid larvae of the *Chironomus plumosus* group has been studied experimentally by Ewer (1942). She compared the oxygen consumption of normal larvae at different pressures of oxygen with that of larvae whose respiratory pigment had been rendered functionless by treatment with carbon monoxide, and from the differences between the two deduced the extent to which the haemoglobin is of functional value to the animal in the transport of oxygen. Her results show that at 17° C. the haemoglobin of *Chironomus* larvae (previously kept in aerated water) does not function at air saturation of the water, but only at oxygen concentrations below 3 ml./l. (44% saturation). This can be correlated with the common occurrence of *Chironomus* larvae in stagnant habitats known to be more or less depleted of oxygen from time to time.

The work of Ewer in part repeats, under careful experimental conditions, similar work done by Harnisch (1936), whose experimental technique is open to criticism. Harnisch studied both animals previously kept in oxygen gas and animals which had been subjected to severe oxygen lack by keeping them about 20 hr. in nitrogen gas before experiments. The nitrogen-treated larvae when replaced in aerated water in a Barcroft manometer had an oxygen uptake 160% that of the oxygen-treated larvae. This indication of the repayment of an oxygen debt persisted for about 3 hr. The increased oxygen uptake of the nitrogen-treated animals was observed not only in aerated water but also at lower oxygen pressures, even at 14% air saturation. The oxygen consumption of nitrogen-treated larvae which had also been subjected to carbon monoxide was, however, lower than that of nitrogen-treated larvae whose haemoglobin had not been converted into carboxyhaemoglobin; this was so in water at all oxygen pressures at and below air saturation. Harnisch concluded that when the metabolic rate rises in recovering from oxygen lack the haemoglobin becomes functional in oxygen transport at all pressures of oxygen, and that the increased oxygen consumption of recovering larvae is, thanks to their haemoglobin, maintained irrespective of the oxygen content of the water. That the larvae, which must frequently experience anaerobic conditions in nature, should thus be able to recover from oxygen lack even in water containing very little oxygen, Harnisch considers to be of paramount importance in assessing the function of the haemoglobin. This

work is, however, open to criticisms, some of which have been made by Ewer (1942). The temperature varied from 16 to 23° C. in experiments supposed to be comparable and Harnisch used carbon monoxide pressures high enough to have had an inhibitory effect on cellular oxidations. He dismisses this possibility with the statement that although Warburg (1926) had shown such a concentration to have an inhibitory effect on yeast he himself considers that 'bei der Hefe der Kontakt zwischen dem Giftgas und den Zellen doch wohl wesentlich inniger war als bei meinen Tieren'! He applies no statistical tests to his data, and two series of estimations of the metabolism in aerated water of animals after nitrogen treatment (Table 2, p. 396 and Table 6, p. 406) give significantly different averages.

For these reasons a further investigation of the metabolism of chironomid larvae after oxygen lack was considered necessary.

## MATERIAL AND METHODS

The chironomids used were the final instar larvae of *Chironomus plumosus* L. collected from the mud of Regent's Park Lake, London. The species was identified by means of Edwards's key (1929) from adults which emerged in the laboratory, and checked by comparison with the collection at the British Museum. I wish to thank Dr J. Smart for allowing me access to this collection. Larvae were collected 1 or 2 days before experiments and were kept in the laboratory in aerated water in shallow dishes with a thin layer of mud, under which conditions they remained healthy, and successfully pupated and emerged.

The respiration of larvae was measured in an apparatus in which the larvae were subjected to a continual slow stream of water, their oxygen consumption being calculated from the difference in oxygen content of the water before and after passing through the respiratory chamber. A diagram of the apparatus is given in Fig. 1. A 5 l. aspirator (*a*), containing aerated buffered water (0·004 *N* solution of $NaHCO_3$) was connected by way of a water-mixer (*b*), to the respiratory chamber (*c*). This consists of an 11 cm. length of wide glass tubing, 1·5 cm. diam., blackened on the outside except for a longitudinal unpainted slit about 5 mm. wide through which the behaviour of the larvae could be observed without exposing them to excessive light, which has an activating effect. Perforated porcelain disks on the rubber bungs prevented the larvae from crawling out of the respiratory chamber. The three-way tap (*f*) and capillary outflow tube (*d*) allowed samples of the water flowing into and out of the respiratory chamber being taken for analysis of dissolved oxygen. This was determined by the syringe-pipette micro-Winkler method described by Fox & Wingfield (1938). The nozzle of the syringe-pipette was inserted into the rubber tubing at (*f*) or (*d*) and water samples of 1·4 ml. thus removed. The rate of flow through the apparatus was kept steady by maintaining a constant level in the aspirator (*a*) by means of a continuous inflow from flask (*g*) and a simple overflow device, and by firmly clamping the whole system of tubing to prevent any shift in position. The rate was determined by the length of time taken by the outflowing drops to fill a given volume. The respiratory chamber, together with the inflow and outflow tubes, were supported in a large thermostatic water bath.

Between thirty and forty larvae were used in each experiment. With a water flow of 1·3 ml./min. these larvae lowered the oxygen content of the water by about 20% at 17° C. From the difference in oxygen content of the water before and after passing through the respiratory chamber and the rate of flow of the water, the oxygen consumption of the larvae was calculated in cu.mm. oxygen/g. (wet weight)/hr.

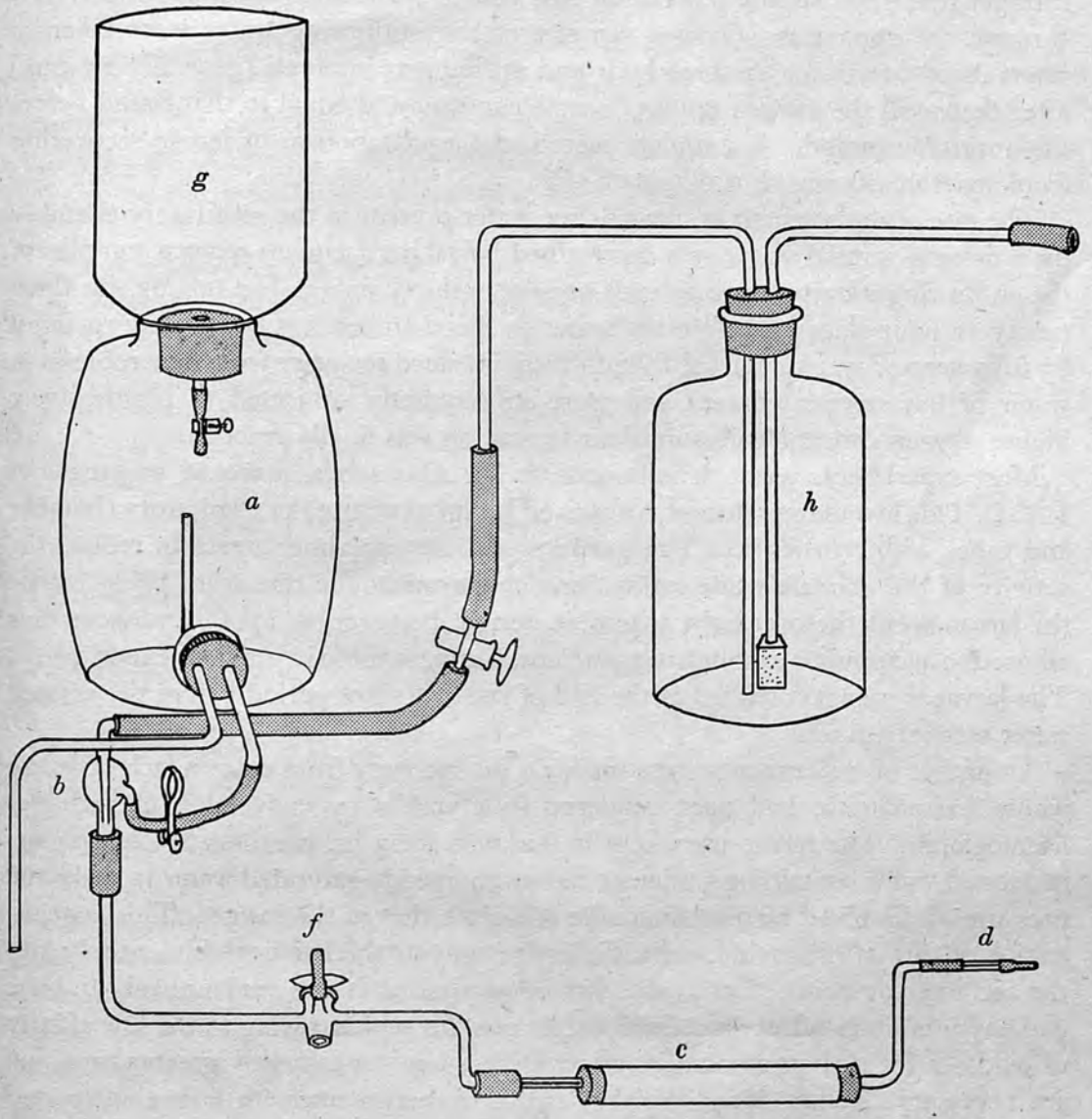



Fig. 1. Apparatus for measuring the oxygen consumption of *Chironomus* larvae. For explanation see text.

The 3 l. bottle (*h*) contained nitrogenated water (oxygen content less than 0·1 ml./l.); this could be driven through the respiratory chamber by the pressure of gas from a nitrogen cylinder, the larvae being in this way subjected to a period of oxygen lack.

Experiments were of about 24 hr. duration. Larvae were put into the respiratory chamber in flowing aerated water and left for about 2 hr. in order to reach a steady

state of activity. The oxygen contents of inflowing and outflowing waters were then determined at intervals of approximately 45 min. for the next 3 hr., and from these determinations the average metabolic rate of the animals was determined. The inflow of aerated water was then stopped and nitrogenated water slowly forced over the animals. The larvae were left thus for 16 hr., at the end of which period the nitrogenated water supply was cut off and aerated water once again allowed to flow through the apparatus. Oxygen samples of the outflowing water were taken at intervals of 5 min. for the next hour and at frequent intervals (generally 15 min.) after that until the oxygen uptake became constant and equal to that found before the anaerobic period. A complete record of the metabolism of larvae recovering from anaerobiosis was thus obtained.

The rate of replacement of oxygen-free water present in the respiratory chamber by inflowing aerated water was determined by taking frequent oxygen samples of the outflowing water with no animals present in the chamber. The mixing was slow, nearly an hour elapsing before the water in the chamber was completely replaced by fully aerated water. Larvae therefore commenced recovery from anaerobiosis in water of low oxygen content and were subsequently subjected to progressively higher oxygen concentrations until air saturation was finally reached.

Most experiments were carried out at 17° C. One series, however, was made at 1–2° C. This low temperature was obtained by surrounding the respiratory chamber and tubes with crushed ice. The purpose of these experiments was to reduce the activity of the animals while estimations of the metabolic rate were being made: the larvae spent the overnight anaerobic period, however, at 17° C. and were thus allowed to accumulate as much oxygen debt, if any, as those in previous experiments. The larvae were again chilled at the end of the overnight period before the aerated water was turned on.

A number of experiments were made on the recovery from oxygen lack of larvae whose haemoglobin had been rendered functionless by conversion to carboxy-haemoglobin. The larvae were kept in darkness for 3 hr. previous to experiments in aerated water containing sufficient carbon monoxide-saturated water to make the pressure of dissolved carbon monoxide one-sixth that of the oxygen. This concentration of carbon monoxide was sufficient to convert the haemoglobin entirely into the carboxy-compound, but at this low concentration it was very unlikely to have had any inhibitory effect on cellular oxidations, for which, owing to the low affinity of oxidases for carbon monoxide, relative pressures considerably greater than one are necessary (Keilin, 1929; Wolsky, 1938). Experiments with larvae so treated were carried out in the same manner as those with normal larvae, except that the water contained 0·2 ml./l. carbon monoxide to prevent dissociation of the carboxy-haemoglobin. The small amount of light introduced through the slit in the respiratory chamber did not cause any dissociation. Individual experiments with carbon monoxide-treated animals were alternated with control experiments with normal animals. This was a necessary precaution to ensure that larvae in the same physiological state were being compared, since seasonal differences in metabolic rate were found to occur.

At the end of all experiments the larvae were dried on filter paper and weighed. In the carbon monoxide experiments a drop of the animal's blood was then examined with a hand spectroscope: the absence of fading of the absorption bands after the addition of sodium hydrosulphite indicated that no dissociation of carboxyhaemoglobin had occurred during the experiment.

## RESULTS

The results of a series of seven experiments on normal animals at 17° C. are given in Table 1. Fig. 2 shows graphically the changes in metabolic rate during the course of the experiments. The oxygen uptake of an average basal value of $191{\cdot}7 \pm 7{\cdot}8$* cu.mm./g. (wet weight)/hr. before nitrogen subjection rose within 5 min. of the introduction of aerated water to a significantly higher value, the

Table 1. *Oxygen consumption of larvae of* Chironomus plumosus *L. at* 17° C. *before and after* 16 *hr. in nitrogenated water. Normal animals, May–June*

| Time before anaerobiosis | Oxygen concentration ml./l. | Oxygen consumption, cu.mm./g. (wet weight)/hr. | | |
|---|---|---|---|---|
| | | Separate values | Mean | Grand mean and S.E. |
| 4–3 hr. | 6·5 | 293, 158, 179, 163, 188, 184 | 194 | $191{\cdot}7 \pm 7{\cdot}8$ |
| 3–2 | 6·6 | 209, 150, 164, 168, 274, 198 | 194 | |
| 2–1 | 6·6 | 197, 166, 163, 179, 243, 187, 252, 163 | 194 | |
| 1–0 | 6·6 | 155, 148, 156, 176, 158, 204, 262, 280, 173, 156 | 187 | |
| Time after anaerobiosis | | | | |
| 0–5 min. | 0·4 | 12, 16, 33 | 20 | |
| 5½–10 | 4·2 | 284, 227, 147, 288, 298, 231, 307 | 255 | $235{\cdot}0 \pm 13{\cdot}8$ |
| 10½–15 | 5·2 | 155, 210, 210, 230, 224, 243 | 212 | |
| 15½–20 | 5·8 | 172, 137, 117, 243, 235, 198 | 184 | $204{\cdot}5 \pm 9{\cdot}3$ |
| 20½–25 | 6·2 | 204, 129, 242, 237, 280, 185 | 213 | |
| 25½–30 | 6·5 | 267, 108, 212, 213, 161, 249, 228 | 205 | |
| 30½–40 | 6·5 | 98, 187, 271, 217, 208, 243 | 204 | |
| 40½–60 | 6·4 | 143, 238, 236, 217, 260 | 219 | |
| 1–1½ hr. | 6·5 | 84, 224, 216, 235 | 190 | 188·4 |
| 1½–2 | 6·4 | 22, 210, 217, 273, 215 | 187 | |

average metabolic rate 5–15 min. afterwards being $235{\cdot}0 \pm 13{\cdot}8$. This marked increase only lasted for about 10 min., subsequent values from 20 min. to 1 hr. after the end of anaerobiosis being only slightly greater than the basal rate of the previous day. The statistical significance of this slight increase is very doubtful: the averages are $204{\cdot}5 \pm 9{\cdot}3$ after, as compared with $191{\cdot}7 \pm 7{\cdot}8$ before, anaerobiosis, with the large probability value ($p$) of 0·27. Further, an analysis of variance of these data did not indicate a significant increase in metabolic rate. At the end of 1 hr. the oxygen consumption had returned to the basal rate.

The initial rise in oxygen consumption was accompanied by great activity on the part of the animals: the larvae, which had been completely inactive in nitrogenated water, started violent undulatory movements of the body upon the introduction of aerated water. It seemed likely that the increased oxygen uptake was due to this

* Standard error.

activity rather than to the repayment of an oxygen debt; to test this experiments were made in which the activity changes were eliminated by chilling the animals. The results of six such experiments are given in Table 2 and Fig. 3. The basal metabolic rate of such larvae was 25·0 ± 2·4 cu.mm./g./hr. After the 16 hr. anaerobic period the metabolism rose slowly during the first 25 min. to a value about 160% of the basal rate and maintained this high level for at least 2 hr. The difference in metabolic rate before and 10 min. to 2 hr. after the anaerobic period is highly significant.

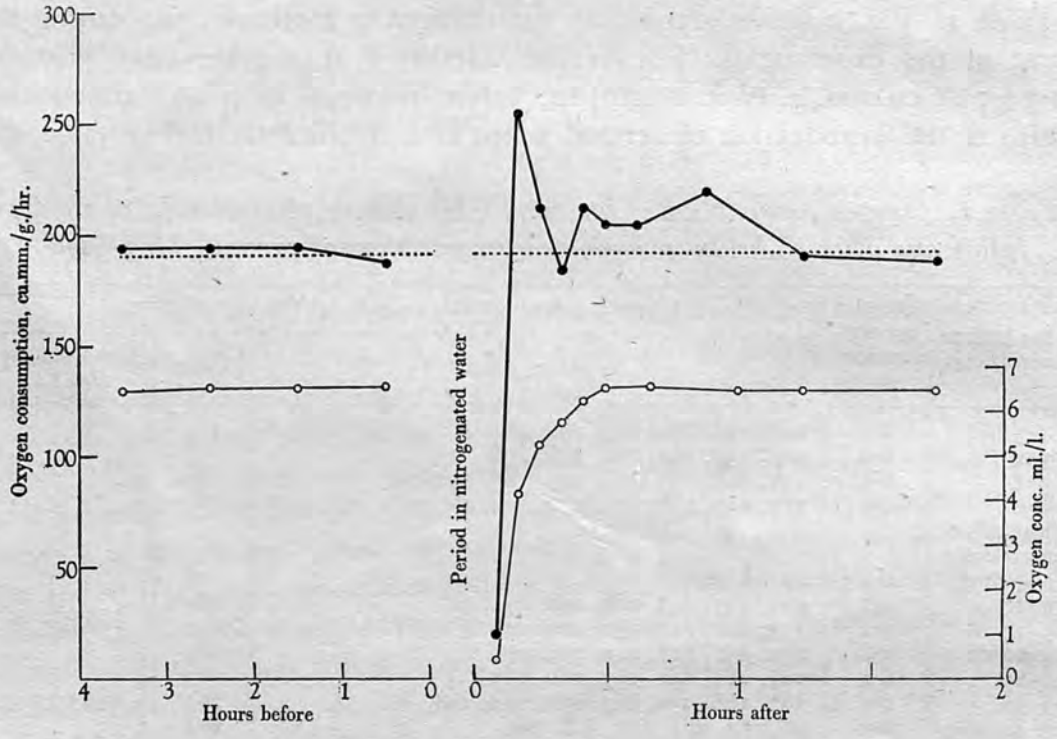



Fig. 2. Rates of oxygen consumption at 17° C. of *Chironomus plumosus* larvae before and after a 16 hr. period of anaerobiosis. Normal animals, May–June. ●, oxygen consumption; ○, oxygen concentration in respiratory vessel. The broken line represents the average metabolism before nitrogen subjection. Data from Table 1.

In interpreting the results at 17° C., therefore, the initial increase in oxygen consumption must be attributed to the great increase in activity of the larvae at that time, since immobile, chilled larvae do not show it. Larvae at both temperatures, however, showed a subsequent more prolonged increase in metabolism, and although at 17° C. the increase is so slight that its reality is doubtful, at the lower temperature it is greater.

The results of a third series of experiments in which the recovery from anaerobiosis of normal animals was compared with that of carbon monoxide-treated larvae are given in Tables 3 and 4 and Fig. 4. The metabolism curve is of the same general form as that already described for normal larvae at 17° C. except that the basal level is lower, being 140·3 ± 4·0 cu.mm./g./hr. compared with 191·7 ± 7·8 cu.mm./g./hr. previously. This must be due to a seasonal change, these last series of experiments having been made in late September on animals which would have

over-wintered as larvae, while the first series was made in May and June on larvae of a summer generation.

The basal metabolic rate of larvae with carboxyhaemoglobin is seen to be the same as that of untreated larvae. This confirms Ewer's conclusion that in aerated

Table 2. *Oxygen consumption of larvae of* Chironomus plumosus *L. at* 1° C. *before and after* 16 *hr. in nitrogenated water. Normal animals, July*

| Time before anaerobiosis | Oxygen concentration ml./l. | Oxygen consumption, cu.mm./g. (wet weight)/hr. | | |
|---|---|---|---|---|
| | | Separate values | Mean | Grand mean and S.E. |
| 2–1½ hr. | 5·7 | 35·3, 16·8, 16·0, 18·9, 26·9 | 22·8 | |
| 1½–1 | 5·8 | 52·2, 20·9, 7·3, 24·2, 27·9 | 26·5 | 25·0 ± 2·4 |
| 1–½ | 5·8 | 36·7, 33·6, 19·5, 8·6, 20·6, 21·6 | 23·4 | |
| ½–0 | 5·8 | 31·3, 39·3, 19·5, 22·8 | 28·2 | |
| Time after anaerobiosis | | | | |
| 0–5 min. | 0·3 | 0, 19·3, 9·8, 0, 9·4, 0 | 6·4 | |
| 5½–10 | 1·7 | 42·2, 48·2, 0, 0, 17·7 | 21·6 | |
| 10½–15 | 3·6 | 16·3, 15·7, 18·1, 19·6, 87·9, 38·7 | 32·7 | |
| 15½–20 | 4·6 | 29·2, 14·3, 44·7, 40·0, 49·4, 39·2 | 36·1 | |
| 20½–25 | 5·1 | 6·8, 26·4, 53·0, 52·1, 56·2, 37·8 | 38·7 | |
| 25½–30 | 5·4 | 14·3, 45·0, 56·5, 57·0, 52·8, 37·2 | 43·8 | |
| 30½–40 | 5·6 | 14·3, 47·2, 41·9, 55·8, 54·6, 25·6, 22·2, 48·7, 46·1, 51·6, 61·3, 37·2 | 42·2 | 38·9 ± 2·1 |
| 40½–60 | 5·7 | 29·9, 48·7, 46·8, 46·2, 42·8, 43·3, 41·2, 57·6, 26·9, 41·9, 41·2, 33·3, 52·2, 41·2, 37·6 | 43·4 | |
| 1–1½ hr. | 5·9 | 44·7, 33·4, 46·8, 31·4 | 39·1 | |
| 1½–2 | 5·8 | 51·4, 30·0, 29·3, 22·8, 39·2 | 34·5 | |

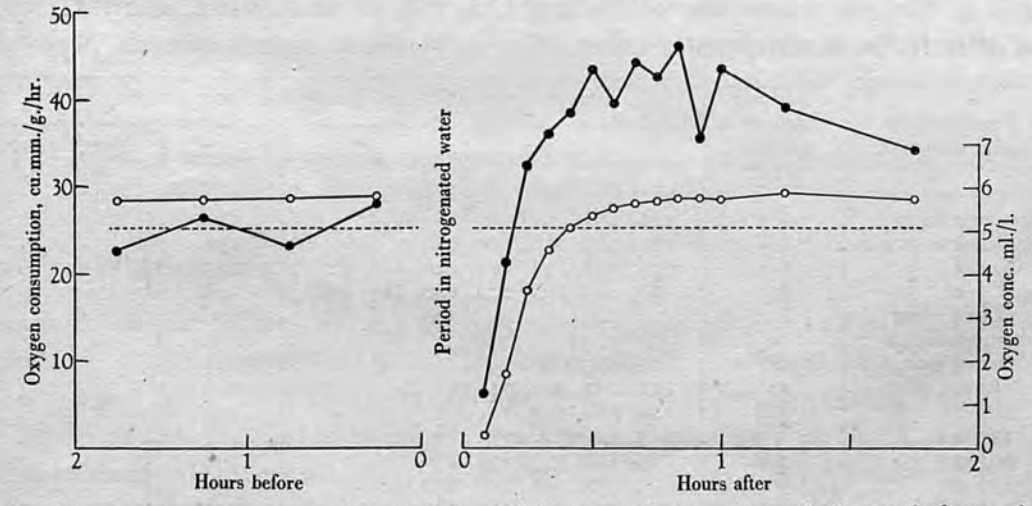



Fig. 3. Rates of oxygen consumption at 1° C. of *Chironomus plumosus* larvae before and after a 16 hr. period of anaerobiosis. Normal animals, July. ●, oxygen consumption; ○, oxygen concentration in respiratory vessel. The broken line represents the average metabolism before nitrogen subjection. Data from Table 2.

water the haemoglobin is not functional, and shows that treatment with carbon monoxide had not affected cell oxidations in the larvae. The metabolism of the treated larvae after the anaerobic period, on the other hand, differed from that of normal larvae. The initial increase in oxygen consumption caused by activity did not occur, although the activity of the treated larvae appeared in no way diminished.

The larvae show this increased activity at a time when, owing to the slow rate of flow through the respiratory chamber, the inflowing aerated water has only partly replaced the nitrogenated water, the oxygen concentration inside the chamber

Table 3. *Oxygen consumption of larvae of* Chironomus plumosus *L. at* 17° C. *before and after* 16 *hr. in nitrogenated water. Normal animals, September*

| Time before anaerobiosis | Oxygen concentration ml./l. | Oxygen consumption, cu.mm./g. (wet weight)/hr. | | |
|---|---|---|---|---|
| | | Separate values | Mean | Grand mean and S.E. |
| 3–2 hr. | 6·8 | 146, 160, 114, 176 | 149 | 140·3 ± 4·0 |
| 2–1 | 6·7 | 143, 162, 148, 156, 95, 121 | 138 | |
| 1–½ | 6·7 | 128, 145, 146, 136, 156, 109 | 137 | |
| ½–0 | 6·7 | 117, 128, 170, 123, 142, 145, 151, 149 | 141 | |
| Time after anaerobiosis | | | | |
| 0–5 min. | 1·0 | 34, 28, 76, 56 | 48 | |
| 5½–10 | 4·3 | 238, 177, 231, 238, 205 | 218 | 189·5 ± 14·0 |
| 10½–15 | 5·1 | 165, 122, 208, 195, 115 | 161 | |
| 15½–20 | 5·7 | 88, 106, 226, 196, 112 | 145 | 141·6 ± 4·0 |
| 20½–25 | 6·0 | 109, 108, 150, 139, 174 | 136 | |
| 25½–30 | 6·3 | 128, 103, 164, 148, 121 | 133 | |
| 30½–35 | 6·3 | 98, 97, 128, 154, 137 | 123 | |
| 35½–40 | 6·5 | 128, 147, 135, 145, 125 | 136 | |
| 40½–50 | 6·6 | 130, 121, 176, 142, 152 | 144 | |
| 50½–60 | 6·6 | 146, 126, 198, 153, 136 | 152 | |
| 1–1½ hr. | 6·7 | 166, 138, 170, 174, 156 | 161 | |
| 1½–2 | 6·7 | 154, 142 | 148 | |

Table 4. *Oxygen consumption of larvae of* Chironomus plumosus *L. at* 17° C. *before and after* 16 *hr. in nitrogenated water. Carbon monoxide-treated animals, September*

| Time before anaerobiosis | Oxygen concentration ml./l. | Oxygen consumption, cu.mm./g. (wet weight)/hr. | | |
|---|---|---|---|---|
| | | Separate values | Mean | Grand mean and S.E. |
| 3–2 hr. | 6·4 | 145, 138 | 142 | 143·3 ± 5·2 |
| 2–1 | 6·5 | 184, 107, 150, 119, 119 | 136 | |
| 1–½ | 6·4 | 154, 135, 131, 142, 126, 168 | 143 | |
| ½–0 | 6·4 | 187, 149, 162, 102, 126, 163, 158 | 149 | |
| Time after anaerobiosis | | | | |
| 0–5 min. | 0·7 | 21, 26, 30, 9 | 21 | |
| 5½–10 | 4·0 | 103, 137, 124, 75, 141 | 116 | 129·0 ± 9·6 |
| 10½–15 | 5·1 | 128, 168, 121, 164 | 145 | |
| 15½–20 | 5·5 | 153, 81, 177, 107, 166 | 137 | 152·0 ± 4·2 |
| 20½–25 | 5·8 | 165, 124, 157, 104, 188 | 148 | |
| 25½–30 | 6·0 | 175, 133, 109, 148, 143 | 142 | |
| 30½–35 | 6·0 | 155, 105, 139, 159, 162 | 144 | |
| 35½–40 | 6·1 | 153, 116, 150, 149, 173 | 148 | |
| 40½–50 | 6·2 | 185, 133, 151, 164, 164 | 161 | |
| 50½–60 | 6·3 | 210, 137, 126, 123, 196 | 158 | |
| 1–1½ hr. | 6·5 | 170, 138, 186, 177, 207 | 176 | |
| 1½–2 | 6·6 | 158, 152, 177 | 162 | |
| 2–3 | 6·5 | 158, 136 | 147 | |

varying from 3 to 5 ml./l. (45–75% saturation) during the 10 min. that the activity is most apparent. Thus the failure of the carbon monoxide animals to increase their oxygen uptake with their increase in activity indicates that within this range of

oxygen pressures the haemoglobin is normally functional, enabling untreated larvae to pick up the extra oxygen demanded by increased activity; in the carbon monoxide larvae the energy for such activity must have been partly derived from anaerobic processes.

Ewer (1942) showed that in *Chironomus dorsalis* at 17° C. the haemoglobin of larvae only became functional at oxygen concentrations of 3 ml./l. (44% air saturation) or less; the above experiments indicate that the haemoglobin of my larvae was being used in oxygen transport at a slightly higher concentration. This difference is probably accounted for by the fact that with *C. plumosus* the increased oxygen

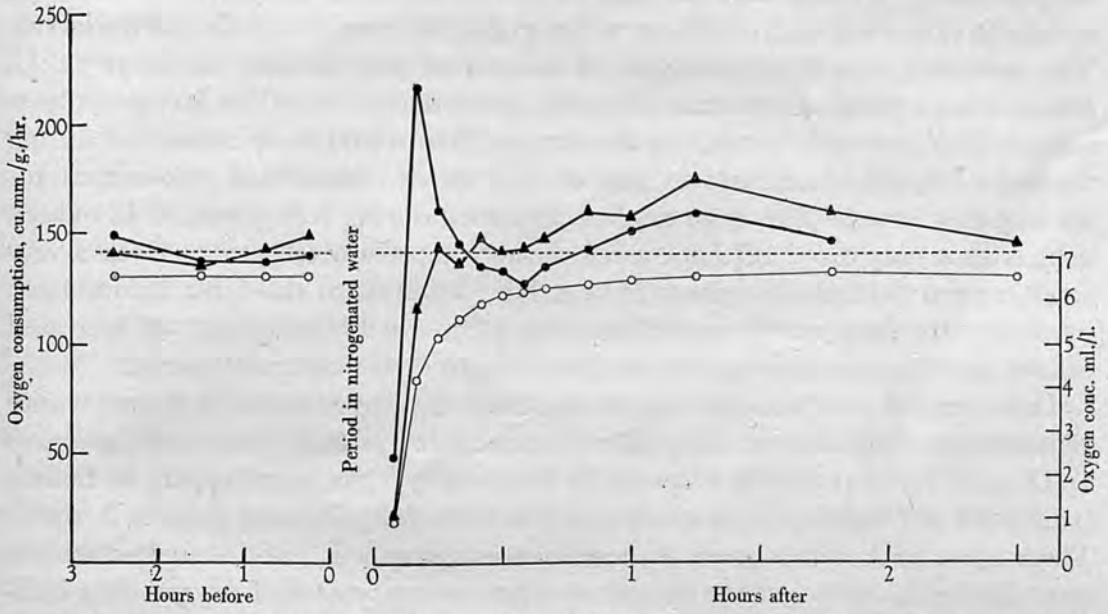



Fig. 4. Rates of oxygen consumption at 17° C. of *Chironomus plumosus* larvae before and after a 16 hr. period of anaerobiosis. September. ●, oxygen consumption of normal animals; ▲, oxygen consumption of carbon monoxide-treated animals; ○, oxygen concentration in respiratory vessel. The broken line represents the average metabolism of normal animals before nitrogen subjection. Data from Tables 3 and 4.

demands of the tissues during the period of great activity extend the range of external oxygen pressures at which the haemoglobin functions. The larger size of *C. plumosus* larvae may also intervene through their relatively smaller surface.

After the first 25 min. of recovery, in other words by the time that the larvae are once again in fully aerated water, the oxygen uptake of the carbon monoxide-treated larvae is equal to that of the untreated animals, and, as with them, is maintained for some time at a level slightly higher than the basal value. With the treated larvae this secondary increase was, in fact, greater than with the untreated animals and continued for a longer time, in some cases for 2 hr. after the beginning of recovery.

## DISCUSSION

In all experiments described the larvae showed an increased metabolic rate during recovery from anaerobiosis. To what extent, however, this represents the repayment of an oxygen debt at 17° C. is uncertain, for the initial well-defined increase in

oxygen consumption is due to an increase in activity of the larvae, while statistical analysis of the subsequent more prolonged increase renders its reality doubtful. At 1° C., however, the rise in oxygen consumption lasting about 2 hr. is statistically significant and must be interpreted as the repayment of an oxygen debt. This may also be the case at 17° C. (without and with carbon monoxide), the statistical improbability being due to the small size of the rise.

It is possible from the experimental data to calculate the extent of such a debt in terms of cu.mm. oxygen consumed by recovering larvae over and above that which they would normally consume in the same time; thus in Figs. 2 and 3 this extra oxygen consumption is represented by the area between the curves of observed metabolic values and the lines drawn at the level of the basal metabolism of the larvae. The increased oxygen consumption of the winter and summer larvae at 17° C. amounts to 12·0 and 14·0 cu.mm. oxygen/g. respectively. Had these larvae not been subjected to anaerobic conditions the oxygen they would have consumed during the anaerobic period amounts to 2240 cu.mm. for the winter and 3080 cu.mm. for the summer larvae. The extra oxygen consumed during repayment of an oxygen debt is thus only 0·5% of that missed during the anaerobic period. Such a very small oxygen debt of *Chironomus* is in striking contrast to the debts accumulated by vertebrate tissues after anaerobic activity, in which the amount of increased oxygen consumption is proportional to the length of the anaerobic period.

The extent of repayment of oxygen debts among invertebrates, however, varies considerably. Well-defined debts have been recorded in *Periplaneta* and *Lumbricus* by Davis & Slater (1928), in *Planorbis* by Borden (1931), in grasshoppers by Bodine (1928) and in *Tenebrio*, *Cryptocercus* and *Zootermopsis* by Gilmour (1940*a*, *b*, 1941). There is evidence that in some of these animals a proportion of the organic acids is resynthesized to carbohydrate: in others a greater repayment of oxygen debt indicates their complete removal by oxidation. In yet other invertebrates oxygen debts are clearly not repaid in full; thus the ciliate *Tetrahymena gelei* only repays 25% (Thomas, 1942, cited in von Brand, 1945), and *Eustrongylides ignotus* 30% (von Brand, 1942) of the debts incurred. Both these animals are known to excrete organic acids and in this way lessen the need for repayment. Excretion of the waste products formed may also account for the small size and variability of the oxygen debts recorded in *Planaria* (Lund, 1921), *Nereis* (Hyman, 1932), *Urechis* (Hall, 1931) and *Tubifex* (Dausend, 1931; Harnisch, 1935, 1936). Finally, Harnisch (1942) could find no evidence of any repayment of oxygen debt in the larvae of *Chironomus bathophilus*.

Studies of the anaerobic glycogen metabolism of invertebrates which successfully withstand periods of anaerobiosis in nature have shown that various higher and lower fatty acids are formed by glycogen fermentation (for literature see von Brand, 1945). In intestinal parasites these fatty acids are either converted to non-toxic fat, and stored, or, more commonly, excreted by the animal. In their ability to excrete the products of anaerobic metabolism they are thus adapted to withstand prolonged periods of oxygen lack. That free-living animals which live in oxygen-poor surroundings should also be able to remove the products of carbohydrate fermentation

in this way is not surprising, their respiratory problems being of the same nature as those of internal parasites. In aquatic invertebrates the elimination of such waste products might be either by excretory organs, or in the case of small animals by diffusion across the surface of the body, provided that this is permeable to them. The repayment of oxygen debts by animals such as *Mya* and *Anodonta* (van Dam, 1938) is possibly made necessary by the shutting of the valves of the shell during anaerobiosis, which would limit the ready diffusion of waste products out of the body. The accumulation of toxic waste products within the body, and the need for oxygen to remove these in the repayment of an oxygen debt, is most apparent in animals least adapted to withstand oxygen lack, such as terrestrial invertebrates and the vertebrates.

It seems probable therefore that the absence of an oxygen debt at all proportional to the length of the anaerobic period in *Chironomus* larvae is due to the removal, either by diffusion or excretion, of the waste products as they are formed. With this in view the water in which a number of larvae had been kept anaerobically overnight was tested for lactic acid. This was found to be present. Although lactic acid is only one of several organic acids produced by the incomplete breakdown of glycogen, it at any rate was removed from the larvae as such and not retained in the body. The observed slight increase in oxygen consumption after anaerobiosis would thus account only for the transformation of those products which remained within the body owing to their indiffusability. In this case the greater repayment of debt by chilled animals could be accounted for by a greater accumulation of such products within the body due to a reduced rate of diffusion or excretion. The fatty acids formed during anaerobiosis may also to some extent be converted into fat within the body, as in certain endoparasites, and the need for repayment of oxygen debt further reduced in this way. Evidence supporting this is given by Harnisch (1939) who found that towards the end of a long anaerobic period (23–24 hr.) the production of fatty acids decreases and fat is formed in the bodies of the larvae.

It is interesting that Harnisch (1942) could find no evidence of repayment of oxygen debt after anaerobiosis in *Chironomus bathophilus*, a species closely related to *C. plumosus* and capable of living in oxygen-poor lakes. Harnisch interprets this absence of oxygen debt as indicating that the larvae respire anaerobically at all times, even when oxygen is present. It may, however, be that in this euroxybiotic species the mechanisms for the rapid elimination of metabolic waste products have been perfected.

Harnisch (1936) has recorded considerably greater repayment of debt in *Chironomus thummi* than I found in *C. plumosus*. The average metabolism of his normal animals lay between 260 and 278 cu.mm./g./hr. while that of animals after 15 hr. in nitrogen varied in different experiments from 368 to 444 cu.mm./g./hr. Thus there was a 41–59% increase in metabolic rate, persisting for two or more hours. Correlated with this increased metabolism he noted that the larvae showed regular undulatory movements which continued for many hours. Since larvae previously kept in oxygenated water did not show such activity he regarded the movements as indicating 'dass im Körper der Tiere Produkte anoxybiotischen Stoffwechsels

vorhanden sind und somit gesteigerter Sauerstoffverbrauch ("sekundäre Oxybiose") besteht'. An alternative interpretation, not suggested by Harnisch, is that the increased metabolism is a direct result of the increased activity rather than of an oxygen debt. The fact that he sometimes found larvae which had previously been kept in oxygenated water to be making vigorous respiratory movements, in which case the animals also showed a high oxygen consumption, supports this interpretation. Harnisch's increased oxygen uptake would then be parallel to that recorded during the short period of intense activity in my experiments. That his larvae remained active much longer than mine may be due to a specific difference. In view of his lack of appreciation of the activity factor, his results should be treated with caution.

Harnisch also conducted a series of experiments on carbon monoxide-treated larvae after oxygen lack. He found these larvae to have a lower oxygen consumption than untreated larvae at all oxygen pressures at and below air saturation of water, and from this he concluded that when their metabolism is raised in paying back an oxygen debt the haemoglobin is functional all the time. During preliminary carbon monoxide treatment, however, he notes a reduction in the activity of the animals, their typical undulatory movements being no longer apparent, which is perhaps not surprising since he used pure carbon monoxide! The differences which he subsequently records between treated and untreated animals may well be caused merely by this activity difference.

In my series of experiments with carbon monoxide-treated larvae the animals, without functional haemoglobin, were capable of maintaining a metabolic rate greater than normal in aerated water, so that the respiratory pigment is of no significance in paying back a small debt in aerated water. That my larvae with carboxyhaemoglobin should actually have a slightly higher oxygen consumption than normal larvae, appearing thus to pay back a greater debt, is curious; it can hardly be attributed to the fact that during their short activity period the movements were partly anaerobic, for any such additional debt incurred in only 10 min. must have been very small.

Harnisch's conclusion that after oxygen lack the larvae use their haemoglobin at all oxygen pressures is thus not confirmed by my experiments. The difference between our results may in part be due to the activity difference between the two species of larvae, the increased oxygen demand of the tissues of his active animals extending the range of function of the pigment so that it is used even at high oxygen pressures. During the short time that my larvae were most active the haemoglobin was functional, but this period coincided with a low oxygen pressure in the water, at which in any case the pigment would be used (Ewer, 1942). In both Harnisch's larvae and mine the possession of haemoglobin enabled the larvae to show an increased degree of activity aerobically.

The functional significance of the immediate violent activity of the larvae after anaerobiosis when water containing oxygen was introduced is only apparent when the animals are considered in their natural habitat. *Chironomus* larvae live in U-shaped tubes in mud and remain in their tubes during periods of oxygen lack.

Violent undulatory movements upon the reappearance of oxygen in the overlying water will have the effect of producing a current of water through the tube, thus both washing away any accumulated carbon dioxide or organic acids present in the tube and providing a continuous supply of water from which oxygen may be extracted. Since in nature the oxygen content of water previously depleted of oxygen is unlikely to rise as rapidly as in the experiments the maintenance of a ventilation current is important. Moreover, thanks to the haemoglobin, the undulatory activity necessary to set up such a current can be maintained aerobically in water containing little oxygen, enabling the animals to re-establish full aerobic metabolism as quickly as possible after a period of oxygen lack.

In this connexion I have now undertaken a study of the behaviour of larvae in their tubes in as natural conditions as possible. It may well be that their responses to oxygen lack in such circumstances are different from those under the very artificial experimental conditions described above.

## SUMMARY

1. When *Chironomus plumosus* larvae receive aerated water after a period of anaerobiosis their oxygen consumption increases at once to a value well above the normal. This initial increase lasts for about 10 min. at 17° C., after which the oxygen consumption falls but continues to be slightly above normal for 1 hr.

2. The initial sudden considerable rise in oxygen consumption is due to increased activity of the larvae; it is absent in larvae made inactive by a low temperature of 1° C. Such larvae, however, maintain an oxygen consumption above the normal value for about 2 hr.

3. The increased oxygen consumption lasting for 1–2 hr. after anaerobiosis represents the repayment of an oxygen debt, but this debt is only 0·5% of the oxygen which would have been consumed had the anaerobic period been an aerobic one.

4. The small size of the oxygen debt suggests that most of the products of anaerobic metabolism are excreted.

5. The haemoglobin of the larvae does not function during the repayment in fully aerated water of the small oxygen debt. This is shown by experiments with and without carbon monoxide.

6. During the initial short considerable rise in oxygen consumption at 17° C. due to larval activity after anaerobiosis the haemoglobin is functional. This activity occurs while the water is not yet fully aerated, and so the haemoglobin enables the larvae to maintain their activity aerobically in water containing little dissolved oxygen.

7. In nature the undulatory activity of the larvae immediately after anaerobiosis must serve to ventilate the U-shaped tubes in which they live in the mud, and so to fill the tubes with aerated water.

I wish to thank Prof. H. Munro Fox, F.R.S., in whose laboratory this work was done, for advice and help.

## REFERENCES


BODINE, J. H. (1928). Insect metabolism. The anaerobic metabolism of an insect (Orthoptera). *Biol. Bull. Woods Hole*, **55**, 395.

BORDEN, M. A. (1931). A study of the respiration and of the function of haemoglobin in *Planorbis corneus* and *Arenicola marina*. *J. Mar. Biol. Ass. U.K.* **17**, 709.

BRAND, T. VON (1942). Physiological observations upon a larval *Eustrongylides*. *Biol. Bull. Woods Hole*, **82**, 1.

BRAND, T. VON (1945). The anaerobic metabolism of invertebrates. *Biodynamica*, **5**, 165.

DAM, L. VAN (1938). *On the Utilization of Oxygen and Regulation of Breathing in some Aquatic Animals*. Groningen.

DAUSEND, K. (1931). Über die Atmung der Tubificiden. *Z. vergl. Physiol.* **14**, 557.

DAVIS, J. G. & SLATER, W. K. (1928). The aerobic and anaerobic metabolism of the common cockroach (*Periplaneta orientalis*), III. *Biochem. J.* **22**, 331.

EDWARDS, F. W. (1929). British non-biting midges (Diptera, Chironomidae). *Trans. Ent. Soc., Lond.*, **77**, 279.

EWER, R. F. (1942). On the function of haemoglobin in *Chironomus*. *J. Exp. Biol.* **18**, 197.

FOX, H. M. & WINGFIELD, C. A. (1938). A portable apparatus for the determination of oxygen dissolved in a small volume of water. *J. Exp. Biol.* **15**, 437.

GILMOUR, D. (1940*a*). The anaerobic gaseous metabolism of the roach *Cryptocercus punctulatus* Scudder. *Biol. Bull. Woods Hole*, **79**, 297.

GILMOUR, D. (1940*b*). The anaerobic gaseous metabolism of the termite *Zootermopsis nevadensis* Hagen. *J. Cell. Comp. Physiol.* **15**, 331.

GILMOUR, D. (1941). Anaerobic metabolism in the larvae of *Tenebrio molitur* L.: gaseous metabolism and changes in glycogen, sugar, fat and lactic acid. *J. Cell. Comp. Physiol.* **18**, 93.

HALL, V. E. (1931). The muscular activity and oxygen consumption of *Urechis caupo*. *Biol. Bull. Woods Hole*, **61**, 400.

HARNISCH, O. (1935). Versuch einer Analyse der Sauerstoffverbrauchs von *Tubifex tubifex* Müll. *Z. vergl. Physiol.* **22**, 450.

HARNISCH, O. (1936). Primäre und sekundäre Oxybiose der Larve von *Chironomus thummi*. *Z. vergl. Physiol.* **23**, 391.

HARNISCH, O. (1939). Studien zum Anaeroben und Erholungstoffwechsel der Larve von *Chironomus thummi*. II. Säurebildung und 'Notoxybiose'. *Z. vergl. Physiol.* **27**, 275.

HARNISCH, O. (1942). Zur Analyse der Oxybiose der Larve von *Chironomus bathophilus* K., einer *Chironomus*-Larve der Seentiefe. *Zool. Anz.* **139**, 1. (Seen in abstract only.)

HYMAN, L. H. (1932). Relation of oxygen tension to oxygen consumption in *Nereis virens*. *J. Exp. Zool.* **61**, 209.

KEILIN, D. (1929). Cytochrome and respiratory enzymes. *Proc. Roy. Soc.* B, **104**, 206.

LUND, E. J. (1921). Oxygen concentration as a limiting factor in the respiratory metabolism of *Planaria agilis*. *Biol. Bull. Woods Hole*, **41**, 203.

THOMAS, J. O. (1942). The anaerobic carbohydrate metabolism of *Tetrahymena gelei*. Dissertation, Stanford University.

WARBURG, O. (1926). Über die Wirkung des Kohlenmonoxyds auf dem Stoffwechsel der Hefe. *Biochem. Z.* **177**, 471.

WOLSKY, A. (1938). The effect of carbon monoxide on the oxygen consumption of *Drosophila melanogaster* pupae. *J. Exp. Biol.* **15**, 225.

REPRINTED FROM THE JOURNAL OF EXPERIMENTAL BIOLOGY,
VOL. 24, NOS. 3 & 4, PP. 343–351, DECEMBER 1947.
[*All rights reserved.*]
PRINTED IN GREAT BRITAIN

# THE FUNCTION OF HAEMOGLOBIN IN *TANYTARSUS* (CHIRONOMIDAE)

BY BARBARA M. WALSHE
*Zoology Department, Bedford College, University of London*

(*Received* 13 *May* 1947)

(With One Text-figure)

Eighty years ago Lankester (1867) identified spectroscopically the red blood pigment of the larvae of *Chironomus plumosus* as haemoglobin. Subsequently (1873) he correlated its presence with the fact that they inhabit stagnant ponds and putrescent mud where the amount of accessible oxygen must often be small. Since then it has been generally assumed that their haemoglobin enables the larvae to live in surroundings deficient in oxygen, and experimental evidence for this has lately been supplied by Harnisch (1936) and by Ewer (1942). These workers compared the metabolism of normal larvae with that of larvae whose haemoglobin had been made functionless by conversion to carboxyhaemoglobin. They found that the pigment was only used as an oxygen carrier at low pressures of oxygen; it is used to the greatest extent in water which is 22% saturated with air at 17° C. Ewer also made a series of measurements of the oxygen content of the pond from which her larvae were obtained and found oxygen concentrations corresponding to 32% air saturation or less for periods of at least 16 consecutive hours. Thus it may be assumed that in nature the haemoglobin of the species with which Ewer worked is of functional value.

The larvae of several chironomid genera other than *Chironomus*, however, contain haemoglobin, and it has frequently been pointed out (Malloch, 1915; Harnisch, 1930) that the possession of haemoglobin by a larva is no proof that it can live in situations poor in oxygen. Thus, although *C. plumosus* is known to be very euroxybiotic the almost identical *C. bathophilus* is less so, while *Tanytarsus* species are markedly sensitive to low oxygen concentrations and only occur in well-aerated waters (Thienemann, 1923). The wealth of limnological data on the distribution of chironomids provides many other instances of stenoxybiosis in larvae with haemoglobin. In such forms the haemoglobin can scarcely function in the same manner as it does in the *Chironomus* species studied by Harnisch and by Ewer, otherwise they would be capable of maintaining a normal aerobic metabolic rate at low oxygen concentrations. The functional significance of the haemoglobin of such oxygen-needy chironomids has never been satisfactorily studied. Harnisch (1930, 1933, 1937) made a comparative study of *Chironomus*, *Prodiamesa* and *Tanytarsus*, three chironomid genera with different capacities of withstanding low oxygen concentrations in nature, but he was mainly concerned with their respiration after periods of anaerobiosis and did not attempt to assess the mode of functioning of the haemoglobin in the stenoxybiotic species.

I therefore chose *Tanytarsus* larvae for an experimental study of the function of the haemoglobin. These small larvae, which contain sufficient haemoglobin to make them red in colour, are very sensitive to oxygen lack and occur in nature in oligotrophic lakes, the oxygen content of which never falls below about 50% air saturation and in running water. The principle and techniques employed were those of Ewer (1942): the rates of oxygen uptake of normal larvae at different oxygen concentrations were compared with those of larvae whose haemoglobin had been converted into carboxyhaemoglobin and was thus incapable of transporting oxygen.

Final instar larvae (7–8 mm. in length) of *Tanytarsus brunnipes* (Zett.) were collected from a small stream in Cambridge. The species was determined from adults which emerged in the laboratory, the identification being checked by comparison with British Museum specimens. Larvae were collected a day or two before experiments and were kept in running aerated water in shallow dishes with a thin layer of mud.

The method used to determine the metabolic rate was that described by Ewer (1942), with minor modifications. Larvae were enclosed in 10 ml. glass syringes in distilled water buffered with sodium bicarbonate (normality 0·004). A small glass bead was put into each syringe to ensure adequate mixing of the water. Four syringes were used at a time, each containing between fifteen and twenty *Tanytarsus* larvae. They were clipped on to a large wheel rotating in a thermostatic water bath at 17° C. At intervals of approximately 1 hr. a sample of water was withdrawn from each syringe and its oxygen content determined by the syringe-pipette micro-Winkler method described by Fox & Wingfield (1938). Each experiment lasted about 3 hr.; during this time the pH of the water altered less than 0·2 unit, and the oxygen concentration fell from air saturation (6·75 ml./l.) to about 2 ml./l., or, in experiments at a lower range of oxygen contents, from 4 ml./l. to about 0·7 ml./l. At the end of the experiment the animals were removed from the syringes, dried on filter-paper and weighed. The oxygen consumption for each hour of the experiment was calculated in cubic millimetres of oxygen per gram wet weight per hour. The average of the oxygen contents of the water at the beginning and end of each hour's interval gave the oxygen content to which the oxygen consumption rate was referred.

The method of treatment with carbon monoxide was as follows. The animals were kept in a 170 ml. bottle in aerated water to which sufficient carbon monoxide-saturated water had been added to make the pressure of carbon monoxide one-sixth of that of the dissolved oxygen. They were then left in the dark until their haemoglobin was entirely converted into carboxyhaemoglobin. The conversion was tested spectroscopically and was found to require 1 hr. It was calculated that the removal of oxygen by the larvae during this time did not increase the relative pressure of carbon monoxide to more than one-fifth. Experiments using animals with carboxyhaemoglobin were made in water containing 0·2 ml./l. carbon monoxide to prevent dissociation of the carboxy-compound. At the end of these experiments the blood was tested spectroscopically to ascertain that the haemoglobin was still in the form of carboxyhaemoglobin; this was judged by the failure of the absorption bands to fade on the addition of sodium hydrosulphite. Experiments with carbon monoxide-

treated animals were alternated with those using normal animals. This ensured that larvae in the same physiological state were compared, since work on *Chironomus* (Walshe, 1947) had shown that seasonal differences in metabolic rate occur.

The utilization of haemoglobin at very low oxygen pressures was also tested in another way: the times of death of larvae with and without haemoglobin in water of low oxygen content were compared. Larvae were enclosed in 1 l. bottles containing water of known low oxygen content (ranging from 9 to 23 % air saturation) and kept in the dark at room temperature, the numbers of dead being noted at intervals. The experimental bottles were set up in pairs: one bottle with normal untreated larvae and the other with larvae previously treated with carbon monoxide, as already described. In all experiments except one (Exp. 3, Table 3) ten larvae were used in each bottle; in Exp. 3, twenty larvae were used. At the end of each experiment the oxygen content of the water was estimated.

The metabolic rate of *Tanytarsus* at 17° C. at various concentrations of dissolved oxygen is given in Table 1 and Fig. 1. The mean rate of oxygen consumption in fully

Table 1. *Oxygen consumption of* Tanytarsus brunnipes *larvae at* 17° C. *at various concentrations of dissolved oxygen*

| Oxygen concentration (ml./l.) | Oxygen consumption (cu.mm./g. (wet weight)/hr.) | |
|---|---|---|
| | Separate values | Mean and S.E. |
| 6·00–5·01 | 630, 507, 590, 494, 532, 528, 424, 522, 541, 544, 469, 552, 489, 553, 454 | 525 ± 13 |
| 5·00–4·01 | 343, 440, 442, 435, 117, 322, 305, 231, 299, 424, 358, 450, 415 | 352 ± 27 |
| 4·00–3·01 | 505, 435, 424, 421, 415, 376, 435, 73, 51, 101, 326, 288, 344, 318, 367, 352 | 327 ± 34 |
| 3·00–2·01 | 309, 362, 316, 248, 223, 357, 298, 240, 354 | 301 ± 18 |
| 2·00–1·61 | 229, 246, 319, 330, 334, 379, 316, 219, 295, 228, 183 | 279 ± 18 |
| 1·60–0·90 | 224, 194, 224, 262, 273, 357, 299, 271, 298 | 267 ± 16 |

aerated water is 525 ± 13 cu.mm./g. (wet weight)/hr., but falls considerably at lower oxygen values. It is seen from the curve that the metabolism is dependent on the oxygen pressure in the water at all pressures below that corresponding to air saturation. This is contrary to Harnisch's statement that the oxygen consumption of *Tanytarsus* is constant over a wide range of oxygen pressures and only begins to decline at the low pressure of 1–2 % of oxygen (equivalent to 7 % air saturation) (Harnisch, 1929). The data from which he draws this conclusion, however, are so badly presented in his paper that it is impossible to estimate their validity. Using a Warburg manometer he plots the decline in pressure in the apparatus caused by the oxygen consumption of the larvae in a series of graphs lacking both ordinates and abscissae, and he judges the effects on the larvae of various gas mixtures by changes in the rate of decrease of the gas pressure. He selects certain experiments as examples for discussion and gives no idea of the actual numbers of experiments made, or of the range of gas mixtures used. The temperature for one experiment was 'etwa 20° C.', that of the others is not given. In comparing the effect of low oxygen

pressures on *Eutanytarsus inermipes* (= *Tanytarsus brunnipes*) with *Chironomus* he states: 'Ich habe leider keinen Versuch, der die Stelle klar trifft, an der *Eutanytarsus* vor *Chironomus* knickt. Sie wird sich aber noch finden lassen, da die Abknickung der Atmungskurve von *Eutanytarsus* stets deutlich stärker ist als die von *Chironomus thummi*.' This conclusion he subsequently quotes (1930) as an established fact.

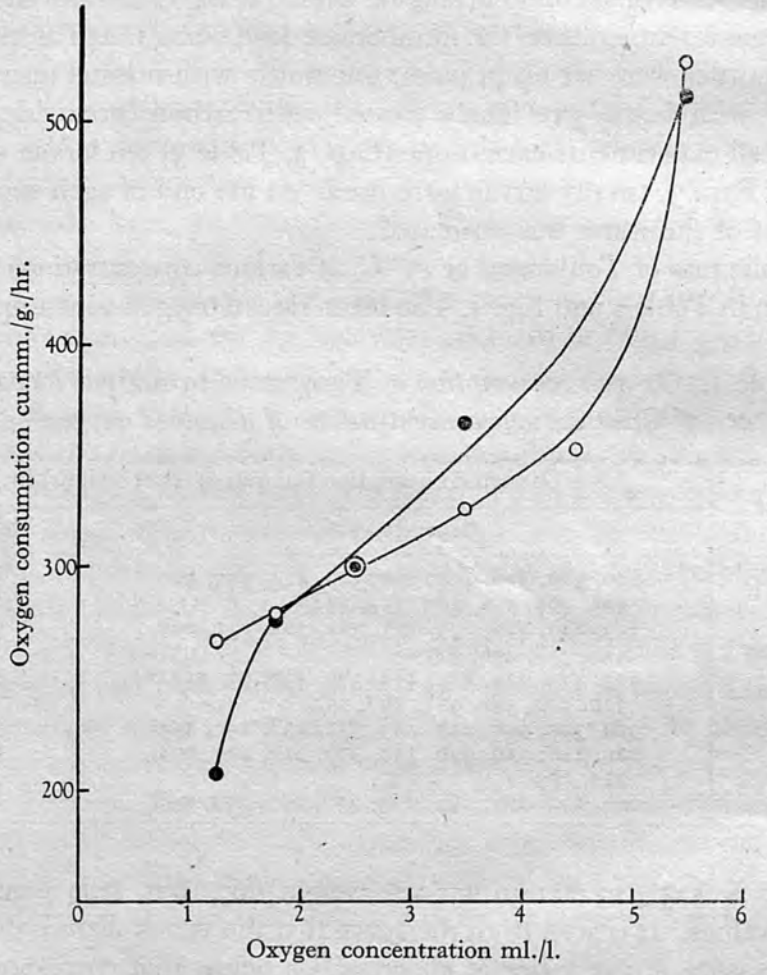



Fig. 1. Rates of oxygen consumption at 17° C. of *Tanytarsus* at various concentrations of dissolved oxygen. ○, normal animals; ●, animals with carboxyhaemoglobin. Data from Tables 1 and 2.

The general significance of dependence or independence of the oxygen consumption of animals on oxygen pressure has been very intensively discussed (Henze, 1910; Amberson, Mayerson & Scott, 1924; Rashevsky, 1933; Maloeuf, 1937*a*, *b*) and will not be elaborated here. Harnisch's theory (1937), however, that a dependent type of curve is an indication of a repayment of oxygen debt by the animals cannot be the explanation in the case of my larvae. In order to avoid the possibility of the accumulation of an oxygen debt before experiments, Harnisch kept his experimental animals in running water in wide glass tubes with bolting silk ends. I repeated this technique in a number of my experiments, but the subsequent

metabolism of larvae so treated was still just as dependent on the oxygen content of the water as without this treatment.

The oxygen consumption at various concentrations of dissolved oxygen of *Tanytarsus* larvae with carboxyhaemoglobin is given in Table 2 and Fig. 1. As in the case of the untreated animals, the oxygen consumption is high in fully aerated water and falls with declining oxygen pressures. A statistical comparison of the metabolic rates of treated and untreated larvae shows that between 6·0 and 1·6 ml./l. oxygen (90%–24% air saturation), the slight differences recorded are not significant. Below 1·6 ml./l. the oxygen consumption of carbon monoxide-treated larvae drops to 78% that of normal larvae: this decrease is statistically significant. It indicates that below this oxygen value the haemoglobin is functional in the normal animal in picking up oxygen.

Table 2. *Oxygen consumption of* Tanytarsus brunnipes *larvae with carboxyhaemoglobin at* 17° C. *at various concentrations of dissolved oxygen*

| Oxygen concentration (ml./l.) | Oxygen consumption (cu.mm./g. (wet weight)/hr.) | | |
|---|---|---|---|
| | Separate values | Mean and S.E. | % normal rate |
| 6·00–5·01 | 513, 524, 424, 622, 394, 547, 547, 455, 689, 531, 471, 478, 630, 663, 412, 283, 639, 411, 369 | 505 ± 25 | 96 |
| 5·00–4·01 | 564, 509, 451, 504, 412, 356, 345, 254, 336, 442, 265, 370, 299, 419, 273 | 387 ± 25 | 109 |
| 4·00–3·01 | 322, 564, 370, 286, 498, 408, 421, 605, 477, 167, 360, 127, 169, 376, 448, 235 | 365 ± 25 | 112 |
| 3·00–2·01 | 427, 494, 246, 291, 313, 358, 277, 249, 307, 271, 212, 168 | 301 ± 27 | 100 |
| 2·00–1·61 | 287, 285, 222, 268, 271, 228, 370, 244, 368, 216 | 276 ± 17 | 99 |
| 1·60–0·90 | 178, 206, 238, 204, 273, 163, 241, 205, 191, 330, 163, 190, 204, 126 | 208 ± 14 | 78 |

The function of the haemoglobin at very low oxygen pressures was confirmed and extended by experiments of another type. The reason for the different technique was as follows. The experiments in syringes were unsuitable for detecting the slight differences in metabolic rate between untreated and treated larvae at very low oxygen pressures for the following reason: unless the differences were considerable they might easily be masked at oxygen concentrations of less than about 1 ml./l. by the random error of the Winkler method. This error, being largely determined by the titration end-point and by the introduction of oxygen in the reagents, is independent of oxygen concentration and is therefore relatively greater at low concentrations. For this reason another method was adopted and the times of death of larvae with and without carboxyhaemoglobin in waters of low oxygen contents were studied. A more rapid death of larvae with carboxyhaemoglobin was taken to mean that the haemoglobin of normal larvae functions at that oxygen pressure. The data from a number of such experiments are summarized in Table 3. Exps. 1 and 6 were made in winter at a lower temperature than the others and the absolute values for survival are thus

not comparable throughout the series, but since experiments with and without haemoglobin were always made simultaneously the assessment of the function of the haemoglobin remains valid. Larvae without functional haemoglobin kept in water with an initial oxygen content of less than 15% air saturation (approximately 1 ml./l.) died more rapidly than normal larvae (Exps. 1–3). It follows that at this low oxygen concentration the haemoglobin is of survival value to the larvae.

A more rapid rate of death of carbon monoxide-treated larvae also occurred when the initial oxygen content of the water was greater than 15% saturation (Exps. 4–6), but the increased rate of dying was not apparent in these larvae until many hours after the start of the experiment. Although the larvae were enclosed in a relatively

Table 3. *Rates of death of* Tanytarsus brunnipes *larvae at low oxygen concentrations*

| Exp. no. | Temp. (° C.) | Larvae with haemoglobin | | | | Larvae with carboxyhaemoglobin | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Oxygen, % air saturation | | Hr. after enclosure | % alive | Oxygen, % air saturation | | Hr. after enclosure | % alive |
| | | Initial | Final | | | Initial | Final | | |
| 1 | 13–14 | 9 | 7 | 44 | 90 | 9 | 8 | 44 | 80 |
| | | | | 52 | 90 | | | 52 | 70 |
| | | | | 68 | 80 | | | 68 | 40 |
| | | | | 75 | 80 | | | 75 | 20 |
| 2 | 18–20 | 12 | 6 | 21 | 100 | 12 | 6 | 21 | 100 |
| | | | | 28 | 100 | | | 28 | 70 |
| | | | | 50 | 100 | | | 50 | 0 |
| 3 | 18–20 | 14 | 5 | 20 | 100 | 14 | 8 | 20 | 100 |
| | | | | 45 | 95 | | | 45 | 0 |
| 4 | 18–20 | 15 | 11 | 20 | 100 | 15 | 12 | 20 | 100 |
| | | | | 27 | 100 | | | 27 | 100 |
| | | | | 43 | 90 | | | 43 | 90 |
| | | | | 49 | 90 | | | 49 | 60 |
| 5 | 18–20 | 20 | 15 | 21 | 90 | 22 | 15 | 21 | 100 |
| | | | | 28 | 90 | | | 28 | 100 |
| | | | | 50 | 90 | | | 50 | 60 |
| 6 | 13–14 | 23 | 15 | 44 | 100 | 25 | 15 | 44 | 100 |
| | | | | 52 | 100 | | | 52 | 100 |
| | | | | 68 | 90 | | | 68 | 80 |
| | | | | 75 | 90 | | | 75 | 70 |
| | | | | 97 | 80 | | | 97 | 40 |

large volume of water their metabolism appreciably reduced its oxygen content during the course of the experiment (as is shown in Table 3) and it is therefore not possible from these experiments to determine precisely the highest oxygen concentration at which the haemoglobin is functional. Since, however, the bottles were opened and their oxygen contents determined as soon as an increased rate of dying was apparent, the critical oxygen value at which the haemoglobin becomes of significance for survival would seem to be about 15% air saturation (1·1 ml./l. at 14° C., 0·9 ml./l. at 19° C.). Above 15% saturation it is possible that the haemoglobin is also of value in oxygen transport and therefore in ultimately increasing the length of survival, but that the fatal effects of a slightly inadequate oxygen supply take some hours to become apparent.

The lower limit of the range of oxygen pressures at which the haemoglobin functions can more easily be determined. To do so larvae were enclosed in water of low oxygen content in small specimen tubes and the state of their haemoglobin was observed with a spectroscope. When the haemoglobin became fully deoxygenated the oxygen concentration of the water was determined. The average oxygen concentration at which this occurred was 5% air saturation. At and below this concentration, therefore, their haemoglobin can have no functional value.

The range of oxygen pressures over which the larvae of *Tanytarsus* use their haemoglobin at 17° C. is thus from 5 to less than 25% air saturation, while that of *Chironomus* is from 9 to 37% air saturation (Ewer, 1942). The haemoglobin therefore functions at lower oxygen pressures in *Tanytarsus* than in *Chironomus*. In normal *Chironomus* larvae the oxygen uptake at 17° C. is constant and independent of the oxygen concentration of the water down to about 15% air saturation. Above 37% air saturation of the water the animal's oxygen requirements are met by oxygen in physical solution in the blood, but between 37 and 15% air saturation of the water the animal's oxygen uptake owes its independence of external oxygen pressure to a functional haemoglobin. The haemoglobin is thus of functional value to the animal in making available enough oxygen for normal metabolism at oxygen pressures too low to allow of this in the absence of haemoglobin.

In *Tanytarsus*, however, it is meaningless to talk of the normal oxygen requirements of the larvae, since their oxygen uptake varies with the external oxygen pressure and one cannot therefore say what is normal. Even when the haemoglobin is functioning at low oxygen pressures it only raises the metabolic rate a little compared with larvae lacking haemoglobin. The haemoglobin still leaves the oxygen uptake considerably below that of animals in higher oxygen pressures. If a dependence of oxygen uptake on oxygen pressure indicates inadequacy of oxygen transport to the tissues (Henze, 1910), then at all oxygen concentrations below air saturation* *Tanytarsus* suffers partial oxygen lack and the haemoglobin does nothing to alleviate this condition except at very low oxygen pressures, and even then the extra oxygen picked up by the haemoglobin only slightly increases the metabolic rate. The tissues of *Tanytarsus*, in fact, have such a high oxygen demand that they suffer oxygen lack at oxygen pressures of the blood far higher than those which will cause dissociation of a haemoglobin with a high oxygen affinity, such as that of *Tanytarsus*; the oxygen linked with the haemoglobin cannot be of use to the oxygen-greedy tissues.

Is it, however, legitimate to assume that at all oxygen pressures at which the oxygen uptake is dependent the animal is necessarily suffering real oxygen shortage? It may be that animals with this type of metabolism have a surfeit of the intracellular oxidation-reduction systems, enabling them to show a high metabolic rate when plentifully supplied with oxygen, but not necessarily being adversely affected at lower oxygen pressures by an incapacity to maintain this rate. This hypothesis could only be tested by determining the lowest oxygen pressure at which such an animal could live indefinitely and normally. This, to my knowledge, has never been

* And possibly above it. My data, not extending above air saturation, do not settle this point.

done. It is known that *Tanytarsus* is very sensitive to poorly aerated water and Thienemann (1928) says that it is not found in nature in water below 50% air saturation. This is well above the upper limit at which the haemoglobin is used. In fact, the haemoglobin of *Tanytarsus* functions by transporting to the tissues an inadequate amount of oxygen, at an external oxygen pressure which in any case is probably ultimately lethal. The most it can do is to delay death a little. From this it is tempting to conclude that the haemoglobin plays no significant part as an oxygen carrier in the life of *Tanytarsus* in nature, in which case its presence may perhaps be due to some quality other than its oxygen transporting ability.

It should, however, be remembered that the animal lives in conditions very different from those presented to it in these experiments. *Tanytarsus* lives in mud tubes in well-aerated water, but nothing is known of the range of oxygen concentrations it actually encounters in these tubes, nor of its metabolic rate or response to declining oxygen pressures under these conditions.*

Evidence of this nature must come before an evaluation can be made of the normal significance of the haemoglobin of *Tanytarsus*.

## SUMMARY

1. The metabolic rate of *Tanytarsus* larvae is higher in air-saturated water than at lower oxygen concentrations; the oxygen consumption is thus dependent on the external oxygen pressure.

2. The haemoglobin in the blood of the larvae does not function in oxygen transport when the larvae are in water which is between 25 and 100% saturated with air at 17° C. Below 25% air saturation of the water the metabolic rate of larvae without functional haemoglobin (i.e. treated with carbon monoxide) is lower than that of normal larvae. When kept in water below this oxygen concentration larvae with carboxyhaemoglobin also die quicker than normal larvae.

3. The external oxygen concentration at which the haemoglobin in the blood of the larvae becomes deoxygenated is 5% air saturation.

4. The range of oxygen concentrations over which the larvae of *Tanytarsus* use their haemoglobin at 17° C. is thus from 5 to 25% of air saturation.

5. The doubtful significance of the haemoglobin in the life of *Tanytarsus* in nature is discussed.

This investigation was made in the Department of Prof. H. Munro Fox.

* Hyman (1932) records that *Nereis* in tubes has a lower metabolism, more independent of declining oxygen concentrations, than free animals.

## REFERENCES


AMBERSON, W. R., MAYERSON, H. S. & SCOTT, W. J. (1924). The influence of oxygen tension upon metabolic rate in Invertebrates. *J. Gen. Physiol.* **7**, 171.

EWER, R. F. (1942). On the function of haemoglobin in *Chironomus*. *J. Exp. Biol.* **18**, 197.

FOX, H. M. & WINGFIELD, C. A. (1938). A portable apparatus for the determination of oxygen dissolved in a small volume of water. *J. Exp. Biol.* **15**, 437.

HARNISCH, O. (1929). Verbreitung und ökologische Bedeutung des Hämoglobins bei den Chironomidenlarven. *Verh. internat. Zoologenkongr. X. Budapest*, p. 345.

HARNISCH, O. (1930). Daten zur Respirationsphysiologie Hämoglobin-führender Chironomidenlarven. *Z. vergl. Physiol.* **11**, 285.

HARNISCH, O. (1933). Respirationsphysiologische Grundlagen der Ökologie der Chironomidenlarven. *Verh. dtsch. zool. Ges.* **35**, 209.

HARNISCH, O. (1936). Primäre und sekundäre Oxybiose der Larve von *Chironomus thummi*. *Z. vergl. Physiol.* **23**, 391.

HARNISCH, O. (1937). *Chironomus* und *Tanytarsus*. *Biol. Zbl.* **57**, 628.

HENZE, M. (1910). Über den Einfluss des Sauerstoffdruckes auf den Gaswechsel einiger Meerestiere. *Biochem. Z.* **26**, 255.

HYMAN, L. H. (1932). Relation of oxygen tension to oxygen consumption in *Nereis virens*. *J. Exp. Zool.* **61**, 209.

LANKESTER, E. R. (1867). Preliminary notice of some observations with the spectroscope on animal substances. *J. Anat., Lond.*, **2**, 114.

LANKESTER, E. R. (1873). A contribution to the knowledge of haemoglobin. *Proc. Roy. Soc.* **140**, 70.

MALLOCH, J. R. (1915). The Chironomidae, or midges, of Illinois, with particular reference to the species occurring in the Illinois River. *Bull. Ill. Lab. Nat. Hist.* **10**, 275.

MALOEUF, N. S. R. (1937*a*). Studies on the respiration (and osmoregulation) of animals. I. Aquatic animals without an oxygen transporter in their internal medium. *Z. vergl. Physiol.* **25**, 1.

MALOEUF, N. S. R. (1937*b*). Studies on the respiration of animals. II. Aquatic animals with an oxygen transporter in their internal medium. *Z. vergl. Physiol.* **25**, 29.

RASHEVSKY, N. (1933). Note on the mathematical theory of oxygen consumption at low oxygen pressures. *Protoplasma*, **20**, 125.

THIENEMANN, A. (1923). Die beiden Chironomusarten der Tiefenfauna der Norddeutschen Seen. *Ver. int. Ver. Limnol. Kiel. Stuttgart*, **1**, 108.

THIENEMANN, A. (1928). *Die Binnengewässer*. Stuttgart.

WALSHE, B. M. (1947). On the function of haemoglobin in *Chironomus* after oxygen lack. *J. Exp. Biol.* **24**, 329.

REPRINTED FROM THE JOURNAL OF EXPERIMENTAL BIOLOGY,
VOL. 25, NO. 1, PP. 35–44, MARCH 1948.
[*All rights reserved.*]
PRINTED IN GREAT BRITAIN

# THE OXYGEN REQUIREMENTS AND THERMAL RESISTANCE OF CHIRONOMID LARVAE FROM FLOWING AND FROM STILL WATERS

BY BARBARA M. WALSHE, *Bedford College, University of London*

(*Received* 25 *July* 1947)

(With Three Text-figures)

Extensive surveys of the distribution of chironomid larvae have established the fact that the majority of species are strikingly restricted in their habitat: the causes of this specialization, however, remain largely undetermined. For a few species comprehensive ecological work has made it possible to deduce the factors important in determining distribution (Thienemann, 1923, 1928; Lenz, 1925), but an experimental attack on the physiology and behaviour of larval and adult chironomids in relation to their choice of habitat remains almost a virgin field.

In other groups of aquatic invertebrates experimental work has indicated that related forms from different habitats may have well-marked physiological differences. Various species of ephemerid nymphs and caddis larvae from streams, for instance, have higher metabolic rates than related species from stagnant water, and the former are more sensitive to oxygen lack than the latter (Fox & Simmonds, 1933; Fox, Simmonds & Washbourn, 1935). May-fly nymphs from swift streams are less resistant to high temperatures than those from slow-moving waters (Whitney, 1939), and the heat tolerance of other aquatic organisms has been shown to be adapted to the temperature conditions of their environment (Plateau, 1872; Mason, 1939).

In order to find the extent to which the physiological requirements of chironomid larvae are related to their special habitats I have made measurements of certain physiological processes in the larvae of a number of species from different types of environment. The rate of oxygen consumption, capacity for anaerobiosis and resistance to high temperatures were measured. As far as possible closely related species with different habitats were selected for comparison.

## (1) OXYGEN CONSUMPTION

The oxygen requirements of chironomid larvae of four species were studied. The larvae fell into two comparable pairs, the members of each pair being closely related and of about the same size. One member of each pair occurs in a stream and the other in a still-water environment. The following species were used: *Anatopynia varia* (Fabr.) (Tanypodinae) from a stagnant ditch; *A. nebulosa* (Mg.) from a stream. *Chironomus longistylus* Goet. (Chironominae) from a stagnant ditch; *Tanytarsus brunnipes* (Zett.) (Chironominae) from a stream. Final instar larvae of all species were used, except *Chironomus longistylus* in which case young larvae

6–7 mm. in length, were selected in order to be more nearly comparable with the 7 mm. larvae of *Tanytarsus*. The three last-mentioned species have sufficient haemoglobin in the blood to make the larvae appear distinctly red; *Anatopynia varia*, however, contains much less, the blood being only pale pink, and in some individuals almost colourless. The larvae were usually collected on the day before the experiments and were kept overnight in running water in shallow dishes with a thin layer of mud. Larvae which had been more than 2 days in the laboratory were never used.

The oxygen consumption of the larvae was determined by the method described by Ewer (1942). Between twelve and twenty *Chironomus* or *Tanytarsus* larvae, or six *Anatopynia*, were used each time.

The results of these experiments are given in Tables 1–4 and Fig. 1. The ditch and stream species differ strikingly in their oxygen requirements. Both *Tanytarsus brunnipes** and *Anatopynia nebulosa*, from streams, have a higher metabolic rate at

Table 1. *Oxygen consumption of young* Chironomus longistylus *Goet. larvae at* 17° C.

| Oxygen concentration (ml./l.) | Oxygen consumption (cu.mm./g. (wet wt.)/hr.) | |
|---|---|---|
| | Separate values | Means |
| 6·01–7·00 | 444, 331, 417, 200, 342, 219, 140, 337, 452 | 320 ± 37 |
| 5·01–6·00 | 366, 335, 311, 280, 308, 366, 360, 394, 296, 396, 342, 323, 543, 375, 304, 202, 107 | 330 ± 21 |
| 4·01–5·00 | 292, 266, 247, 312, 259, 379, 244, 284, 381 | 296 ± 9 |
| 3·01–4·00 | 309, 252, 313, 310, 258, 244, 505, 328, 390, 316 | 313 ± 22 |
| 2·01–3·00 | 268, 236, 297, 240, 245, 356, 294, 359, 315, 287, 324, 196, 314, 225, 440, 464 | 304 ± 11 |
| 1·01–2·00 | 350, 219, 379, 215, 279, 330, 315, 267, 291, 277, 213, 377, 266, 264, 184, 332, 283, 295 | 285 ± 13 |
| 0·51–1·00 | 168, 220, 247, 245, 297, 205, 228, 213, 159 | 220 ± 13 |
| 0·00–0·50 | 45, 62, 205, 68, 185 | 117 |

Table 2. *Oxygen consumption of* Tanytarsus brunnipes (*Zett.*) *larvae at* 17° C.

| Oxygen concentration (ml./l.) | Oxygen consumption (cu.mm./g. (wet wt.)/hr.) | |
|---|---|---|
| | Separate values | Means |
| 6·01–7·00 | 364, 484, 360, 270, 434, 356, 394, 590, 666, 531, 700, 482 | 469 ± 38 |
| 5·01–6·00 | 500, 451, 486, 304, 233, 336, 313, 344, 565, 621, 466, 440, 635, 448, 301 | 430 ± 31 |
| 4·01–5·00 | 305, 418, 361, 354, 363, 210, 371, 440, 435, 467, 238, 399, 315, 320, 404, 296, 467, 436 | 367 ± 9 |
| 3·01–4·00 | 318, 286, 287, 341, 331, 219, 232, 315, 378 | 301 ± 17 |
| 2·01–3·00 | 297, 366, 280, 327, 218, 310, 422, 390, 381, 368, 303, 268, 307, 294, 350, 443, 426 | 342 ± 8 |
| 1·01–2·00 | 250, 119, 329, 248, 251, 315, 237, 263, 226, 224 | 246 ± 16 |
| 0·50–1·00 | 47, 146, 128, 176, 70, 198, 201, 162 | 141 ± 20 |

* Other data of the oxygen consumption at various oxygen pressures of *T. brunnipes* have already been published (Walshe, 1947). The experiments giving these data were made on larvae from a different habitat and at a different season: these factors must account for the slight differences between the two sets of data.

Table 3. *Oxygen consumption of* Anatopynia varia (*Fabr.*) *larvae at* 17° C.

| Oxygen concentration (ml./l.) | Oxygen consumption (cu.mm./g. (wet wt.)/hr.) | |
|---|---|---|
| | Separate values | Means |
| 6·01–7·00 | 348, 156, 300, 428, 503, 503, 428, 287, 214, 207 | 337 ± 40 |
| 5·01–6·00 | 402, 304, 263, 324, 326, 430, 511, 271, 413 | 360 ± 28 |
| 4·01–5·00 | 96, 357, 115, 256, 307, 353, 273, 356, 340, 276, 198, 224, 275, 242 | 262 ± 22 |
| 3·01–4·00 | 218, 194, 231, 394, 247, 199, 167, 426, 386, 296, 413 | 288 ± 30 |
| 2·01–3·00 | 340, 357, 350, 414, 195, 247, 200, 381, 429, 441 | 335 ± 29 |
| 1·01–2·00 | 67, 216, 132, 251, 339, 244, 314, 167, 134, 255, 268, 210, 286, 221, 182 | 220 ± 20 |
| 0·00–1·00 | 75, 1, 38, 120, 195, 179, 92, 33, 46, 225, 88, 335, 352, 457 | 160 ± 36 |

Table 4. *Oxygen consumption of* Anatopynia nebulosa (*Mg.*) *larvae at* 17° C.

| Oxygen concentration (ml./l.) | Oxygen consumption (cu.mm./g. (wet wt.)/hr.) | |
|---|---|---|
| | Separate values | Means |
| 6·01–7·00 | 519, 253, 542, 371, 423, 346, 440, 370, 324, 454, 466, 612, 668, 223 | 429 ± 34 |
| 5·01–6·00 | 510, 183, 474, 264, 395, 209, 231, 206, 258, 277, 337, 474, 388 | 324 ± 33 |
| 4·01–5·00 | 194, 228, 232, 268, 306, 305, 412, 235, 288, 324, 197, 213, 279, 301, 221, 224, 479, 300, 315, 170 | 275 ± 16 |
| 3·01–4·00 | 134, 267, 299, 174, 157, 232, 293, 261, 215, 290, 232, 129, 197, 250, 173, 118, 453, 611, 131 | 243 ± 27 |
| 2·01–3·00 | 179, 169, 67, 228, 362, 208, 247, 275, 134, 196, 140, 162, 208, 189 | 197 ± 19 |
| 1·01–2·00 | 85, 67, 176, 20, 125, 24, 138, 152, 104 | 99 ± 18 |
| 0·00–1·00 | 86, 39, 132, 98, 106 | 92 |

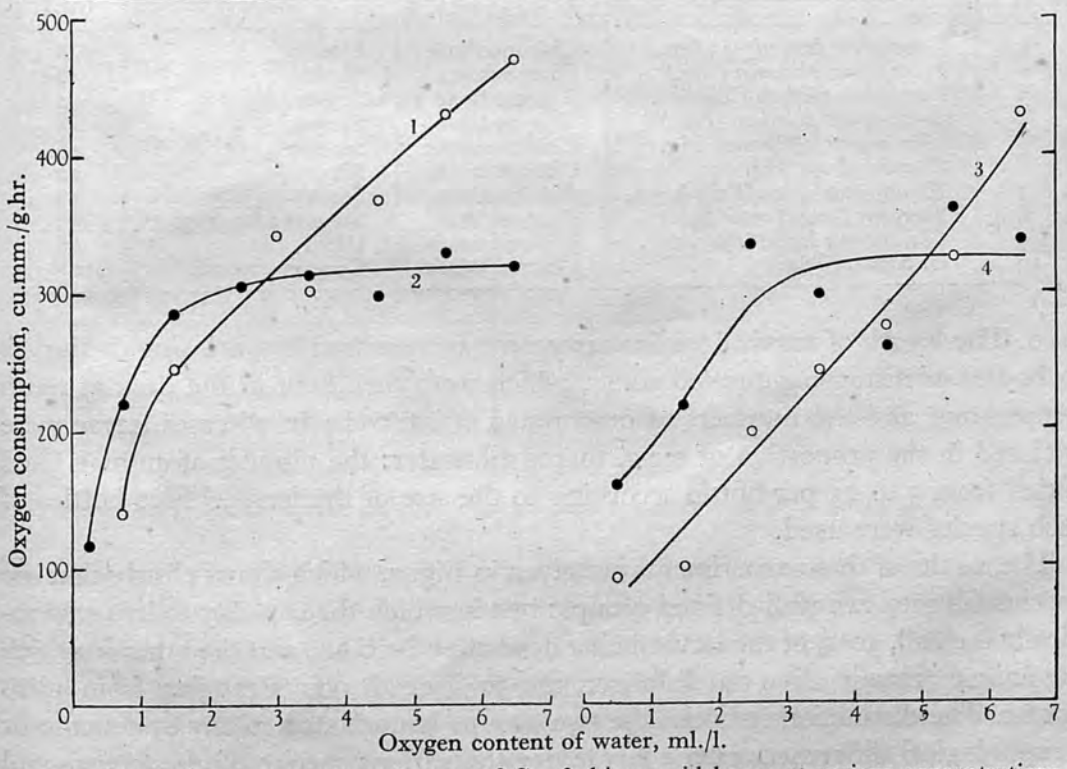



Fig. 1. Rates of oxygen consumption at 17° C. of chironomid larvae at various concentrations of dissolved oxygen. (1) *Tanytarsus brunnipes* (stream); (2) *Chironomus longistylus* (ditch); (3) *Anatopynia nebulosa* (stream); (4) *A. varia* (ditch). Data from Tables 1–4.

air saturation of the water than the corresponding ditch species; but at lower oxygen contents the oxygen consumption of the stream species falls rapidly, the relation between oxygen consumption and oxygen content of the water being roughly linear. The rate of oxygen consumption of the two stagnant water species, however, is almost independent of the oxygen content until this falls to the value of 3 ml./l. (44% air saturation).

The rates of heart beat, in fully aerated water at 17° C., of the four species were found to be $32{\cdot}4 \pm 1{\cdot}1$ beats per min. in *Anatopynia varia* as compared with $32{\cdot}4 \pm 1{\cdot}0$ in *A. nebulosa*, and $72{\cdot}9 \pm 5{\cdot}1$ in *Chironomus longistylus* compared with $64{\cdot}4 \pm 2{\cdot}1$ in *Tanytarsus brunnipes*. There is thus no significant difference in the rate of blood circulation in the comparable ditch and stream species in spite of the differences in oxygen consumption. This is unlike the case of the ephemerid nymphs *Cloeon* and *Baetis* (Fox & Simmonds, 1933).

### (2) SURVIVAL UNDER ANAEROBIC CONDITIONS

A comparative study was made of the capacity of larvae from different habitats to live under anaerobic conditions. A list of the species used and the habitats from which they were obtained is given in Table 5. In all cases final instar larvae were

Table 5. *Survival times of various chironomid larvae under anaerobic conditions*

| No. on Fig. 2 | Species | Subfamily | Habitat | 50% alive (hr.) |
|---|---|---|---|---|
| 1 | *Tanytarsus brunnipes* (Zett.) | Chironominae | Stream | 8 |
| 2 | *Prodiamesa olivacea* (Mg.) | Diamesinae | Stream | 10 |
| 3 | *Procladius choreus* (Mg.) | Tanypodinae | Stream | 12 |
| 4 | *Anatopynia nebulosa* (Mg.) | Tanypodinae | Stream | 14 |
| 5 | *Chironomus albimanus* Mg. | Chironominae | Stream | 16 |
| 6 | *C. rubeculosus* Mg. | Chironominae | River | 20 |
| 7 | *Anatopynia varia* (Fabr.) | Tanypodinae | Ditch | 46 |
| 8 | *Tanypus punctipennis* Mg. | Tanypodinae | Stagnant backwater | 50 |
| 9 | *Chironomus longistylus* Goet. | Chironominae | Ditch | 68 |
| 10 | *C. paganus* Mg. | Chironominae | Concrete trough | 101 |

used. The length of survival without oxygen was measured by enclosing the larvae in bottles of nitrogen-saturated water, which were then kept in the dark at room temperature and the numbers of dead noted at intervals. In all cases larvae were enclosed in the proportion of 0·2 g. to 400 ml. water: the number of animals used varied from 5 to 25 per bottle according to the size of the larvae. Five bottles of each species were used.

The results of these experiments are given in Fig. 2, which shows clearly that the species fall into two well-defined groups: one in which the capacity to live anaerobically is small, 50% of the larvae being dead after 8–20 hr., and the other in which the animals remain alive much longer, the 50% death points ranging from 46 to 101 hr. The distinction between the two groups is unrelated to any systematic or morphological differences in the larvae, for the various species of *Anatopynia* and *Chironomus* are seen to have very different susceptibilities. On the other hand, the

distinction between the two groups can clearly be correlated with differences in habitat. The group with a slight capacity for anaerobiosis is composed of species taken from running waters, while the larvae in which the capacity is greater are all from stagnant water environments. This is comparable with the case of the ephemerid nymphs and caddis larvae of Fox *et al.* (1933, 1935).

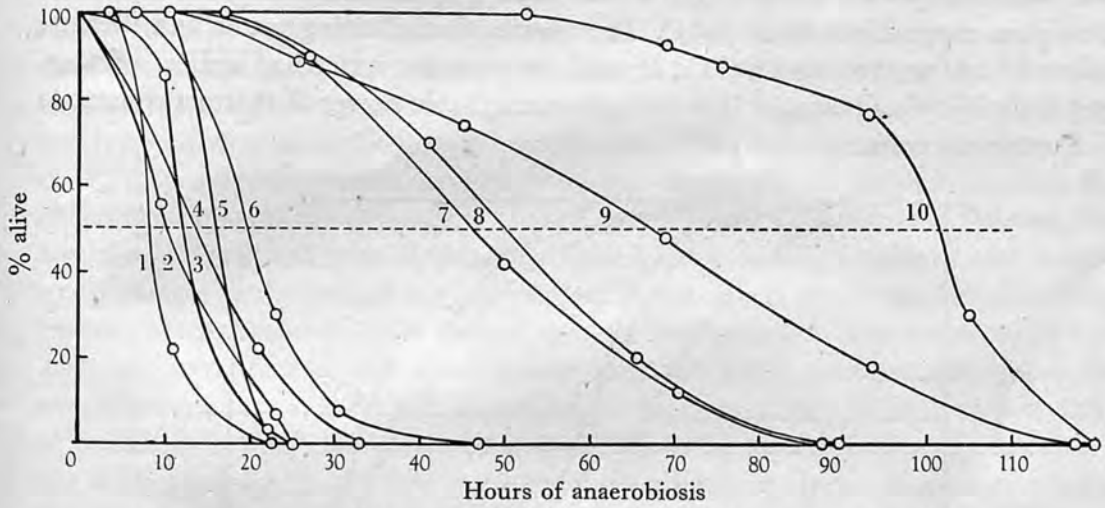



Fig. 2. Duration of life under anaerobic conditions of various chironomid larvae. Species used (nos. 1–10) given in Table 5.

## (3) THERMAL RESISTANCE

Experiments were made to determine the heat tolerance of larvae from different habitats. Table 6 gives a list of the seven species with their habitats; fully grown larvae were used. The thermal resistance was determined by placing twenty

Table 6. *Heat resistance of various chironomid larvae*

| No. on Fig. 3 | Species | Subfamily | Habitat | Thermal index (° C.) |
|---|---|---|---|---|
| 1 | *Tanytarsus brunnipes* (Zett.) | Chironominae | Stream | 29 |
| 2 | *Prodiamesa olivacea* (Mg.) | Diamesinae | Stream | 30 |
| 3 | *Anatopynia nebulosa* (Mg.) | Tanypodinae | Stream | 30·5 |
| 4 | *Chironomus riparius* Mg. | Chironominae | Concrete trough | 34·5 |
| 5 | *C. albimanus* Mg. | Chironominae | Stream | 35 |
| 6 | *C. longistylus* Goet. | Chironominae | Ditch | 35·5 |
| 7 | *Anatopynia varia* (Fabr.) | Tanypodinae | Ditch | 38·8 |

larvae in an open glass vessel containing 100 ml. of air-saturated water, and keeping them in a thermostat at an appropriate temperature for 22 hr. The number which died during that time was then counted. Temperatures between 23 and 39° C. were used. The water was not aerated during the experiments; consequently less oxygen was available at the higher temperatures. Even at the highest temperatures, however, the diminution of dissolved oxygen could not alone have been responsible for the death of the larvae, but it possibly modified their capacity to resist high

temperatures.* In nature, however, high temperatures would almost always be accompanied by diminished oxygen concentrations: the larvae would thus be influenced by a combination of the two factors.

Fig. 3 shows the decline in numbers of larvae remaining alive after 22 hr. at various temperatures. The capacity to withstand high temperatures varies: taking as a thermal index the temperature at which 50% of the larvae are dead in 22 hr., the values range from 29 to 39° C. The species from flowing water had thermal indices of 29, 30, 30·5 and 35° C., those from stagnant water had indices of 34·5, 35·5 and 38·8° C. Of special interest is the remarkable power of thermal resistance of *Anatopynia varia*.

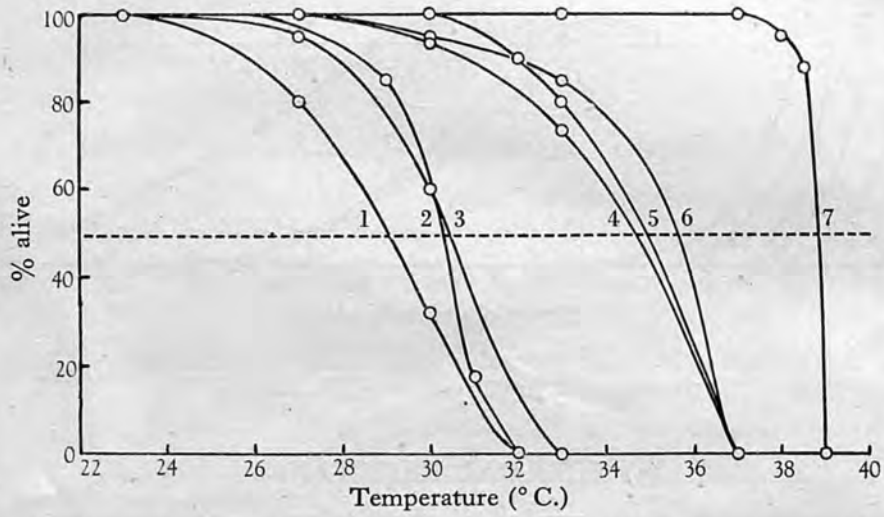



Fig. 3. Thermal resistance of various chironomid larvae. Species used (nos. 1–7) given in Table 6.

## (4) DISCUSSION

Of the chironomid larvae whose oxygen requirements have here been studied, the two species from a stream have higher metabolic rates in aerated water than the corresponding species from still water. The similarity in size, structure and activity of the members of the two pairs of larvae eliminates the possibility that the metabolic differences are due to these factors. The fact that relatively high metabolic rates have also been found in stream species of other aquatic arthropods (Fox *et al.* 1933, 1935) suggests that this property may be a general characteristic of animals living in streams. The evidence of Washbourn (1936) that the oxygen consumption of trout fry increases with the current velocity in which they are reared shows that the metabolic difference between fast- and slow-water animals can be acquired during individual life. Although in the case of the trout fry the direct effect of current may well have been the causal factor influencing the metabolic rate, with chironomid larvae, which in their mud tubes are protected from the current, this cannot be so

* Whitney (1939) tested this possibility when working with the nymphs of Ephemeroptera. He found the extra survival in oxygenated water as compared with aerated water never greater than 14%, which was not sufficient to obscure the specific differences in thermal resistance.

and the significant cause seems more likely to be a high oxygen pressure, if indeed the high metabolism is individually acquired. On the other hand, it may be innate.

The high metabolic rate of the stream chironomid larvae is only maintained in fully aerated water, the relation between oxygen concentration and oxygen consumption being approximately a linear one. This contrasts with the non-linear relationship between the two variables in the larvae from stagnant waters. Such differences in animals' responses to low oxygen concentrations have been detected in a wide variety of organisms (Hyman, 1932; Tang, 1933) and have given rise to much speculation as to the general significance of the dependence or independence of oxygen consumption on oxygen pressure. It should be pointed out that the difference between the dependent type of curve shown by the stream chironomids and the independent type of the other chironomids is only a relative one, related to differences in the critical oxygen pressure below which the normal metabolic rate cannot be maintained. This critical oxygen concentration lies between 15 and 44% air saturation of the water in the stagnant water chironomids, and at, or possibly even above, 100% air saturation in the stream ones. That it should differ so markedly in such closely related animals makes the assumption reasonable that it has some special ecological or physiological significance. In the chironomid larvae the differences are related to the habitat of the animals, but Fox *et al.* (1937), measuring the oxygen consumption of various ephemerid nymphs, found that although the nymph with the most dependent type of metabolism (*Baetis* sp.) came from a stream and that with the most independent type (*Cloeon dipterum*) from a pond, the correlation between degree of dependence and habitat did not hold for the other species studied.

The main interest of such comparative studies of metabolism lies in the fact that very closely related and structurally similar animals do show these extensive differences in types of metabolic response to oxygen. In these animals different degrees of dependence cannot be the result of differences in the efficiency of oxygen transport to the tissues, since in the chironomid larvae, at any rate, the size and the speed of blood circulation are the same in both members of the contrasted pairs. The larvae which show dependence are, however, those with a high metabolic rate in aerated water, suggesting a difference in cellular respiratory systems.

The differences in type of metabolism shown by these closely related larvae raises the question of the possible function in respiration of the haemoglobin they contain. Ewer (1942) showed that the haemoglobin of the larvae of *Chironomus riparius* and *C. cingulatus* (two very similar species), while functionless as a transporter of oxygen in air-saturated water, enables them to maintain a normal metabolic rate at lower oxygen concentrations: in other words, by lowering the critical oxygen pressure the haemoglobin was to a large extent responsible for their independent type of metabolism curve. One is probably justified in assuming a similar function of the haemoglobin in the closely allied *C. longistylus*, but the independent curve of *Anatopynia varia* can scarcely be the result of the possession of a functional

haemoglobin since the blood of this species contains very little of the pigment. Conversely, the strikingly dependent *Tanytarsus* and *Anatopynia nebulosa* contain as much haemoglobin as *Chironomus longistylus*. In *Tanytarsus* the haemoglobin is only used in oxygen transport at oxygen concentrations below 25% air saturation (Walshe, 1947), at which concentration the larvae, even with the help of their haemoglobin, are only capable of maintaining a metabolic rate less than half that which they have in fully aerated water. To what extent the haemoglobin is really of use to a chironomid with a dependent type of metabolism curve is therefore doubtful: it certainly does not equip them to continue a normal aerobic metabolism at low oxygen pressures.

From an ecological point of view the differences are more easily interpreted. A high metabolic rate and a need for complete air saturation of the water to maintain this rate imply that the stream larvae are only fitted for life in situations where water movement ensures adequate aeration. The oxygen content of the water just above the mud in the brook from which the two stream chironomids were collected was measured at intervals throughout the year and never fell below 78% air saturation, whereas the oxygen concentration in the ditch containing the other two species was always much lower than this. Oxygen samples were taken in this ditch at intervals during one day in summer and even at its highest point the oxygen immediately above the animals' tubes only rose to 58% saturation and throughout the hours of darkness was at the very low value of 2% saturation. The still-water species therefore have to endure very low pressures of oxygen, at any rate in summer, and their ability to maintain a normal metabolic rate at low oxygen pressures fits them for their life in such an environment. It would appear that in such species adaptation to environment has proceeded to enable the larvae to live in habitats deficient in oxygen whereas the stream chironomids are unmodified in this respect, no selection for such an adapted metabolism having been necessary.

With one exception, the larvae of stream chironomids are much less resistant to high temperatures than still-water forms. Whitney (1939) also found that ephemerid nymphs from slow-flowing or still waters had a greater heat tolerance than those from swift streams, and he correlated these differences with greater temperature fluctuations in the former environments. A similar correlation can be made for the chironomids: temperatures in the stream never rose above 15° C. during the summer in which these experiments were made, whereas a water temperature of 20° C. was recorded in the still-water habitats. In general, the thermal resistance of animals is closely related to the temperature conditions of their environment, the maximum temperature for normal life being little above the highest temperature to which the organism is normally subjected, although the maxima can be artificially raised by acclimatization. To what extent the thermal resistance of an animal in its natural state represents an individual acclimatized condition, and to what extent it is the genetical result of adaptation by selection of physiological processes which have temperature optima most fitted to the temperature ranges experienced, is as yet unknown. In this connexion it was interesting to find *Chironomus albimanus* (the stream chironomid with the highest thermal resistance) also living in a stagnant water

environment in association with *Chironomus plumosus* and *Anatopynia varia*. Its choice of habitat therefore seems wide and its thermal resistance correspondingly greater than the more typical stream species. This suggests a genetically determined condition.

The maintenance of a normal metabolic rate at low oxygen pressures, and the capacity to withstand both oxygen lack and high temperatures, are features in the physiology of stagnant-water chironomids which enable them to live in such habitats. It is clear that these physiological characters are adaptive, and it is reasonable to suppose that they have been acquired by such chironomids as necessary conditions for life in difficult habitats. On the other hand, because the stream species have a high metabolic rate and are much more susceptible to adverse conditions one may assume that constantly favourable oxygen pressures and temperatures in the stream habitat have made it unnecessary for them to evolve adaptations to meet unfavourable conditions. This does not necessarily imply, as one might be tempted to conclude, that such an environment was the original type to be colonized by chironomids, but rather that stream and pond larvae have evolved along two independent adaptive lines. Adaptation for life in streams demanded modifications mainly in morphology and behaviour as opposed to the profound respiratory specializations evolved by larvae colonizing stagnant habitats.

This investigation was made in the laboratory of Prof. H. Munro Fox.

## SUMMARY

1. The larvae of two chironomid species, *Tanytarsus brunnipes* and *Anatopynia nebulosa*, living in streams consume more oxygen than the closely related *Chironomus longistylus* and *Anatopynia varia* from still water.

2. The oxygen consumption of the two stream species falls as the oxygen content of the water diminishes, whereas that of the two still-water species remains approximately constant until the oxygen content has fallen to a low value.

3. Of the larvae of ten chironomid species, those from streams are much less resistant to anaerobic conditions than those from still water.

4. Stream chironomids have a lower thermal resistance than still-water forms.

## REFERENCES

EWER, R. F. (1942). On the function of haemoglobin in *Chironomus*. *J. Exp. Biol.* **18**, 197.

FOX, H. M. & SIMMONDS, B. G. (1933). Metabolic rates of aquatic arthropods from different habitats. *J. Exp. Biol.* **10**, 67.

FOX, H. M., SIMMONDS, B. G. & WASHBOURN, R. (1935). Metabolic rates of ephemerid nymphs from swiftly flowing and from still waters. *J. Exp. Biol.* **12**, 179.

FOX, H. M., WINGFIELD, C. A. & SIMMONDS, B. G. (1937). The oxygen consumption of ephemerid nymphs from flowing and from still waters in relation to the concentration of oxygen in the water. *J. Exp. Biol.* **14**, 210.

HYMAN, L. H. (1932). Relations of oxygen tension to oxygen consumption in *Nereis virens*. *J. Exp. Zool.* **61**, 209.

LENZ, F. (1925). Chironomiden und Seetypenlehre. *Naturwissenschaften*, **13**.

MASON, I. L. (1939). Studies on the fauna of an Algerian hot spring. *J. Exp. Biol.* **16**, 487.

PLATEAU, M. F. (1872). Recherches physicochimiques sur les articulés aquatiques. Deuxième partie. *Bull. Acad. Belg. Cl. Sci.* **34**, 274.

TANG, PEI-SUNG (1933). On the rate of oxygen consumption by tissues and lower organisms as a function of oxygen tension. *Quart. Rev. Biol.* **8**, 260.

THIENEMANN, A. (1923). Die beiden *Chironomus*-arten der Tiefenfauna der norddeutschen Seen. *Verh. int. Ver. Limnol. Kiel.* **1**, 108.

THIENEMANN, A. (1928). *Die Binnengewässer*. Stuttgart.

WALSHE, B. M. (1947). The function of haemoglobin in *Tanytarsus* (Chironomidae). *J. Exp. Biol.* **24**, 343.

WASHBOURN, R. (1936). Metabolic rates of trout fry from swift and slow running waters. *J. Exp. Biol.* **13**, 145.

WHITNEY, R. J. (1939). The thermal resistance of mayfly nymphs from ponds and streams. *J. Exp. Biol.* **16**, 374.

(*Reprinted from* NATURE, *Vol.* 160, *page* 474, *October* 4, 1947.)

## Feeding Mechanisms of *Chironomus* Larvæ

THE large, hæmoglobin-bearing larvæ of the genus *Chironomus*, commonly known as bloodworms, are the most abundant and widespread members of the bottom mud communities in ponds and lakes, but owing to the obscurity of the medium in which they live little is known about their behaviour. The larvæ live in U-shaped tubes through which an intermittent irrigation current is maintained[1], and are stated to feed on the organic mud around them, eating part of the walls of their burrows or feeding off the surface of the mud[2]. Alsterberg[3], however, considered them to be largely plankton feeders, eating algæ brought in by the irrigation current and trapped by the tube walls or the spines of the anterior proleg. But details of their feeding mechanism or respiratory behaviour are unknown. Accordingly, I have watched the behaviour of larvæ of *Chironomus plumosus* L. under various conditions.

Glass U-tubes, resembling in proportions those which the larvæ construct in Nature, and perforating a small celluloid platform holding a thin layer of mud (Fig. 1), housed the larvæ. Platform and tubes were placed in water in small glass aquaria. In the course of the observations a feeding method was discovered which has not previously been described. The larvæ were seen to feed largely on suspended matter filtered out of a stream of water set up through the tube by their own activity. This feeding is carried out as follows. Maintaining its position in the tube by means of firmly anchored posterior

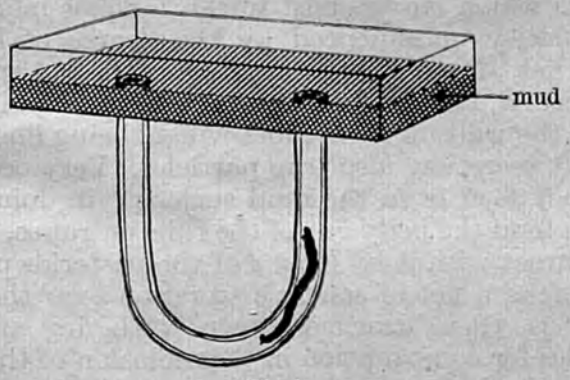



Fig. 1

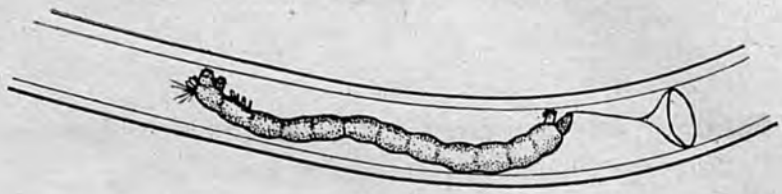


Fig 2.

prolegs, the larva performs a number of rotatory movements of the anterior region of the body, the head describing complete circles of alternating direction round the wall of the tube. While these movements are being made the anterior proleg draws out strands of salivary secretion by rapid approach to, and withdrawal from, the mouthparts, the strands being attached to the walls of the tube and stretched across its lumen to form a loose, saucer-shaped sheet. The larva then withdraws a few millimetres down the tube, dragging with it a salivary thread from the middle of the sheet, which is thus pulled out to form a shallow conical net (Fig. 2). Attached to the thread with its mouthparts, it begins violent antero-posterior undulations of the body with a frequency of about two per second. These have the effect of producing a current of water through the tube, and particles suspended in the water are caught by the conical net. The larva next straightens its body and rapidly eats the cone with the suspended matter on it; it then at once spins another web. The whole process is remarkably rapid, successive cones being formed and eaten at the rate of one every $1\frac{1}{2}$–2 min. Approximately half the time is spent in undulating and the other half in eating and making the net. Although these time intervals vary for individual larvæ their repetition is very regular, and the whole performance forms a very stereotyped behaviour pattern which may persist for an hour or more with a regularity uninfluenced by the degree of loading of the net. More rarely, larvæ omit the net-spinning process and, instead, between the periods of irrigation, scrape the walls of their tubes which, being lined with salivary secretion, also trap particles. Very occasionally they feed from the mud surface; in doing this, they extend the body out of the tube entrance, retaining contact with it by means of the posterior prolegs, and spread a net of salivary secretion over the mud, which is then dragged down with its attached particles for consumption in the seclusion of the tube.

The filtering method is, however, the commonest

feeding mechanism in *C. plumosus* and resembles that of leaf-mining chironomids[2,4], except that in the latter the larva turns round after spinning the salivary cone, which is therefore posterior to the larva with the apex pointing away from the body. It also recalls the feeding mechanism of *Urechis caupo*, in which a long cone is secreted by the echiuroid worm and remains attached to the head during the irrigation of the U-shaped burrow[5]. In this case the cone is of mucus with pores of ultramicroscopic diameter and consequently high filtering efficiency[6].

I estimated the filtering powers of the nets of *Chironomus* by introducing into the inhalant current a suspension of carmine, and measuring the diameter of the particles before and after passing through the tube. The nets retain all particles bigger than 17 $\mu$ in diameter and most of those bigger than 12 $\mu$ in diameter, and would thus trap many planktonic organisms in addition to any particles of detritus, etc., temporarily suspended in the water as a result of agitation of the mud surface. The larvæ are unselective feeders in that they eat indiscriminately all particles attached to the net.

The observations have so far been confined to the larvæ of *Chironomus plumosus*, but it is likely that the other closely related species have the same habits. These larvæ provide one more example of a semi-sedentary animal solving the feeding problem by means of a filter mechanism.

BARBARA M. WALSHE

Bedford College,
University of London. Aug. 1.

[1] Lindroth, A., *Zool. Anz.*, **138**, 224 (1942).
[2] Leathers, A. L., *Bull. U.S. Bur. Fish*, **38**, 1 (1922).
[3] Alsterberg, G., *Arch. Hydrobiol.*, **15**, 291 (1925).
[4] Burtt, E. T., *Proc. Roy. Ent. Soc. Lond.*, **A**, **15**, 113 (1940).
[5] Fisher, W. K., and MacGinitie, G. E., *Ann. Mag. Nat. Hist.*, **10** (1928)
[6] MacGinitie, G. E., *Biol. Bull. Woods Hole*, **88**, 107 (1945).

PRINTED IN GREAT BRITAIN BY FISHER, KNIGHT AND CO., LTD., ST. ALBANS