J. exp. mar. Biol. Ecol., 1980, Vol. 46, pp. 255–277

OXYGEN UPTAKE, THE CIRCULATORY SYSTEM, AND HAEMOGLOBIN FUNCTION IN THE INTERTIDAL POLYCHAETE *TEREBELLA HAPLOCHAETA* (Ehlers)

R. M. G. WELLS, P. J. JARVIS
Department of Zoology, University of Auckland, Auckland, New Zealand

and

S. E. SHUMWAY
Portobello Marine Laboratory, University of Otago, Portobello, New Zealand

Abstract: The intertidal polychaete *Terebella haplochaeta* (Ehlers) shows a high degree of oxyregulation in declining pO_2 when confined to its burrow at low tide. This response is achieved by a number of adaptations to the respiratory system. The worm ventilates its burrow in a headward direction by rhythmical contractions of the body. The rate of these pulsations increases at low pO_2 and assists the circulation of the coelomic and vascular fluids. Haemoglobin in the vessels has a high affinity for oxygen and a sigmoidal equilibrium curve. Both the shape and position of the oxygen-binding curve are sensitive to changes in pH, pCO_2, and temperature in a way that suggests augmentation of oxygen delivery at low tide. The concentration of haemoglobin in the vessels is high and is further raised following warm acclimation, presumably to meet an increase in oxygen demand. The ultrastructure of the gills and blood vessels indicates a design for function at low oxygen tensions where diffusion distances must be short and surface areas large in order to enhance the rate of diffusion of oxygen from the near environment.

INTRODUCTION

The past ten years has seen a growing interest in the respiratory adaptations of marine organisms, particularly with regard to the Polychaeta, Gastropoda, and Crustacea. Studies which have focused on intertidal invertebrates have had to take into account the transition from aquatic to aerial conditions occurring with each phase of the tide. Several reviewers have drawn attention to the difficulties in interpreting the interaction of environmental and organismic factors in the complex intertidal zone (see Mangum, 1970, 1976a, b; Mangum & Van Winkle, 1973; Newell, 1973; Weber, 1978a).

Many intertidal animals possess a respiratory pigment, and among those that do, the Polychaeta frequently have haemoglobin dissolved in the plasma of the vascular system or a cellular haemoglobin contained in the coelom. The oxygen-binding properties of these pigments are now known to be closely correlated with the availability of oxygen in the near environment, even though precise field measurements are often lacking. The recent interest in this aspect of respiratory behaviour is

reflected in the reviews by Mangum (1970, 1976a, b), Wells & Dales (1976), Weber (1978b), and Chung & Ellerton (1979).

Unfortunately, there have been few attempts to describe the adaptations of a whole respiratory system by taking into account the interactions between the parts of the system at a molecular level (the haemoglobin), the systemic level (the circulation), and the organismic level. A notable exception has been the synthetic approach of C. P. Mangum and her colleagues (e.g. Mangum *et al.*, 1975; Mangum, 1976a, b). In the present study, we have attempted to consider some of the components which comprise the respiratory system in the intertidal polychaete *Terebella haplochaeta* (Ehlers).

MATERIAL AND METHODS

Collection of material

Terebella haplochaeta were collected from burrows in sandstone, in the intertidal zone at Cheltenham Beach, Auckland, New Zealand. Whenever possible, individuals were used immediately on return to the laboratory. Animals collected for studies of oxygen uptake were flown to the Portobello Marine Laboratory where they were kept for at least 1 wk prior to experimentation. All animals were maintained in aerated sea water at 16–18 °C.

Collection of blood

Animals were blotted and an incision made on the anterior dorsal surface with care being taken to avoid puncturing the large dorsal blood vessel. Coelomic fluid was collected or discarded. The worm was then rinsed with filtered sea water and again blotted. The dorsal blood vessel was cut and the blood collected into 5-μl graduated glass capillary tubes.

Laboratory reproduction of the habitat

Worms were kept in a dark aquarium with fresh, aerated sea water and when provided with sediment from the natural habitat, built tubes similar to those found on the shore. As one side of the tube comprised the aquarium wall the worms were plainly visible. All observations were made in darkness using an infrared light.

Sea water was siphoned out over a 4-h period (ebbing tide), the burrows were left uncovered for 2 h (slack tide), and then flooded with aerated sea water to simulate a tidal cycle. Samples of burrow water (0.5 ml) were taken into a glass syringe and the oxygen content measured using a Radiometer E5046 electrode. Five worms were introduced into Pasteur pipettes and the animals' irrigatory behaviour was observed in these transparent burrows. This assumes that in nature the tubes are watertight. While we lack direct evidence for this assertion, *T. haplochaeta* does construct

permanent, mucus-lined tubes. Gill colouration was observed over an outgoing tide and estimations of oxyhaemoglobin in the gills were made by comparison with known percentages of human oxyhaemoglobin prepared by anaerobically mixing various ratios of oxy : deoxy haemoglobin in glass capillaries.

When all the water had been siphoned from an aquarium (low tide), estimations of the volume of water trapped in burrows were obtained by extraction into graduated glass syringes.

Irrigatory waves progressing anteriorly up the worm's body were observed during the simulated tidal cycle. Twenty animals were used at oxygen concentrations ranging from 100% to 14.2% saturation. These worms were kept in sealed syringes having an initial capacity of 4 ml of fully oxygenated sea water at 20 °C. The number of waves · min^{-1} were counted every 5 min. The pO_2 of the water was measured with the oxygen electrode used to determine oxygen in burrow water. The end of the control syringe was left open in a beaker of oxygenated sea water in the same water bath.

Histology, morphology, and anatomy

Estimations of body, gill, and blood vessel wall thicknesses were made from slides of sectioned worms using a micrometer eye piece fitted to a compound light microscope. Animals were narcotized using menthol in sea water and then fixed in Bouin's solution for 24 h. Whole worms were embedded in paraffin wax and sectioned with a Jung Rotary Microtome at 5-μm intervals. Every sixth section of the anterior half of a worm was taken for inspection.

Gills were examined for the possible presence and arrangement of capillaries by taking every section. Mallory and Heidenhain's rapid one-step method of staining was used (Humason, 1972).

The fate of the major blood vessels was traced by dissecting freshly collected worms and by examining sectioned worms. Estimation of the volume and surface area of the entire animal was more difficult. Previous work of this nature on annelids has seen the gills treated as cylinders (Mangum *et al.*, 1975). The gills of *T. haplochaeta* are not cylinders and any calculations are, therefore, only rough estimations. For this reason, measurement of each "branchlet" of the gill was taken with a micrometer eye piece and the data used for volume calculation. Surface area calculations of gills also give only approximate estimates and, therefore, finer details of the gill surfaces were noted by means of the compound microscope but were not used in calculation.

Gills from narcotized animals were amputated and fixed with 2% glutaraldehyde in 0.2% cacodylate buffer (1150 mOsm). The tissue was stored in the buffer at 4 °C, then later dehydrated, and dried under CO_2 in a Polaron E3000 Critical Point Drier before examination under a Jeol JSM U3 scanning electron microscope. Prior to examination under the transmission electron microscope (Philips EM301) gills were

fixed in 1% glutaraldehyde (1100 mOsm) for 1–2 h. The tissue was stored in phosphate buffer for 24 h and post-fixed in 4% OsO_4 for 1 h, dehydrated in alcohol and embedded in 812 Epon. Sections were cut with a Sorvall MT-1 ultramicrotome and stained with 4% uranyl acetate for 3 min and lead citrate for 30 s (Venable & Coggeshall, 1965).

Oxygen uptake by whole animals

Oxygen uptake was monitored using a Radiometer E5046 electrode connected through a Radiometer PHM 71 meter to a Smith's Servoscribe chart recorder as described previously (Crisp *et al.*, 1978). Worms of ≈ 1 g wet wt were allowed to deplete the oxygen supply within a 120-ml respiratory chamber.

Spectrophotometric analysis

Samples of vascular blood and coelomic fluid were diluted with distilled water and scanned from 240–700 nm using a Unicam SP1750 recording spectrophotometer. Absorption maxima from the vascular pigment were similar to those obtained from other invertebrate haemoglobins (Chung & Ellerton, 1979) and confirmed the presence of haemoglobin in *T. haplochaeta*. Unlike other terebellids (Wells & Dales, 1975; Mangum *et al.*, 1975), no pigment was found in the coelomic cells of *T. haplochaeta*.

The concentration of haemoglobin was measured on a haem basis using the cyanmethaemoglobin derivative (Dacie & Lewis, 1975). Two μl blood were diluted with 4 ml of Drabkin's reagent and the absorbance measured at 540 nm in a 1-cm cuvette. A population of *T. haplochaeta* was sampled for haemoglobin concentration over two winters and one summer season in order to estimate possible changes in the oxygen-carrying capacity of the pigment.

Blood and coelomic fluid estimation

Coelomic fluid volumes were estimated gravimetrically by draining the coelomic contents; vascular blood volumes were estimated by comparing haemoglobin concentration in whole vascular fluid with that in whole, lacerated worms. The contribution from haemopoietic tissue and myoglobin was assumed to be negligible.

Carbonic anhydrase activity

Coelomic fluid, vascular blood, and various tissue extracts were tested for the presence of carbonic anhydrase by estimating their effect on the rate of carbon dioxide hydration (Wells, 1973).

Oxygen-binding studies

The reactions of haemoglobin with oxygen at various partial pressures of oxygen were measured using an Aminco Hem-O-Scan analyser as follows. A thin blood film was prepared from 2 μl of whole blood on a transparent coverslip and then placed in an optical path which photometrically monitored the percentage saturation. An O_2-electrode monitored the pO_2 of a progressively oxygenated gas mixture passing over the blood film in the humidified optical chamber. Saturation and pO_2 were recorded simultaneously on the Y and X channels of an X–Y recorder to produce a continuous oxygen equilibrium curve in $\approx$15 min. Whole blood curves were obtained at various temperatures and pCO_2, and for haemoglobin solutions buffered to constant pH with 0.05 M Hepes (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid) buffers. Hepes was selected because its pH is relatively insensitive to temperature, it has a negligible effect on ions other than H , and its pK is appropriate to the pH range 6.5–8.5.

RESULTS

TIDAL ACTIVITY

The burrows of *T. haplochaeta* consist of sand grains cemented together by mucus secreted from the worms and the tubes are easily accessible to water when covered by the tide. Thus at high tide the worms are not likely to be isolated in pockets of water having low oxygen tensions as is the case with a number of other crevice-dwelling organisms (Menzies, 1968). The pO_2 of the overlying water column in the

TABLE I

Relationship between state of "tide" on an artificial beach, gill colour, ventilatory contractions, and oxygen content of water in the tubes of *T. haplochaeta*: contraction rates measured over 5 min and nearest whole number recorded; volume of burrow water when tide out, 0.2 to 0.4 ml; observations from five worms at 20 °C.

State of "tide"	Relative gill colour (1-deoxyHb) (4 oxyHb)	Contraction rate (waves · min $^{-1}$)	Oxygen in burrow water (% saturation)
Fully in	4	6	98.0
1 h (tide ebbing)	4	6	94.1
2 h (tide ebbing)	4	8	91.3
3 h (tide ebbing)	3	8	90.6
Fully out	3	15	63.2
1 h out	2	9	40.2
2 h out	1	8	30.6
Tide in	3	7	80.2
0.5 h in	4	6	96.0

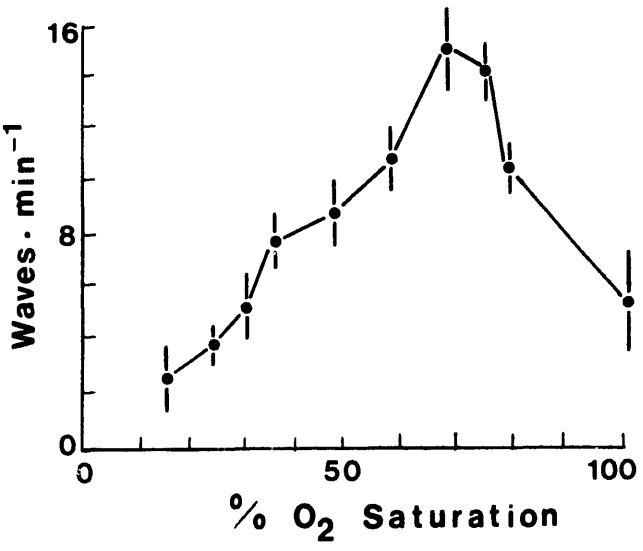
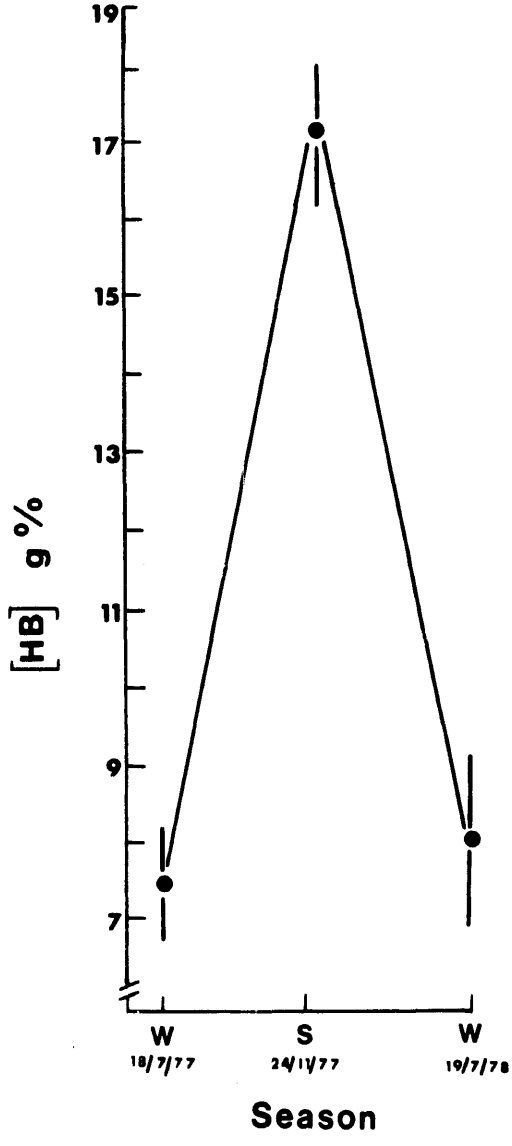


Fig. 1 (above). The effect of oxygen content of burrow water on ventilatory contractions (waves · min $^{-1}$) in *T. haplochaeta* acclimated to 20 °C: error bars show 95% confidence intervals.

Fig. 2 (right). The relationship between the austral seasons and haemoglobin concentrations in the vascular fluid of *T. haplochaeta*: mean monthly air temperatures were 12.8 °C (18.7.77), 24.3 °C (24.11.77), 13.1 °C (19.7.78); error bars show 95% confidence intervals for 10 worms in each group.



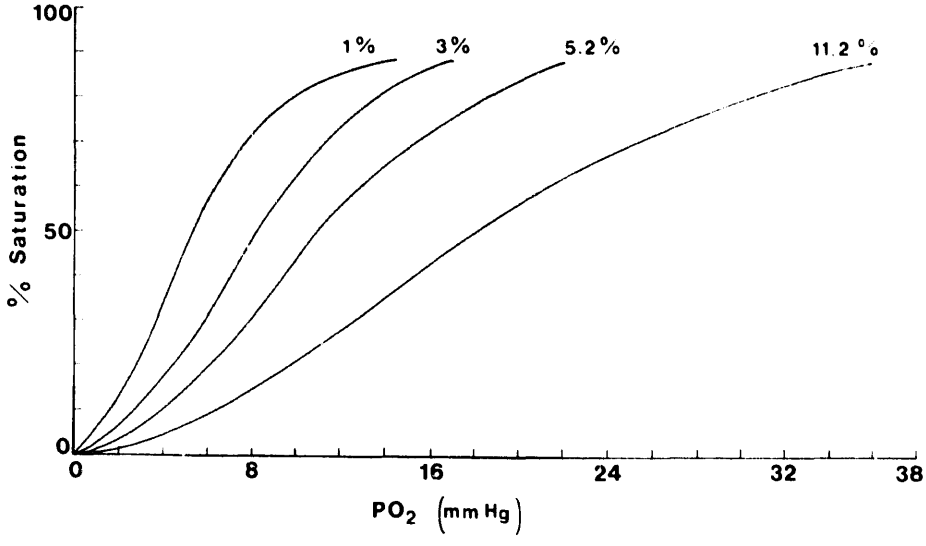


Fig. 3. Oxygen equilibrium curves of whole blood from *T. haplochaeta* at 20 °C equilibrated with 1–11.2% CO_2

washed intertidal zone is close to air saturation but when the tide recedes, the worms are deprived of a renewable supply of oxygenated water and remain trapped in the burrows in a small volume of water (0.2–0.4 ml).

Fig. 1 shows the relation of the rate of the body contractions to the oxygen tension of the overlying sea water. It is clear that at high oxygen tensions the ventilation rate is low (≈ 6 contractions $\cdot$ min^{-1}) and the rate increases with declining oxygen attaining a maximum of 15 contractions $\cdot$ min^{-1} at 70% saturation. Below this tension, the rate falls, but is still higher than at normoxic tensions, down to $\approx 40\%$ oxygen saturation. At high oxygen tensions it is unnecessary to maintain a vigorous ventilatory stream in order to maintain an adequate extraction of oxygen.

Table I summarizes the relative state of blood oxygenation and body contractions in *T. haplochaeta* as a function of the pO_2 of the burrow water during a single tidal cycle. The state of oxygenation of the blood, judged by the colour observed in the gills, follows the pattern of decreasing pO_2 in the burrow water. At high oxygen pressures the blood was fully oxygenated and appeared a scarlet colour. As the pO_2 of the burrow water decreased, the blood assumed a darker colour and appeared to be deoxygenated after 2 h emersion at $\approx 30\%$ saturation of oxygen. The blood within the gills lightened in colour almost immediately on return to oxygenated sea water and immersion of the burrows. It is worth noting, however, that Mangum (1976c) found that samples taken from a ventilated stream tended to over-estimate the amount of dissolved oxygen. While this might also be the case in the present study, the trend in deoxygenation of vascular blood paralleled the falling oxygen tensions as did the rate of ventilatory contractions.

HAEMOGLOBIN

The concentration of haemoglobin in the circulatory system of *T. haplochaeta* varies seasonally and maximum values were obtained in the summer (Fig. 2). A consequence of this is a seasonal shift in the oxygen-carrying capacity of the blood. Assuming that 1 g haem combines with 1.34 ml oxygen (Antonini & Brunori, 1971), then the blood may transport 23.1 vols oxygen per 100 ml blood during the summer, but only 10.3 vols % in winter. It is, however, not sufficient to have enough capacity because the blood must be able to give up as much of its bound oxygen as is demanded by the tissues. In the suite of haemoglobin-oxygen equilibrium curves in Fig. 3 it is evident that the affinity of *T. haplochaeta* haemoglobin decreases in response to a rise in carbon dioxide.

Apart from its effect on *P*50, pH has an influence on the shape of the curve as described by Hill's (1910) sigmoidal coefficient, *n*. The data in Fig. 4 show that in the absence of carbon dioxide, the equilibrium becomes less sigmoidal at low pH and at pH 6.8 the curve assumes a hyperbolic shape as indicated by an *n* value of 1.0. The equilibrium is also sensitive to temperature, the *P*50 rising with increased

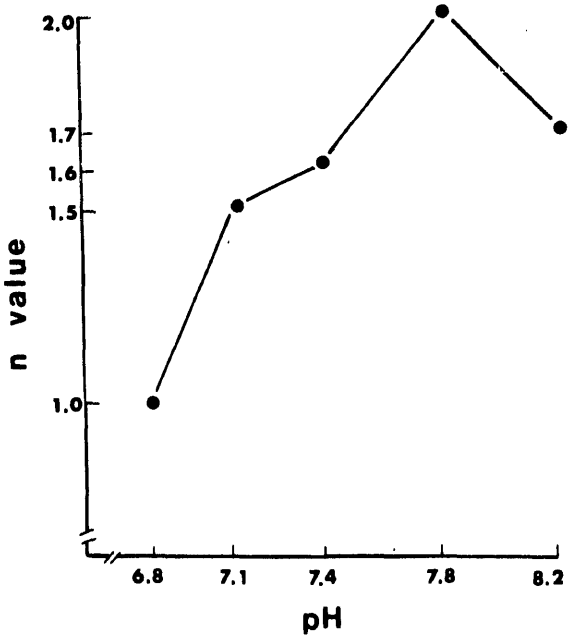


Fig. 4. Plot of the sigmoidal coefficient, n, from the haemoglobin equilibrium data obtained from blood buffered to various pH values and in the absence of carbon dioxide 20 °C.

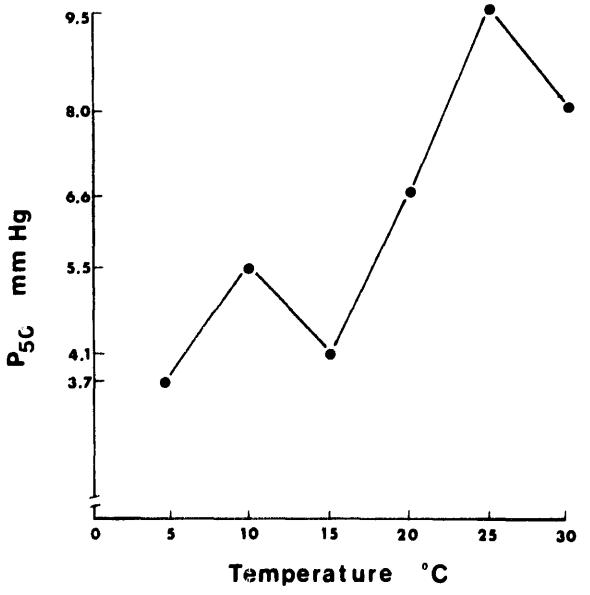


Fig. 5. The effect of temperature on oxygen affinity ($P50$) from the vascular blood of *T. haplochaeta* measured by duplicate points at 20 °C and buffered to pH 7.4.

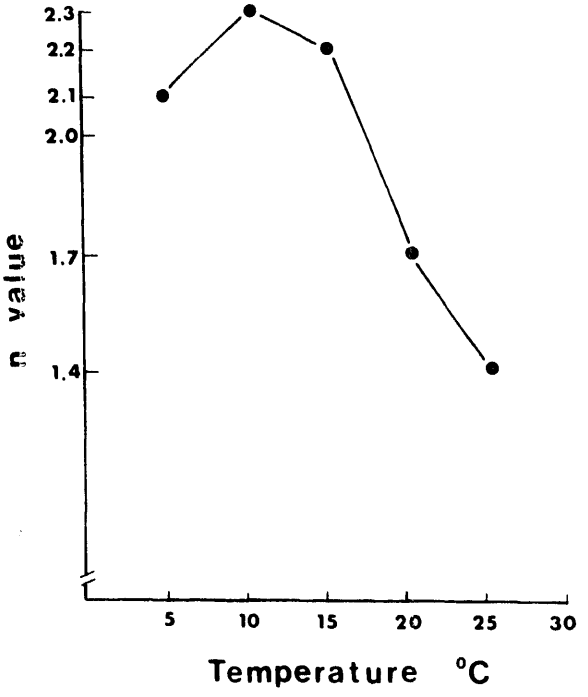


Fig. 6. Plot of the sigmoidal coefficient, n, of *T. haplochaeta* haemoglobin buffered to pH 7.4 at various temperatures.

temperature over the range 5–30 °C (Fig. 5). Deviations from linearity were assumed to be the result of sampling errors. The overall decrease in affinity with temperature is related to the exothermic nature of the oxygenation reaction and the quantitative relationship is given by the Van 't Hoff equation,

$$\Delta H^\circ = 19.159 \frac{T_1 T_2}{T_2 - T_1} \log \frac{P50_1}{P50_2} \text{ J} \cdot \text{mol}^{-1},$$

where ΔH° = heat of oxygenation, T_1, T_2 = lower and higher temperatures in °K, and $P50_1$, $P50_2$ = half saturation pO_2 at T_1 and T_2. Using a regression slope from the data in Fig. 5, ΔH° for *T. haplochaeta* haemoglobin was -21.6 kJ · mol $^{-1}$ which falls within the wide range of -7.5 to -50.2 kJ · mol $^{-1}$ reported for many haemoglobins from ectothermic animals (Rossi-Fanelli *et al.*, 1964).

Aside from oxygen affinity, temperature exerts an effect on the sigmoidal shape of the equilibrium (Fig. 6).

LOCATION AND ACTIVITY OF CARBONIC ANHYDRASE

Using arbitrary units of activity (see Wells, 1973), a high rate of catalysis was detected by duplicate determination in the vascular blood of *T. haplochaeta*, but not in the coelomic fluid. Gill and body wall tissues exhibited minimal activity, probably no more than could be accounted for by extracellular plasma arising from the circulation.

CIRCULATORY SYSTEM

Blood is pumped through the vascular system of *T. haplochaeta* by contractile waves of the coelomic fluid and pulsatile expansion of the large dorsal vessel and gills. The dorsal vessel (Fig. 7) is the most prominent vessel and is sometimes referred to as the heart. This vessel divides anteriorly into three: two afferent branchials and one supra-oesophageal which connects at the other end with the peri-oesophageal ring after running along the side of the oesophagus (Fig. 8). The efferent branchials return from the gills and join to form the ventral vessel, which runs the entire length of the body giving off a series of ventral ring vessels at the gut region. Before joining, however, the efferent branchials give rise to a vessel which divides and runs to the nephridia and mucus glands. Blood from these regions is collected and returned through a vessel which connects to the peri-oesophageal ring. Again, at the level of the ventral ring vessels, these are seen to be interconnected by loops, joining to form a lateral vessel. From the lateral vessel arises a series of dorsal ring vessels which pass into the gut plexus. The gut plexus drains into subintestinals and these in turn run forward to the peri-oesophageal vessel which gives rise to the dorsal vessel and oesophageal vessels.

Deoxygenated blood, or blood which has already circulated, is collected at the peri-oesophageal vessel and returned to the afferent branchials via the dorsal and supra-oesophageal vessels. Newly oxygenated blood is passed from the gills into the ventral vessel. This ventral vessel gives rise to a subneural vessel just before the level of the peri-oesophageal vessel, and runs the remaining length of the body in conjunction with the ventral nerve cord.

As oxygen is obtained only by diffusion from water across a physical barrier it is necessary to have some idea of the thicknesses of tissues across which the oxygen must diffuse. Assuming that the gills are the principal sites of oxygen uptake, it

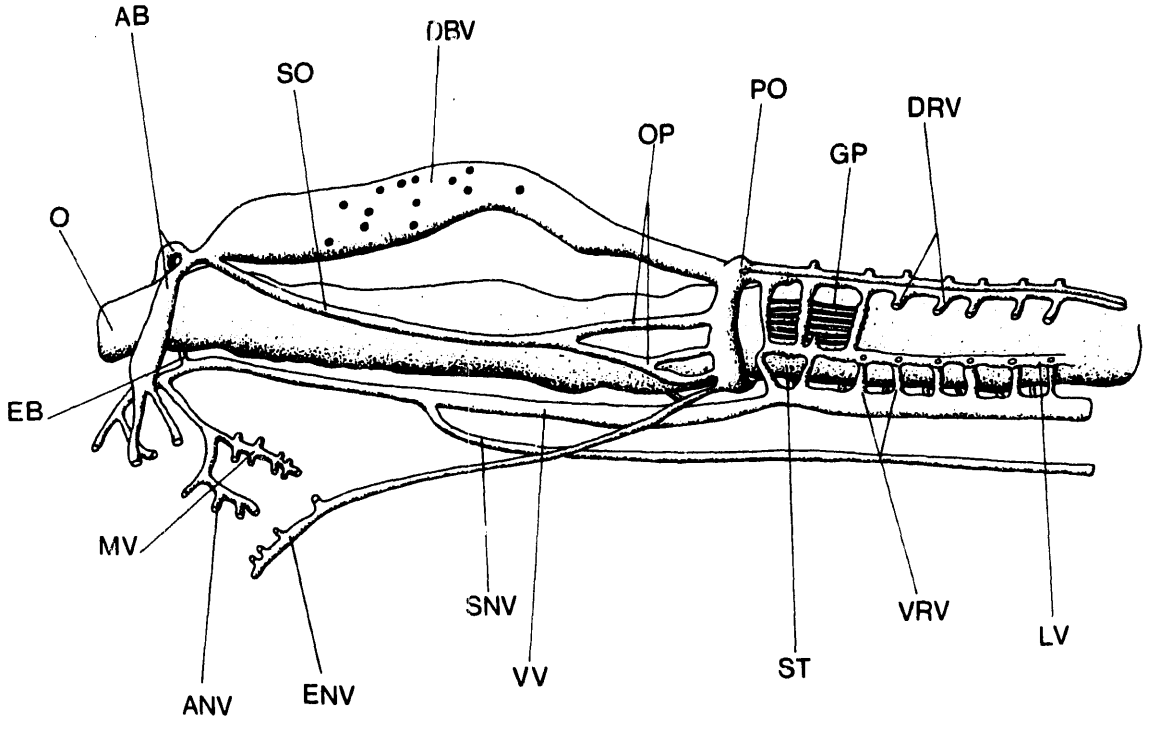


Fig. 7. Diagrammatic representation of the circulatory system in *T. haplochaeta* ($\approx \times 17$) indicating the major blood vessels when viewed from the lateral aspect: O, oesophagus; AB, afferent branchial; SO, supra-oesophageal; DBV, dorsal blood vessel; OP, oesophageal vessels; PO, peri-oesophageal; GP, gut plexus; DRV, dorsal ring vessel; LV, lateral vessel; VRV, ventral ring vessel; ST, stomach; VV, ventral vessel; SNV, supraneural vessel; ENV, efferent nephridial vessel; ANV, afferent nephridial vessel; MV, mucus vessel; EB, efferent branchial.

TABLE II

Estimated thicknesses of body, gill and blood vessel walls in *T. haplochaeta*.

Tissue	Thickness (mm)
Body dorsal wall	4.5×10^{-2} to 1.0×10^{-1}
Body ventral wall	8.5×10^{-2} to 1.0×10^{-1}
Gill wall	8.3×10^{-3} to 1.14×10^{-2}
Gill and body blood vessel wall	7.1×10^{-5} to 1.42×10^{-3}

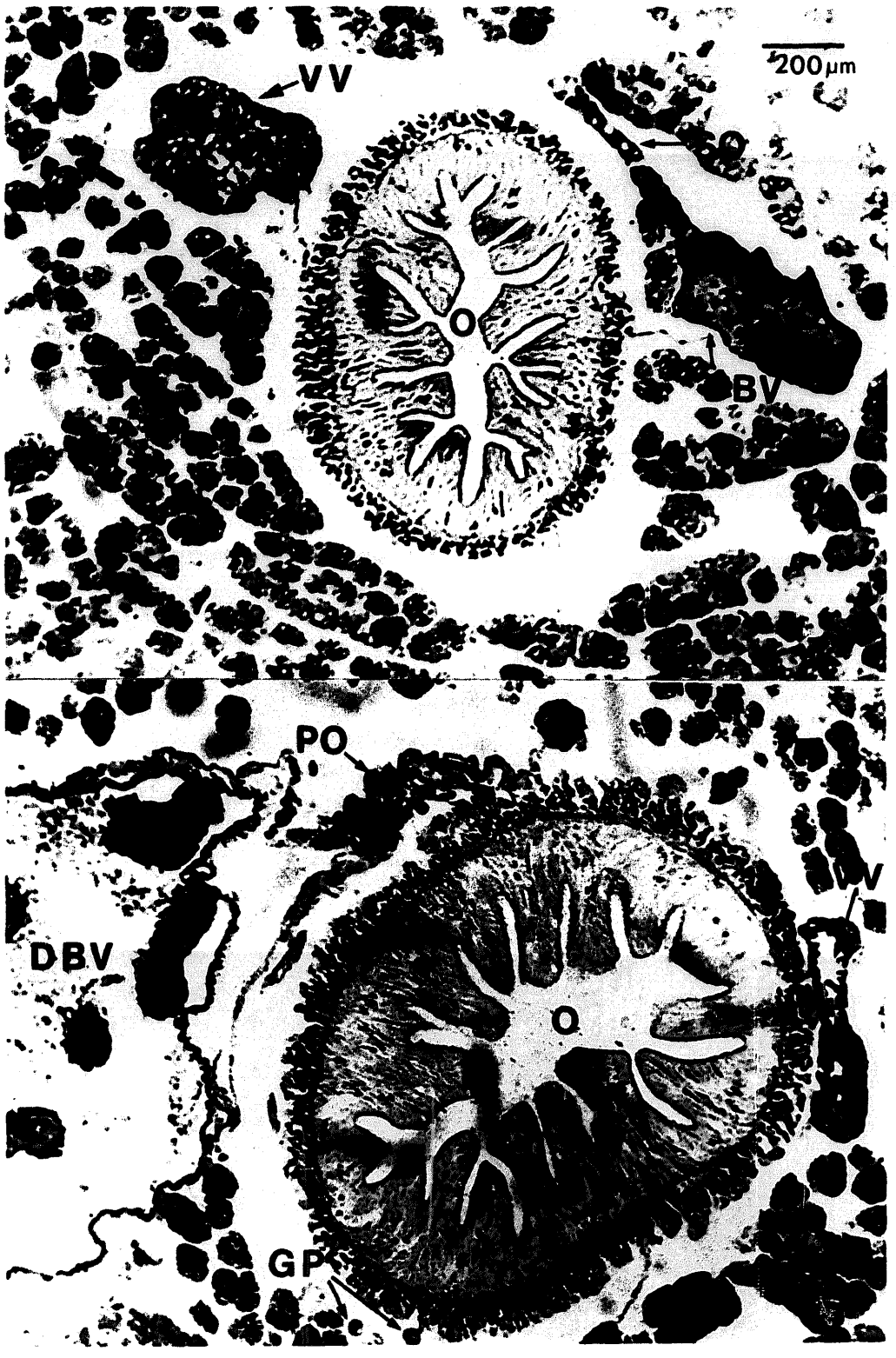


Fig. 8. Sections through the oesophageal regions of *T. haplochaeta* at the junction of the supra-oesophageal and dorsal vessels (above) and through the oesophageal ring vessel (below): O, oesophagus; DBV, dorsal vessel; PO, peri-oesophageal ring; GP, gut plexus; VV, ventral vessel; SO, supra-oesophageal vessel.

TABLE III

Estimated volumes and surface areas of body and gill of *T. haplochaeta*.

	Volume (V)	Surface area (SA)	SA : V
Body	1.62 cm^3	8.79 cm^2	5.4 : 1
Gill	3.32 mm^3	16.58 mm^2	5.0 : 1

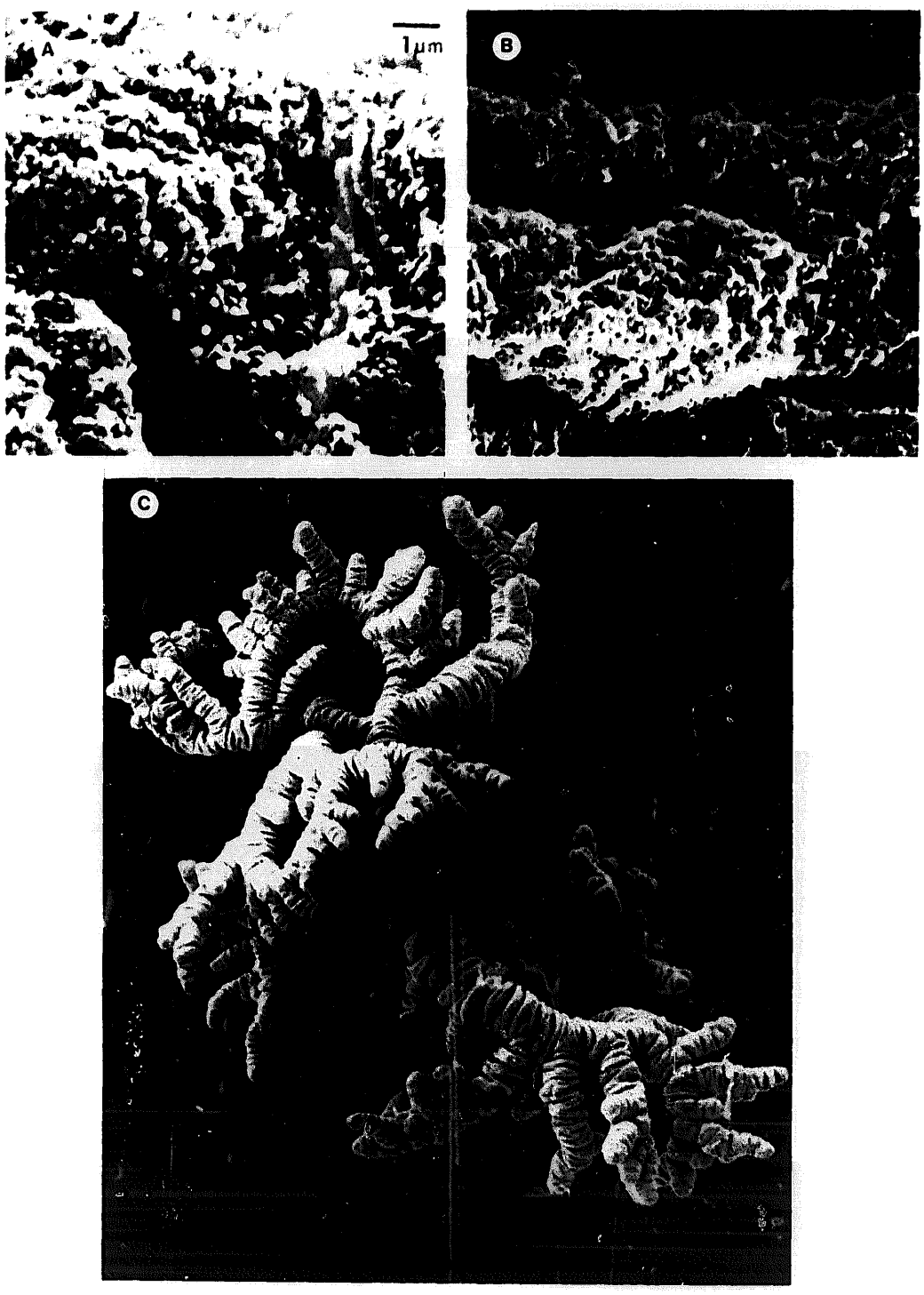


Fig. 9. Scanning electron micrograph of gill microsurface from *T. haplochaeta* at three magnifications showing the folded and microvillous appearance.

seems reasonable to assume that the thickness of the gill wall would be less than other parts of the body. This is shown in Table II where it may be seen that the gill wall is only one tenth the thickness of the ventral body wall. The wall thickness of blood vessels in the gills and body musculature was found to be identical but thinner than the gill wall itself.

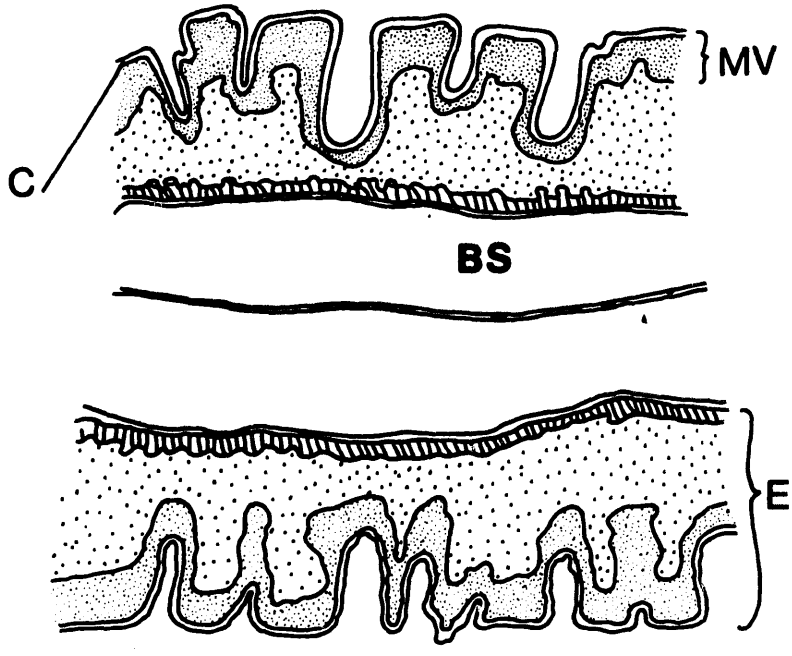


Fig. 10. Diagram of gill sectioned longitudinally showing the arrangement of blood spaces (BS) in relation to the cuticle (C), epithelium (E), and microvillous border (MV): ≈ ×45.

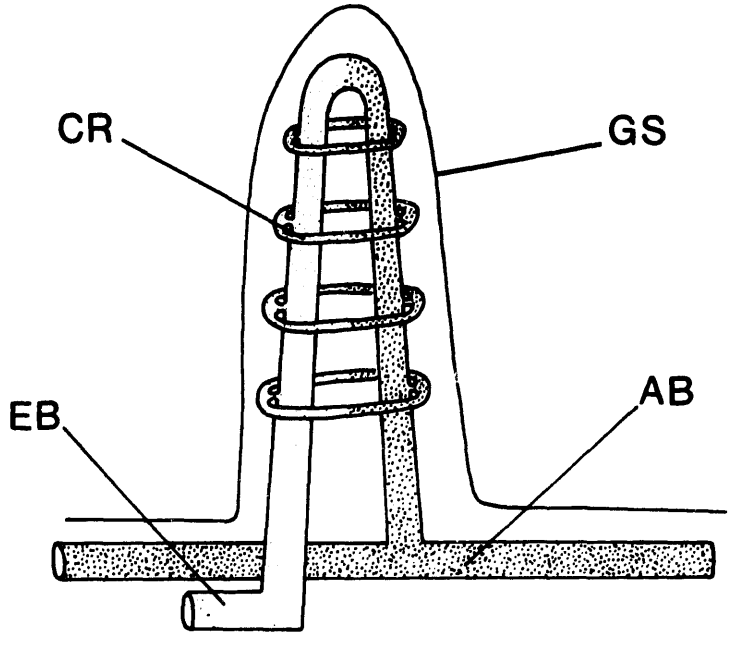


Fig. 11. Diagrammatic representation of the blood vessels and capillaries in a gill of *T. haplochaeta* showing the afferent branchial (AB), efferent branchial (EB), capillary ring (CR), and gill surface (GS): ≈ ×300.

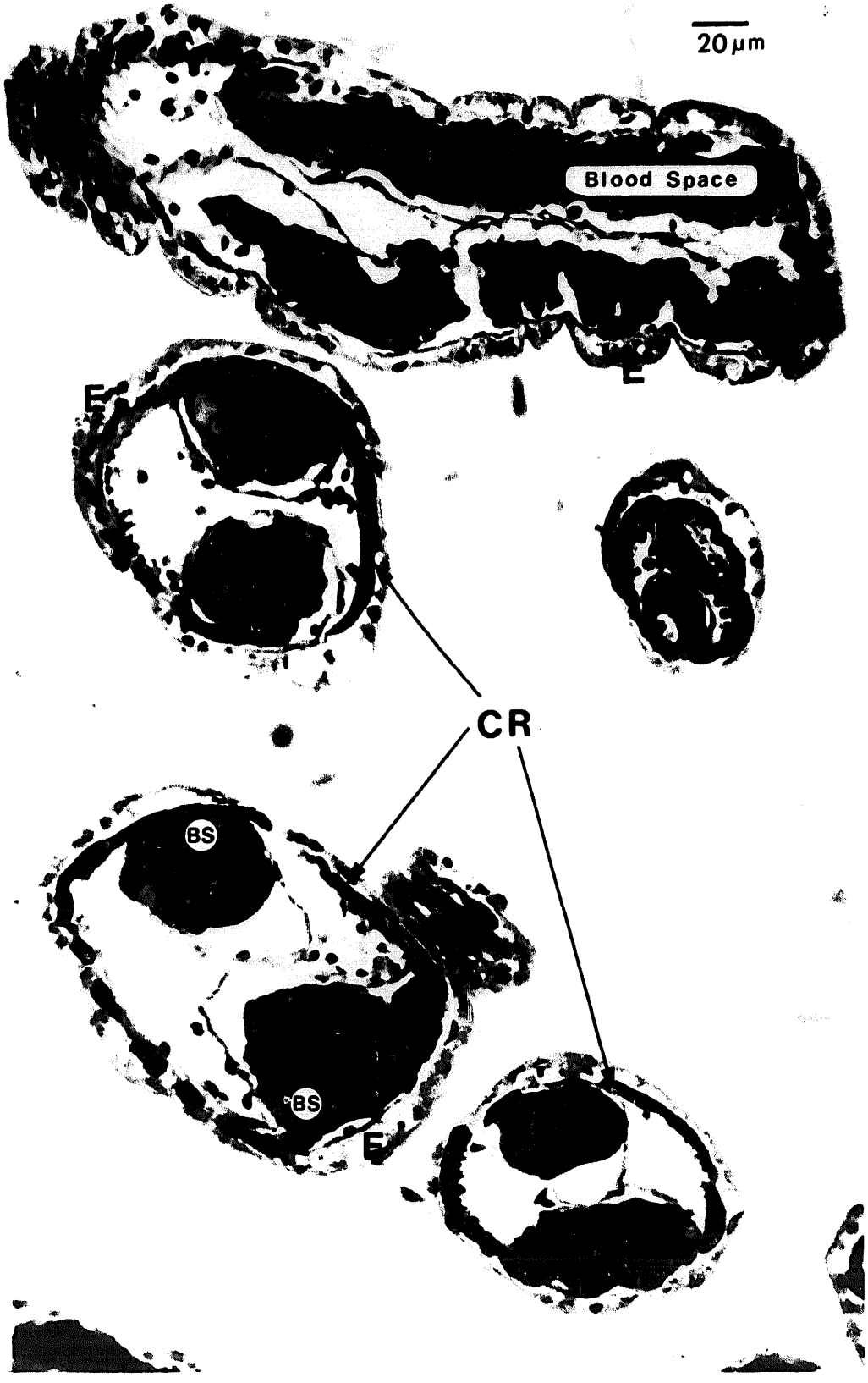


Fig. 12. Section through the gills of *T. haplochaeta* showing the arrangement of blood vessels within the gill branchlets: BS, blood space; CR, capillary ring; E, epithelium.

Estimated volume to surface area ratios obtained from the body and gills of *T. haplochaeta* are given in Table III. The body has the greater surface area to volume ratio and might appear to be a principal site for respiratory exchange. This assumption is, however, based purely on measurements made with the aid of a light microscope. Scanning electron micrographs of the gill surface (Fig. 9) reveal a much convoluted and greatly increased area than was previously observed and thus the contribution of the gill as an exchange surface may be greatly underestimated.

There is no coelomic space within the gill and blood spaces lie immediately adjacent to the epithelial layer (Fig. 10) so that the blood vessels within the gill of *T. haplochaeta* are applied closely to the epidermis. The blood vessels are arranged in the form of a loop, which ends at the tip of each branchia; thus there is almost certainly some mixing of afferent and efferent blood. The afferent and efferent vessels within the branchiae are connected by capillary rings which lie perpendicularly to the axes of the afferent and efferent vessels (Figs. 11 & 12). This arrangement closely resembles that found in the flabelligerid polychaete *Flabelliderma commensalis* (Spies, 1973) although the circulating fluid in the latter contains chlorocruorin rather than haemoglobin.

OXYGEN CONSUMPTION

Fig. 13 shows the relationship between dry tissue weight and oxygen uptake in *T. haplochaeta*. The data were fitted to the equation $Y = aX^b$, where Y is oxygen uptake in ml · h $^{-1}$, X is dry wt in g, b is a slope of the plot of log oxygen uptake against log dry weight and a is a proportionality factor.

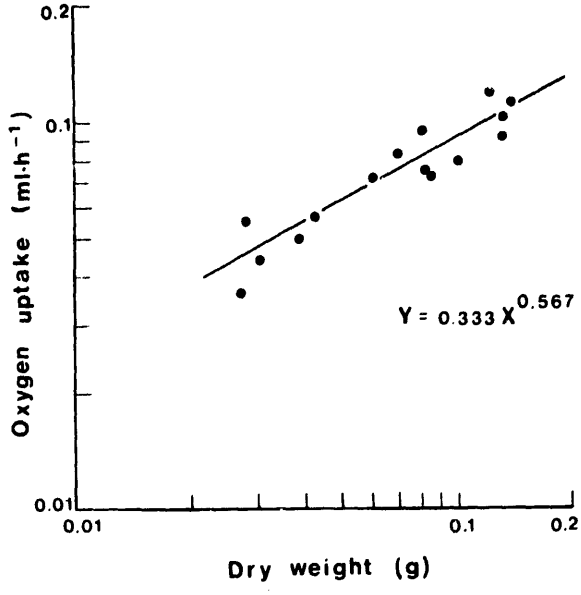


Fig. 13. Relationship between body size and oxygen uptake in *T. haplochaeta* at 20 °C: correlation coefficient r, fitted by least squares = 0.919.

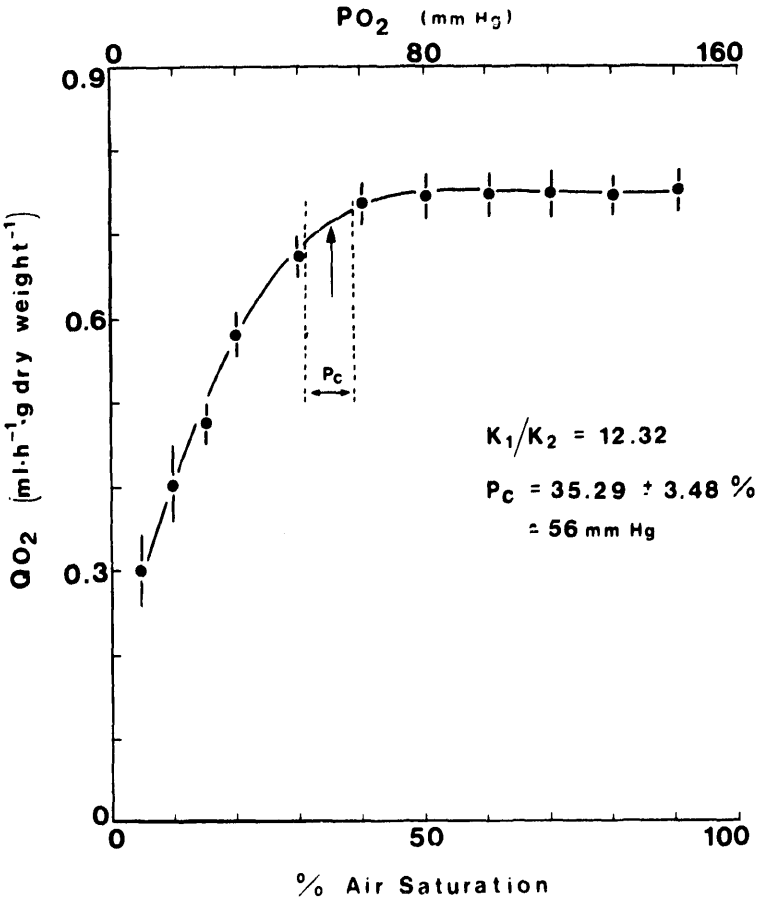


Fig. 14. Response of *T. haplochaeta* to declining oxygen tensions at 20 °C showing a zone of critical pressure (P_c) and the term K_1/K_2 quantifying the degree of oxyregulation (see text for further explanation): results plotted as mean ± SD for 10 worms of ≈0.1 g dry wt.

Fig. 14 shows the effect of declining oxygen tension on oxygen uptake in *T. haplochaeta*. It may be seen that between ≈35 and 100% air saturation the worms are oxygen-independent and that below 35% the animals become oxygen-dependent.

Discussion

When covered by the tide, *T. haplochaeta* exhibits a headward ventilatory stream similar to that seen in another terebellid, *Amphitrite ornata* (Mangum *et al.*, 1975), where peristaltic waves arise at the tail and move in a headward direction resulting in ventilation of the general body surface and gills. Thus the body surface receives oxygen at a high incurrent pO_2 and extracts some of this oxygen, allowing the anteriorly disposed gills to take oxygen from a lower tension in the burrow micro-environment.

Irrigatory behaviour has been shown to be influenced by the oxygen content of the water in several other species of polychaetes. Lindroth (1938) demonstrated that in *Neanthes* (= *Nereis*) *virens* irrigation increased with declining oxygen tensions

and was virtually continuous below ≈30% saturation. With a further decline in oxygen tension a point must eventually be reached at which the oxygen requirements for the energetic cost of irrigation itself are no longer met and there are then two possibilities for the animal: (i) activity may continue, the balance in demand for energy being made up by fermentation of glycogen or (ii) activity may cease, the worm becoming quiescent, so that the demand for oxygen is decreased. In *Neanthes* the rate of oxygen uptake is maintained down to the oxygen tension at which irrigation becomes continuous and below this point the rate of uptake declines along with activity. A similar response is seen in *Arenicola marina* (Wells, 1966) and since both species are intertidal, their respiratory behaviour may be adaptations to intermittent exposure. In addition, *Neanthes* and *Arenicola* may resort to aerial respiration by trapping bubbles of air within the burrows (Dales, 1969). Contrasting situations are seen in glycerid bloodworms which cease both ventilatory and circulatory movements under hypoxic conditions (Hoffman & Mangum, 1970), and in *Chaetopterus variopedatus* which becomes more active when the pO_2 falls below saturation and generates a vigorous ventilatory stream by the action of its broad, paddle-shaped gills (Dales, 1969). *Chaetopterus* is sublittoral and irrigation is prevented only if there is a burrow blockage.

Coyer & Mangum (1973) have shown that the frequency, duration, and amplitude of bursts of ventilation in the terebellid *Amphitrite ornata* change little with oxygen depletion. These ventilatory responses to reduced oxygen do not show close correlations within any particular polychaete family but are more likely to be related to differences in the animals' near environment.

An increase in the transport capacity of the blood in summer is consistent with the increased demand for oxygen at elevated acclimation temperatures which has been noted for a number of polychaetes (Mangum, 1978).

Although the capacity of the blood is high, the contents of the vessels occupy only 9% of the total body volume and are therefore unlikely to serve as a significant store of oxygen which might sustain the worm's respiration during phases of low tide. Unlike other terebellids (cf. Manwell, 1960; Garlick & Terwilliger, 1974; Terwilliger, 1974; Mangum *et al.*, 1975; Wells & Dales, 1975; Weber *et al.*, 1977), *Terebella haplochaeta* has no haemoglobin in its coelomic cells, the coelom occupying ≈28% of the body volume. Thus the coelomic store of oxygen cannot exceed the amount dissolved in physical solution. In this respect, our species closely resembles *Arenicola marina* for which Toulmond (1973) has documented variations in blood pO_2, pCO_2, pH, and oxygen saturation over the tidal cycle. He proposed that the vascular haemoglobin augments tissue oxygenation only during flood tide and is unable to function at low tide when the worm is abruptly cut off from its ventilated stream of oxygenated water.

The sigmoidal nature of the curves allows for the release of a large volume of bound oxygen for a comparatively small drop in pO_2. These properties of the haemoglobin-oxygen equilibrium are characteristic of most haemoglobins which function

in marine organisms inhabiting water of low ambient pCO_2 (reviewed by Mangum, 1976a; Weber, 1978b). Conversely, sigmoidal curves are unsuited for transport under declining oxygen tensions because oxygen is released over too narrow a range of pO_2. In addition, a Bohr effect confers no advantage while ambient carbon dioxide tensions are high. These conditions are likely to occur at low tide when oxygen transport is favoured by a hyperbolic curve and the absence of a Bohr effect (Mangum, 1976a; Weber, 1978b).

While determination of haemoglobin-oxygen equilibrium curves using carbon dioxide is undoubtedly a more accurate reflection of in ivo conditions than determinations in the presence of fixed acids, the influence of molecular carbon dioxide on the equilibrium (the so-called "carbamate" effect) cannot be distinguished from the Bohr effect which arises from the hydration reaction of carbon dioxide and results in a release of hydrogen ions. Although the carbamate effect has not been quantified in the present study, the extent of the Bohr shift was estimated using CO_2-free solutions of haemoglobin buffered to various pH values. Oxygen affinities were estimated by $P50$, which is the pO_2 at which half of the pigment is oxygenated. At pH 7.1 and 7.8 in 0.05 M Hepes buffers at 20 °C, the $P50$ decreased from 6.8 to 4.3 mm Hg. The Bohr coefficient ϕ, $=\Delta\log P50/\Delta pH$ was -0.28. We do not know the pH of the prebranchial blood in *T. haplochaeta* because the small size of these worms precludes such measurements. A pH range of 7.29–7.45 was, however, reported for prebranchial blood sampled from a variety of terebellids (Wells, 1974) and may be assumed to represent at least part of the physiological range.

The effect of pH on the shape of the equilibrium curve is similar to observations reported for the haemoglobins of several marine invertebrates (Weber, 1970, 1975, 1978b; Mangum, 1976a) but the interpretation of these findings does not suggest any clear adaptive significance. It is possible that the transition to a hyperbolic curve at low pH might conserve oxygen at low tide when carbon dioxide and the acidic end-products of glycolysis cannot easily be eliminated.

A low temperature sensitivity of the oxygen-binding reaction in *T. haplochaeta* (-21.6 kJ · mol^{-1}) may be considered as a homeostatic mechanism which secures stability in oxygen delivery over short term temperature fluctuations which might accompany a tidal cycle. It is interesting that a similar value of -28.5 was reported from the intertidal terebellid lugworms *Arenicola marina* (Weber, 1972) and *Abarenicola assimilis* (Chung & Ellerton, 1979). Greater temperature sensitivity has been shown for haemoglobins from the terebellids *Thelepus crispus* (Garlick & Terwilliger, 1974), *Terebella lapidaria* (Wells & Dales, 1975), and some arenicolids (Weber, 1972). Unfortunately it has not been possible to correlate these findings with temperature fluctuations occurring in the micro-environments inhabited by these organisms. It might be predicted that for intertidal invertebrates which are regularly exposed to sharp oscillations in temperature corresponding with the phase of the tide, the haemoglobin-equilibrium may be relatively insensitive to temperature. It is not immediately clear what benefit, if any, is conferred on the worm as the equilibrium

tends to a hyperbola at high temperatures. As with the pH effect on n, however, a hyperbolic curve may help to conserve oxygen when temperatures rise following exposure by the receding tide.

The enzyme carbonic anhydrase is found in various invertebrate tissues where it catalyses the reversible hydration of carbon dioxide (Maren, 1967). The presence of this enzyme is an important factor in determining whether carbon dioxide can be released with sufficient rapidity during the passage of blood through the tissues. The presence of carbonic anhydrase in the blood of the marine polychaetes *Arenicola marina* and *Terebella lapidaria* apparently aids the removal of metabolic carbon dioxide (Wells, 1973; Wells & Dales, 1975) and a similar rôle is postulated in *T. haplochaeta*.

The vascular system in annelids consists basically of a series of segmental vesseis joining two longitudinal vessels. running from one end of the body to the other; one in the mid-dorsal line which flows forward and one in the corresponding ventral position in which the blood flows in the opposite direction. The ventral vessel lies just below the gut and dorsal to the nerve cord, beneath which a smaller (subneural) vessel is sometimes found. The ventral vessel gives rise to capillaries which surround the gut (Dales, 1967). The vascular system of *T. haplochaeta* resembles the vascular system of the terebellid *Amphitrite johnstoni* (= *Neoamphitrite figulus*) where the dorsal vessel seems to have disappeared in the region of the stomach and intestine and is functionally replaced by the lateral or paired ventral or gastric vessels (Dales, 1967). These unite into an enormous dorsal vessel giving rise to afferent branchials anteriorly. Until recently, ultrastructural studies of polychaete gills were lacking. Storch & Alberti (1978) have shown that the polychaete branchiae are true respiratory organs and they differ in this respect from animals such as the fishes and crustaceans where these organs perform both respiratory and ion absorption functions (Welsch & Storch, 1976). According to these studies there is good reason to believe that in different species of polychaetes the branchiae differ in their importance for gas exchange. Thus in the spionid *Malacoceros fuliginosus*, the afferent and efferent blood vessels are closed and there is no mixing of the separate cores of flow.

In other cases, the coelomic space does not project into the gills, thus permitting the blood vessels to come into closer contact with the epidermis (Orrhage, 1964; Siewing, 1976). A further reduction of the diffusion barrier between blood and water occurs in the branchiae of the nereid *Dendronereides heteropoda* (Gravier, 1934) and, on the basis of ultrastructural studies, in the gills of *Terebellides stroemi* (Storch & Alberti, 1978). These cases bear great similarity to the arrangement of blood spaces in the gill of *Terebella haplochaeta*.

Dales (1961) has studied oxygen uptake in three species of terebellids (*Eupolymnia heterobranchia*, *Neoamphitrite robusta*, and *Thelepus crispus*) and reported "b" values ranging from 0.43–0.74. Thus the "b" value reported here of 0.567 is well within this range and is also within the range reported for a variety of other polychaetes (Shumway, 1979).

The proportionality factor (0.333 for *Terebella haplochaeta*) may be used to compare the level of oxygen consumption between species. Shumway (1979) compared the "*a*" values of 13 different species of polychaetes and found that the worms fell into two groups with respect to oxygen uptake, errant polychaetes having a respiratory rate ≈ 2.4 times higher than sedentary ones. These groupings were based on readings taken at, or corrected to, 10 °C assuming a Q_{10} of 2.5. Using this same Q_{10} to correct the value given here for *T. haplochaeta* gives a reading of 0.133 and this value is similar to those reported for other sedentary polychaetes (Shumway, 1979) and in keeping with the tube-dwelling life style of *T. haplochaeta*.

Shumway (1979) reported eight species of polychaetes to be almost perfect oxyconformers. Mangum (1970, *Glycera dibranchiata*), May (1972, *Abarenicola pacifica*), Ewer & Fox (1940, *Sabella pavonina*) have all reported oxygen dependence for polychaetes in declining oxygen tensions. At least partial independence has been shown in the polychaetes *Schizobranchia insignis* (Dales, 1961), *Hyalinoecia tubicola* (Dales *et al.*, 1970), and *Nereis virens* (Hyman, 1932). All of these animals were categorized as oxygen dependent or independent by visual inspection of the graphed data.

Bayne (1971) proposed the use of an oxygen dependence index, K_1/K_2, to express an animal's capability to regulate its rate of oxygen uptake during exposure to declining oxygen tensions. This index is derived from the linear form of the equation for a hyperbola:

$$Q_{O_2} = pO_2/(K_1 + K_2 pO_2),$$

where Q_{O_2} is the weight-specific oxygen uptake in $ml \cdot g^{-1} \cdot h^{-1}$, pO_2 is the oxygen tension in mm Hg, and K_1 and K_2 are the intercept and slope, respectively, of a plot of pO_2 against pO_2/Q_{O_2}. The values of K_1 and K_2 which give the best fit through the points in Fig. 14 define the degree of oxyregulation by the ratio K_1/K_2. As the ratio K_1/K_2 increases, the rate of oxygen consumption becomes more directly proportional to oxygen tension (oxygen dependence) and as the ratio decreases, the rate of oxygen consumption approaches a constant (oxygen independence).

TABLE IV

Calculated K_1/K_2 values for eight species of polychaetes corrected for an animal of 0.1 g dry wt: data taken from Shumway (1979); *N* refers to the number of observations; for further explanation see text.

Species	K_1/K_2	*N*
Abarenicola affinis	119.9	12
Aphrodite aculeata	112.3	9
Arenicola marina	111.8	5
Eulalia heterobranchia	186.7	8
Glycera americana	221.4	10
Nereis diversicolor	170.6	6
Nereis virens	191.3	11
Perenereis nuntia	190.3	8

The oxygen dependence index, K_1/K_2, for *Terebella haplochaeta* of ≈ 0.1 g dry wt was found to be 12.32 ($n = 10$). This is a relatively low value and indicates a high degree of oxygen independence. This index has not yet been calculated for any other polychaetes; however re-calculating the data from Shumway (1979) gives the values shown in Table IV for eight species, all of which show rather high values for K_1/K_2. Several workers have applied this method to data derived from bivalve molluscs (Bayne, 1971, 1973; Taylor & Brand, 1975) and have shown that there is a general trend for increasing oxygen dependence with decreasing likelihood of encountering low environmental oxygen tensions in the natural habitat. Thus, animals which regularly experience low oxygen tensions in their natural habitats show low values for K_1/K_2. *T. haplochaeta* does not fit the general pattern shown by other polychaetes in that it shows good capabilities of oxyregulation; it does, however, lend further support to Bayne's theory in that it has a low K_1/K_2 ratio and is known to encounter low pO_2 levels regularly in this natural environment.

Perhaps the most interesting point to emerge from the investigation of declining oxygen tension on oxygen uptake is the zone of critical pressure (P_c), or the point at which the animal ceases to be oxygen independent and becomes oxygen dependent. In *T. haplochaeta* this was found to be between 30 and 40% of air saturation (calculated mean from data on which Fig. 14 is based is $35.39 \pm 3.5\%$ saturation). It may be that P_c is detected only where there is an irrigatory possibility of Q_{O_2} regulation, and that when worms are deprived of their tubes during respiratory measurements, a wide range of oxygen dependence will result and obscure P_c. It has already been shown that at $\approx 30.6\%$ saturation the worms' haemoglobin is almost totally deoxygenated and that the number of ventilatory waves · min $^{-1}$ has decreased. It appears then, that at oxygen saturation levels $>30\%$ the combination of increased ventilation rate and high affinity haemoglobin provides the animals with enough oxygen to maintain a constant rate of oxygen uptake, whereas in the region of 30–40% saturation when ventilatory movements have decreased the worms are no longer able to obtain the oxygen supply needed to maintain a normal rate of oxygen uptake.

ACKNOWLEDGEMENTS

The authors acknowledge useful discussions with Professor R. P. Dales and Dr. I. D. Marsden. The project was financed by an equipment grant from the Auckland University Research Committee.

REFERENCES

ANTONINI, E. & M. BRUNORI, 1971. *Hemoglobin and myoglobin in their reaction with ligands.* North-Holland, Amsterdam. 436 pp.

BAYNE, B. L., 1971. Oxygen consumption by three species of lamellibranch mollusc in declining ambient oxygen tension. *Comp. Biochem. Physiol.*, Vol. 40A, pp. 955–970.

BAYNE, B. L., 1973. The responses of three species of bivalve mollusc to declining oxygen tension at reduced salinity. *Comp. Biochem. Physiol.*, Vol. 45A, pp. 793–806.

CHUNG, M. C. M. & H. D. ELLERTON, 1979. The physico-chemical and functional properties of extracellular respiratory haemoglobins and chlorocruorins. *Prog. Biophys. molec. Biol.*, Vol. 35, pp. 53–102.

COYER, P. E. & C. P. MANGUM, 1973. Effect of temperature on active and resting metabolism in polychaetes. In, *Effects of temperature on ectothermic organisms, ecological implications and mechanisms of compensation*, edited by W. Wieser, Springer-Verlag, Heidelberg, pp. 173–180.

CRISP, M., J. DAVENPORT & S. E. SHUMWAY, 1978. Effects of feeding and of chemical stimulation on the oxygen uptake of *Nassarius reticulatus* (Gastropoda: Prosobranchia). *J. mar. biol. Ass. U.K.*, Vol. 58, pp. 387–399.

DACIE, J. V. & S. M. LEWIS, 1975. *Practical haematology*. Churchill Livingstone, Edinburgh, 5th edition, 629 pp.

DALES, R. P., 1961. Oxygen uptake and irrigation of the burrow by three terebellid polychaetes: *Eupolymnia*, *Thelepus*, and *Neoamphitrite*. *Physiol. Zoöl.*, Vol. 34, pp. 306–311.

DALES, R. P., 1967. *Annelids*. Hutchinson, London, 200 pp.

DALES, R. P., 1969. Respiration and energy metabolism in annelids. In, *Chemical zoology, Vol. 4*, edited by M. Florkin & B. J. Scheer. Academic Press, New York, pp. 93–109.

DALES, R. P., C. P. MANGUM & J. C. TICHY, 1970. Effects of changes in oxygen and carbon dioxide concentrations on ventilation rhythms in onuphid polychaetes. *J. mar. biol. Ass. U.K.*, Vol. 50, pp. 365–380.

EWER, R. F. & H. M. FOX, 1940. On the function of chlorocruorin. *Proc. R. Soc. Ser. B*, Vol. 129, pp. 137–153.

GARLICK, R. L. & R. C. TERWILLIGER, 1974. Coelomic cell hemoglobin of the terebellid polychaete, *Thelepus crispus* Johnson. Structure and oxygen equilibrium. *Comp. Biochem. Physiol.*, Vol. 47B, pp. 543–553.

GRAVIER, C., 1934. Remarques sur la morphologie du parapode des Néréidiens. *Annls Sci. nat.*, Vol. 17, pp. 347–367.

HILL, A. V., 1910. The possible effects of aggregation of haemoglobin on its dissociation curve. *J. Physiol., Lond.*, Vol. 40, pp. iv–vii.

HOFFMAN, R. J. & C. P. MANGUM, 1970. The function of coelomic cell hemoglobin in the polychaete *Glycera dibranchiata*. *Comp. Biochem. Physiol.*, Vol. 36, pp. 211–228.

HUMASON, G. L., 1972. *Animal tissue techniques*. W. H. Freeman & Co., San Francisco, 641 pp.

HYMAN, L., 1932. Relation of oxygen tension to oxygen consumption in *Nereis virens*. *J. exp. Zool.*, Vol. 61, pp. 209–221.

LINDROTH, A., 1938. Studien über die respiratorischen mechanismen von *Nereis virens* (Sars). *Zool. Bidr. Upps.*, Vol. 17, pp. 367–497.

MANGUM, C. P., 1970. Respiratory physiology in annelids. *Am. Sci.*, Vol. 58, pp. 641–647.

MANGUM, C. P., 1976a. Primitive respiratory adaptations. In, *Adaptation to environment*, edited by R. C. Newell, Butterworths, London, pp. 191–278.

MANGUM, C. P., 1976b. The function of respiratory pigments in estuarine animals. *Estuarine processes, Vol. 1, Uses, stresses, and adaptations to the estuary*, edited by M. Wiley, Academic Press, New York, pp. 356–380.

MANGUM, C. P., 1976c. The oxygenation of hemoglobin in lugworms. *Physiol. Zöol.*, Vol. 49, pp. 85–99.

MANGUM, C. P., 1978. Temperature adaptation. In, *Physiology of annelids*, edited by P. J. Mill, Academic Press, London, pp. 447–478.

MANGUM, C. P. & W. VAN WINKLE, 1973. Responses of aquatic invertebrates to declining oxygen conditions. *Am. Zool.*, Vol. 13, pp. 529–541.

MANGUM, C. P., B. R. WOODIN, C. BONAVENTURA, B. SULLIVAN & J. BONAVENTURA, 1975. The role of coelomic and vascular hemoglobin in the annelid family Terebellidae. *Comp. Biochem. Physiol.*, Vol. 51A, pp. 281–294.

MANWELL, C., 1960. Histological specificity of respiratory pigments. I. Comparisons of the coelom and muscle hemoglobins of the polychaete worm *Travisia pupa* and the echiuroid worm *Arynchite pugettensis*. *Comp. Biochem. Physiol.*, Vol. 1, pp. 267–276.

MAREN, T. H., 1967. Carbonic anhydrase: chemistry, physiology and inhibition. *Physiol. Rev.*, Vol. 47, pp. 595–781.

MAY, D. R., 1972. The effects of oxygen concentration and anoxia on respiration of *Abarenicola*

pacifica and *Lumbrinereis zonata* (Polychaeta). *Biol. Bull. mar. biol. Lab., Woods Hole*, Vol. 142, pp. 71–83.

MENZIES, R. J., 1968. A small basin containing anoxic sediments. *Int. Rev. ges. Hydrobiol.*, Vol. 53, pp. 77–81.

NEWELL, R. C., 1973. Factors affecting the respiration of intertidal invertebrates. *Am. Zool.*, Vol. 13, pp. 513–528.

ORRHAGE, L., 1964. Anatomische und morphologische Studien über die Polychaetenfamilien Spionidae, Disomidae und Poecilochaetidae. *Zool. Bidr. Upps.*, Vol. 36, pp. 335–405.

ROSSI-FANELLI, A., E. ANTONINI & A. CAPUTO, 1964. Hemoglobin and myoglobin. *Adv. Protein Chem.*, Vol. 19, pp. 74–222.

SHUMWAY, S. E., 1979. The effects of body size, oxygen tension and mode of life on the oxygen uptake rates of polychaetes. *Comp. Biochem. Physiol.*, Vol. 64A, pp. 273–278.

SIEWING, R., 1976. Probleme und neuere Erkenntnisse in der Großsystematik der Wirbellosen. *Verh. dt. zool. Ges.*, Vol. 69, pp. 59–83.

SPIES, R. B., 1973. The blood system of the flabelligerid polychaete *Flabelliderma commensalis* (Moore). *J. Morph.*, Vol. 139, pp. 465–490.

STORCH, V. & G. ALBERTI, 1978. Ultrastructural observations on the gills of polychaetes. *Helgoländer wiss. Meeresunters.*, Vol. 31, pp. 169–179.

TAYLOR, A. C. & A. R. BRAND, 1975. The effects of hypoxia and body size on the oxygen consumption of the bivalve *Arctica islandica*. *J. exp. mar. Biol. Ecol.*, Vol. 19, pp. 187–196.

TERWILLIGER, R. C., 1974. Oxygen equilibria of the vascular and coelomic hemoglobins of the terebellid polychaete, *Pista pacifica*. Evidence for an oxygen transfer system. *Comp. Biochem. Physiol.*, Vol. 48A, pp. 745–755.

TOULMOND, A., 1973. Tide-related changes of blood respiratory variables in the lugworm *Arenicola marina* (L.). *Respir. Physiol.*, Vol. 19, pp. 130–144.

VENABLE, J. H. & R. COGGESHALL, 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.*, Vol. 25, pp. 407–413.

WEBER, R. E., 1970. Relations between functional and molecular properties of annelid haemoglobins. Interaction between haems in the haemoglobin of *Arenicola marina*. *Comp. Biochem. Physiol.*, Vol. 35, pp. 179–189.

WEBER, R. E., 1972. On the variation in oxygen-binding properties in haemoglobins of lugworms (Arenicolidae, Polychaeta). In, *Proc. 5th Europ. mar. Biol. Symp.*, edited by B. Battaglia, Piccin, Padova, pp. 231–243.

WEBER, R. E., 1975. Respiratory properties of haemoglobins from eunicid polychaetes. *J. Comp. Physiol.*, Vol. 99, pp. 297–307.

WEBER, R. E., 1978a. Respiration. In, *Physiology of annelids*, edited by P. J. Mill, Academic Press, London, pp. 369–392.

WEBER, R. E., 1978b. Respiratory pigments. In, *Physiology of annelids*, edited by P. J. Mill, Academic Press, London, pp. 393–446.

WEBER, R. E., C. P. MANGUM, H. STEINMAN, C. BONAVENTURA, B. SULLIVAN & J. BONAVENTURA, 1977. Hemoglobins of two terebellid polychaetes: *Enoplobranchus sanguineus* and *Amphitrite ornata*. *Comp. Biochem. Physiol.*, Vol. 56A, pp. 179–187.

WELLS, G. P., 1966. The lugworm (*Arenicola*) – a study in adaptation. *Neth. J. Sea Res.*, Vol. 3, pp. 294–313.

WELLS, R. M. G., 1973. Carbonic anhydrase activity in *Arenicola marina* (L.). *Comp. Biochem. Physiol.*, Vol. 46A, pp. 325–331.

WELLS, R. M. G., 1974. Hydrogen ion activity in polychaete body fluids. *Comp. Biochem. Physiol.*, Vol. 49A, pp. 567–574.

WELLS, R. M. G. & R. P. DALES, 1975. Haemoglobin function in *Terebella lapidaria* L., an intertidal terebellid polychaete. *J. mar. biol. Ass. U.K.*, Vol. 55, pp. 221–220.

WELLS, R. M. G. & R. P. DALES, 1976. Subunit organization in the respiratory proteins of the Polychaeta. *Comp. Biochem. Physiol.*, Vol. 54A, pp. 387–394.

WELSCH, U. & V. STORCH, 1976. *Comparative animal cytology and histology*. Sidgwick & Jackson, London, 343 pp.