THE RESPIRATORY FUNCTION OF THE HAEMOGLOBIN OF THE EARTHWORM[1]

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(With Two Text-figures)

It was claimed by Davy as early as 1862 that the haemoglobin in the blood of the earthworm has a respiratory function, but subsequent studies have given conflicting results. In the more recent investigations advantage has been taken of the fact that haemoglobin has a greater affinity for carbon monoxide than for oxygen; the oxygen consumption of normal worms is compared with that of worms whose haemoglobin has been put out of action by saturation with carbon monoxide. Using this method, Jordan & Schwarz (1920) concluded that haemoglobin functions as a transporter of oxygen only at low pressures of oxygen (23–30 mm. mercury).[2] Dolk & van der Paauw (1929) criticized this work on the grounds that the pure carbon monoxide to which the worms were subjected for some hours might have affected the respiratory enzymes as well as the haemoglobin, and that because of individual variation it was not possible to compare directly the oxygen consumption of normal and carbon monoxide-treated worms. They repeated the work with modifications, and again found that haemoglobin was functional as an oxygen transporter only at low pressures of oxygen, below 57 mm. mercury. Their work was done on narcotized animals, and was therefore criticized by Thomas (1935), who, moreover, considered that the conclusions drawn by Dolk & van der Paauw were not justified by the experimental data they published. Thomas reinvestigated the matter, and came to the conclusion that haemoglobin has no respiratory function at oxygen pressures lower than 114 mm., for the oxygen consumption of normal and carbon monoxide-treated worms was the same. Above 114 mm. oxygen pressure the oxygen uptake of normal worms rose sharply, while that of carbon monoxide-treated worms remained constant, but Thomas did not attribute this difference in behaviour to haemoglobin, and one is left with the impression that in the earthworm haemoglobin is functionless as an oxygen transporter at all pressures of oxygen. Thomas's results must, however, be accepted with reserve, for he gives data of experiments on only two normal worms and three carbon monoxide-treated worms.

[1] A preliminary account of these results has been published elsewhere (Fox, 1940).

[2] In the paper of Jordan & Schwarz, and in some other papers subsequently discussed, the amounts of oxygen in the respiratory medium are given as percentages, the gas mixtures being at atmospheric pressure. In order to make comparison with other results easier, I have in this paper expressed these percentages as pressures of oxygen in mm. of mercury.

In 1938 a brief report was published by Krüger of work on the function of earthworm haemoglobin. Krüger found that haemoglobin transports oxygen at atmospheric and also at lower partial pressures of oxygen; for the oxygen uptake of carbon monoxide-treated worms was lower than that of normal worms at pressures of 152, 114, 76 and 38 mm. Unfortunately in this report the experimental method used is not described and no detailed data are given (for instance, the actual rates of oxygen consumption are not stated), so it is impossible to evaluate the evidence on which Krüger's conclusion is based. The subject was thus left in an unsatisfactory and contradictory state, and, because of its bearing on our understanding of the function of blood pigments in general, it seemed necessary to reinvestigate it, using a rigorously controlled technique.

METHOD

In my experiments large specimens (2·5–5 g.) of *Lumbricus herculeus* Savigny (commonly known as *L. terrestris*) were used. They were kept in damp soil and darkness in a thermostat at 10° C. for at least 3 days, sometimes several weeks, before the experiments. The oxygen consumption of the worms was measured at atmospheric pressure in gas mixtures of oxygen and nitrogen containing 20, 10, 5, 2·5 and 1 % of oxygen, i.e. at oxygen partial pressures of about 152, 76, 38, 19 and 8 mm. mercury. In the carbon monoxide experiments carbon monoxide was added to the gas mixtures in amounts (Table 1) sufficient to saturate at least 90 % of the haemoglobin of the blood within half an hour. The oxygen consumption was measured in a Barcroft differential respirometer (Dixon, 1934) with modified respiration chambers. The respiration chambers were cup-shaped and had a side tube with a tap. The worms lay on a disk of perforated zinc which was supported by peg-like projections from the wall of the cup. Gas mixtures were passed in through the side tap, entering the chamber below the zinc grid; the gas passed around the worms and out through the tap at the end of the manometer. The gas mixtures were made up from cylinders of compressed gas, 2 l. at a time, with a gas burette. They were collected over water and were shaken with a little water before being passed over the worms. Two worms at a time were used in the respirometer.

The respirometer was kept during the experiments in a water thermostat at 10° C. The chambers were hooded in black velvet in order to prevent, in the carbon monoxide experiments, the dissociation of carboxyhaemoglobin by light; this procedure also tended to standardize conditions, for the worms are strongly affected by light.[1] At the end of the experiment, the worms, which were always lively and in good condition, were removed from the apparatus, quickly washed free of soil, dried gently with a cloth, and weighed.

Estimations were made of the amount of carboxyhaemoglobin present in the blood of carbon monoxide-treated worms at the end of the respiration experiments,

[1] In two experiments, the worms were first exposed to darkness and then to diffuse daylight; their rate of oxygen consumption rose by about 30 %. Davis & Slater (1928) found that the rate was doubled in bright light.

lean rates of oxygen consumption, etc. at 10° C. *at various oxygen and carbon monoxide pressures; P, pro*
the bracketed means are of samples of the same population

of ri- ts	Partial pressures of oxygen and carbon monoxide, mm. mercury		Rates of oxygen consumption c.mm. at N.T.P. per g. per hr.			Tests of signi of difference betw B_2 by analysis of
			1st hr.	2nd hr.		
	1st hr.	2nd hr.	Mean	Mean	P	Adjusted mean (log)
	152 O_2	152 O_2	A_1 45·2 ± 4·8	B_1 38·7 ± 4·3	0·1	1·2698 ± 0·0166
	152 O_2	152 O_2 + 76 CO	A_2 43·5 ± 4·0	B_2 29·6 ± 3·3		1·1565 ± 0·0177
	76 O_2	76 O_2	A_1 42·5 ± 2·1	B_1 35·2 ± 1·8	< 0·001	1·2650 ± 0·0133
	76 O_2	76 O_2 + 76 CO	A_2 46·2 ± 1·8	B_2 25·2 ± 1·7		1·0775 ± 0·0144
	38 O_2	38 O_2	A_1 25·0 ± 1·7	B_1 23·3 ± 1·3	< 0·001	1·2916 ± 0·0139
	38 O_2	38 O_2 + 38 CO	A_2 28·2 ± 0·8	B_2 15·7 ± 0·6		1·0705 ± 0·0158
	19 O_2	19 O_2	A_1 16·7 ± 1·3	B_1 15·4 ± 1·2	0·3	1·2521 ± 0·0158
	19 O_2	19 O_2 + 19 CO	A_2 17·8 ± 2·3	B_2 12·9 ± 3·0		1·1448 ± 0·0204
	8 O_2	8 O_2	A_1 7·2 ± 1·0	B_1 6·7 ± 0·8	0·8	1·1908 ± 0·0288
	8 O_2	8 O_2 + 19 CO	A_2 7·6 ± 0·8	B_2 7·0 ± 0·7		1·1980 ± 0·0177

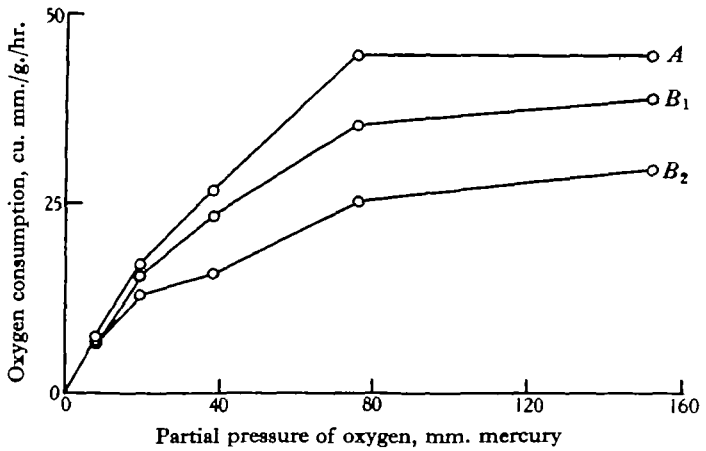

Fig. 1. Mean rate of oxygen consumption of earthworms at 10° C. at different oxygen pressures (experimental results). A during the first hour, in absence of carbon monoxide. B_1 during the second hour, in absence of carbon monoxide. B_2 during the second hour, in presence of carbon monoxide.

and also of worms exposed for only half an hour to the gas mixtures containing carbon monoxide which were used in experiments, since the measurements of respiration began after half an hour's exposure. In all cases at least 90 % of the haemoglobin was in the form of carboxyhaemoglobin.

The amount of carboxyhaemoglobin was estimated with a Hartridge reversion spectroscope. A calibration curve showing the relation between the spectroscope reading and the percentage of carboxyhaemoglobin in the blood had previously been made in the way described by Hartridge (1912, 1913) and Frederick (1931, 1937). For this purpose blood was extracted from several worms and diluted so that, when the blood was examined in the troughs with the spectroscope, the α-band appeared as broad as the space between the α- and β-bands. For the estimation of the amount of carbon monoxide in the blood of the experimental worms, the ordinary technique used for human blood, which involves dilution, was avoided because dilution (with air-saturated water) causes dissociation of earthworm carboxyhaemoglobin. That the relative affinity of earthworm haemoglobin for carbon monoxide and oxygen is less than that of human haemoglobin may be deduced from the fact that the 'span' of earthworm haemoglobin is short (Anson *et al.* 1924). The spectroscope was therefore fitted to a microscope and an undiluted drop of blood was examined with it. The blood, removed quickly with a pipette from the pseudo-hearts, was put on a slide with a coverslip which could be screwed up and down. In this way the thickness of the film of blood could be adjusted so that its absorption spectrum had the same intensity as that used for calibration.

The simplest method of assessing the importance of haemoglobin in oxygen consumption is to compare, in any one experiment, the oxygen consumption of worms, before, during, and after exposure to carbon monoxide. This was found to be impracticable, however, for two reasons. Firstly, it was found that when readings were taken continuously for some hours, the rate of oxygen uptake was not constant, but declined gradually. This fall in respiratory rate with time is presumably due to the gradual recovery of the worms from the disturbance caused by setting up the experiment, and is comparable with that found in fishes by Keys (1930). Secondly, when a worm had absorbed carbon monoxide, it retained the gas for some hours after it had been put into an atmosphere free of carbon monoxide. This method would therefore require that the worms should be left for many hours in the apparatus, but when this is done the worms become limp, and it is inadvisable to measure their respiration in this condition. An alternative method is to compare the mean rate of oxygen consumption of two lots of worms; one in the absence and one in the presence of carbon monoxide. The individual variation in rate of oxygen consumption among the worms is, however, so great that an excessively large number of experiments would have to be made to get a reliable result. A modification of this method was therefore used which allowed the comparison of normal and carbon monoxide-treated worms in quite similar conditions, and which, as described below, also has statistical advantages.

The worms were placed in the apparatus, and 1 l. of the required mixture of oxygen and nitrogen, which had first circulated through a coil of tubing in the

thermostat, was then passed through both chambers of the respirometer, the passage of the gas taking half an hour. Five minutes after the flow of gas had stopped, the taps were closed, and manometer readings were taken every 10 min. during 1 hr. Then another litre of gas was passed through, again taking half an hour. This second lot of gas was either identical with the first (in the control experiments), or it contained carbon monoxide. After an interval of 5 min. the taps were again closed and readings were taken every 10 min. for another hour.

RESULTS

The experimental results are given in Table 1 and Fig. 1, and from them the following conclusions can be drawn. Firstly, the rate of oxygen consumption during the first hour (i.e. when no carbon monoxide is present) is the same at 152 mm. as at 76 mm., while below 76 mm. it falls sharply. This is shown clearly by the values of A, the mean rates of oxygen consumption of all worms during the first hour, which are obtained from the means of A_1 and A_2 (Table 1) weighted for the number of experiments. The values of A are, in c.mm. of oxygen per g. per hr., $44{\cdot}4 \pm 3{\cdot}0$ at 152 mm. pressure, $44{\cdot}4 \pm 1{\cdot}4$ at 76 mm., $26{\cdot}4 \pm 1{\cdot}1$ at 38 mm., $17{\cdot}1 \pm 1{\cdot}2$ at 19 mm., and $7{\cdot}5 \pm 0{\cdot}6$ at 8 mm. (Fig. 1).

Secondly, as stated above, even without carbon monoxide the mean rate of oxygen uptake is lower in the second hour (B_1) than in the first hour (A_1).

Thirdly, Table 1 and Fig. 1 show that the mean respiratory rate in the second hour in the presence of carbon monoxide (B_2) is lower than that in its absence (B_1) at all oxygen partial pressures above 8 mm. The coefficients of variation of the rates of oxygen consumption are, however, high, and while the difference between the means in the absence (B_1) and presence (B_2) of carbon monoxide, as indicated by the probability P given by the t test (Fisher, 1938), are seen from the table to be significant at 76 and 38 mm. of oxygen, at the other pressures they are not significant.

The large variation in B_1 and B_2 is partly correlated with variation in initial oxygen consumption (i.e. in first hour rates) and with variation in weight of worms (the oxygen consumption per g. per hr. shows a significant negative correlation with the weight of the worms, r being $-0{\cdot}8$). Taking advantage of this it is possible to test the significance of the difference between the means in the absence and in the presence of carbon monoxide in a more refined way, by means of the technique of analysis of covariance. I am much indebted to Prof. R.A. Fisher and to Dr W.L. Stevens for pointing this out, and for indicating the procedure necessary. By applying this technique, the effect of presence or absence of carbon monoxide in the second-hour measurements can be estimated without the obscurity produced in the original data by the sources of variation referred to above. In making the analysis, the log oxygen consumption in the second hour is taken as the dependent variate, y, and the log oxygen consumption in the first hour and log weight as independent variates, x_1 and x_2 respectively. From the regression equation $y = a + b_1 x_1 + b_2 x_2$ an adjusted mean, the mean of a, is obtained for each set of

experiments, and this depends only on whether carbon monoxide is present or absent, and on oxygen pressure. These adjusted means (which are of course in the form of logarithms) are set out with their standard errors in Table 1. At each oxygen pressure the difference between the adjusted means of the carbon monoxide-treated and control sets are compared with the standard error of the difference. In all cases except at 8 mm. the differences prove highly significant, as indicated by the values of P given by the t test, shown in Table 1. The elimination of extraneous variability by this technique makes it clear, therefore, that haemoglobin functions as an oxygen transporter at 152, 76, 38 and 19 mm. pressure of oxygen, but not at 8 mm.

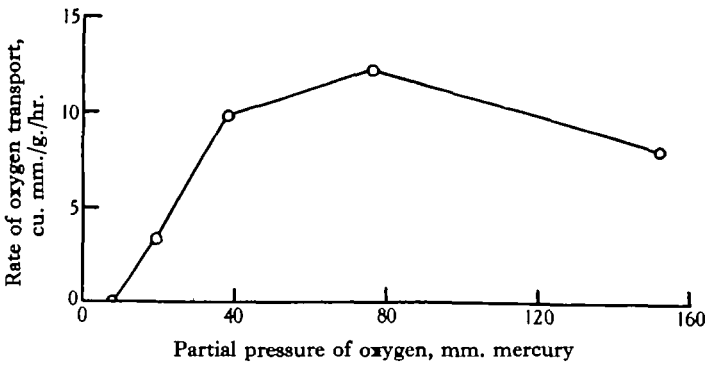

Fig. 2. Rate of oxygen transport by haemoglobin during the second hour at different oxygen pressures (10° C.).

An estimate can be made of the oxygen consumption in c.mm. per g. of worm per hr. which is mediated by haemoglobin at different oxygen pressures, and of the percentage this represents of the total oxygen consumption. An adequate estimate cannot be made directly from the original data because of the variation in initial oxygen consumption (first-hour rate) and in weight. A more reliable estimate can, however, be obtained by adding to the adjusted values used above for each experimental set the values of b_1x_1 and b_2x_2, taking for x_1 the mean log oxygen consumption in the first hour at the given oxygen pressure, and for x_2 the mean log weight of worms in all the experiments. The difference at any oxygen pressure between the sets treated with carbon monoxide and those not treated shows the amount of oxygen transported by haemoglobin at this pressure, when allowance has been made for difference in initial oxygen consumption and weight.

The values with approximate standard errors thus obtained for the oxygen consumption in c.mm. per g. of worm per hr. mediated by haemoglobin at the different oxygen pressures studied are as follows: $8{\cdot}0 \pm 1{\cdot}7$ at 152 mm., $12{\cdot}3 \pm 1{\cdot}3$ at 76 mm., $9{\cdot}8 \pm 0{\cdot}9$ at 38 mm., $3{\cdot}4 \pm 0{\cdot}8$ at 19 mm. and 0 at 8 mm. (Fig. 2). Below 38 mm., therefore, the effectiveness of the haemoglobin decreases rapidly. This can be explained only by assuming that the pigment is not completely saturated at the surface of the body at 19 mm. The loading pressure (pressure at which the pigment is 95 % saturated with oxygen) of earthworm haemoglobin must therefore

be higher than 19 mm. This is much higher than the figure (5 mm.) found for *Arenicola* haemoglobin by Barcroft & Barcroft (1924).

The percentage of the total oxygen consumed by the worm at a given pressure which is transported by haemoglobin can also be obtained from the estimates given above. These values and their approximate standard errors are $23 \pm 5{\cdot}0$ % at 152 mm., $35 \pm 3{\cdot}7$ % at 76 mm., $40 \pm 3{\cdot}8$ % at 38 mm., $22 \pm 5{\cdot}3$ % at 19 mm., and 0 at 8 mm. Krüger's figures are similar to these (27 % at 152 mm., 34 % at 114 mm., 36 % at 76 mm., and 31 % at 38 mm.).

Two qualifications should be made of these estimates of the respiratory significance of earthworm haemoglobin. In the first place it should be pointed out that these experiments were made on quiescent worms at 10° C., a comparatively low temperature. In conditions of higher metabolic rate the importance of haemoglobin as an oxygen transporter would presumably be greater, at least at the higher pressure of oxygen. Secondly, the amount of oxygen transported by haemoglobin as estimated by the carbon monoxide method will be a little too low, for, as is shown below, carbon monoxide increases slightly the rate of respiration of the tissues.

OXYGEN CONSUMPTION OF TISSUE SLICES

The above experiments have shown that the oxygen uptake of earthworms is lowered by the presence of carbon monoxide. Before ascribing this effect to the elimination of haemoglobin alone, it is necessary to determine whether carbon monoxide depresses also the activity of the respiratory enzymes, although the relative proportion of carbon monoxide to oxygen was low enough to make this improbable.

The oxygen consumption of slices of earthworm tissue was therefore measured in the presence and absence of carbon monoxide. The experiments were made at temperatures between 15·2 and 17·7° C. and at the highest partial pressure of oxygen (152 mm.) used in the experiments of whole worms, in order to examine the effect of carbon monoxide when the rate of oxygen consumption was high. The gas mixture containing carbon monoxide consisted of 20 % carbon monoxide, 20 % oxygen and 60 % nitrogen, and for the control experiments, a mixture of 20 % oxygen and 80 % nitrogen was used. Thus the relative proportion of carbon monoxide to oxygen was the same as that used for the experiments on the whole worms at 76, 38 and 19 mm. partial pressure of oxygen, and was twice as high as that used at 152 mm. pressure of oxygen.

The technique used was similar to that employed by Ewer & Fox (1940) for their experiments on tissue slices of *Sabella*. The oxygen consumption was measured in Barcroft differential respirometers the flasks of which were provided with side-taps so that a stream of gas could be passed over the tissues. The slices were put in the respiration flasks in 3 c.c. of frog's phosphate-Ringer solution at *p*H 7·4. This is a suitable medium for earthworm tissue, for Wu (1939) found that the activity of the earthworm gut continued for many hours when bathed in frog's Ringer solution.

Slices of earthworm tissue were prepared in the following manner. The body of the worm posterior to the clitellum was opened longitudinally and the gut contents washed out with frog's Ringer solution. The body wall, which is about 1 mm. thick, was cut into strips about 1 cm. long and not more than 1 mm. thick (see Ewer & Fox, 1940, for justification of the use of slices of this thickness).

Two respirometers were used in each experiment. The tissue in one apparatus was subjected to carbon monoxide, while that in the other was used as a control. Tissue from two worms was used in each experiment, and the tissue slices were thoroughly mixed and then divided into two lots, one of which was put in each respirometer. By using tissue slices of the same origin in both the carbon-monoxide experiment and the controls, the effect of individual variation was eliminated.

One litre of the gas mixture was passed through both flasks of the respirometers while the apparatus was being shaken in the thermostat. After the gas mixture has passed for ½ hr. the apparatus was left shaking for a further 10 min. to allow for temperature and pressure equilibration. The taps were then closed and the rate of respiration was measured. The rate of respiration was found to be constant for at least 1½ hr., after which it decreased slightly in some cases. At the end of the experiment the tissue slices were filtered off in a Gooch crucible, washed, dried at 107° C. for 24 hr. and weighed. The average dry weight of tissue used in each respirometer was 83 mg. The apparatus was shaken at a rate of 120 oscillations per min. with an excursion of 5 cm. An increase in the rate of shaking did not cause an increase in the rate of movement of the fluid in the manometer. The flasks of the respirometers were covered with black velvet to exclude light.

Table 2. *Oxygen consumption of tissue slices of earthworm in* 20 % *oxygen with and without* 20 % *carbon monoxide*

Exp. no.	Temp. (° C.)	Oxygen consumption c.mm. per g. (dry)* per hr.		Rate in presence of carbon monoxide as % of rate in absence
		Without carbon monoxide	With carbon monoxide	
1	15·2	328	432	132
2	15·6	462	502	109
3	15·9	579	676	117
4	15·9	630	728	116
5	16·1	499	587	118
6	16·3	707	723	102
7	16·4	584	649	111
8	16·4	629	667	106
9	16·7	700	715	102
10	16·7	914	944	103
11	16·9	622	620	100
12	16·9	630	662	105
13	17·1	551	663	120
14	17·2	609	654	107
15	17·5	697	881	126
16	17·7	966	863	89
				Mean 110·2 ± 2·7

* 0·106 g. (dry) of worm tissue is equivalent to 1 g. (wet) of whole worm (average of measurements of eight worms).

The results of the experiments are summarized in Table 2. Since in each experiment the tissue in the two respirometers was of the same origin the rate of respiration in the presence of carbon monoxide can be compared with that of its control in the absence of carbon monoxide. In fourteen of the sixteen experiments the rate in carbon monoxide is higher than that in its absence, the average rate in carbon monoxide being 110 % of that of the controls. This difference is significant ($t=3{\cdot}80$, $p=0{\cdot}001$). The increase in rate of respiration may be due either to the burning of carbon monoxide by the tissues, as has been found to be the case in muscle and other tissues of vertebrates (Fenn & Cobb, 1932) or to the stimulation by carbon monoxide of oxygen consumption, as in yeast (Stannard, 1936).

From these experiments on tissue slices it can be concluded that the lowered rate of oxygen consumption found in the worms in the presence of carbon monoxide is due to the elimination of haemoglobin as an oxygen carrier, and not to a depressive effect of the gas on the respiratory enzymes of the tissues.

DISCUSSION

The results of this work, which agree with that of Krüger but not with those of other workers, show conclusively that the haemoglobin of the earthworm transports oxygen not only at low oxygen pressures, but also in air. This does not correspond with the generally accepted view as to the functioning of invertebrate respiratory pigments.

Lankester (1872) pointed out that haemoglobin is found in those invertebrates which are particularly active (e.g. the actively burrowing *Solen legumen*) or in those subject to low oxygen pressures (*Planorbis*, *Chirocephalus*, *Chironomus*, *Tubifex*). In general (see reviews by Barcroft, 1925; Redfield, 1933) it has been thought that among invertebrates haemoglobin is not concerned in transporting oxygen at ordinary oxygen pressures, when enough oxygen is carried by the blood in physical solution, but becomes effective only when the oxygen pressure of the medium is low. It is also held that the pigment may act as a reservoir for oxygen when animals are subjected to a deficiency of oxygen, as in burrowing marine forms at low tide. The evidence supporting these two views is usually, however, of a very indirect nature, and sometimes one view is held only because the other cannot be substantiated.

Leitch (1916) stated that the haemoglobins of *Planorbis* and of *Chironomus* larvae do not act as stores of oxygen, but act as oxygen transporters when the oxygen pressure of the environment is for *Planorbis* below 53 mm., and for *Chironomus* below 7 mm. Since this work is often quoted as showing that the haemoglobin of these animals is used only at low pressures, it should be pointed out that this conclusion is hardly justified. Leitch examined the foot of *Planorbis* with the spectroscope and found that the haemoglobin is always saturated with oxygen when the pressure of oxygen in the environment is above 58 mm., but is reduced when the pressure is below 55 mm., from which she concluded that the haemoglobin is not used at pressures higher than 58 mm. With this technique, in which the estimation of the amount of reduced haemoglobin depends on the fading of the

α- and β-bands of oxyhaemoglobin, it would be extremely difficult to be sure when no reduced haemoglobin was present; and indeed in other experiments on the blood in vitro (that is, in easier experimental conditions) Leitch states that estimations of the amount of oxyhaemoglobin present were accurate only up to 70 %. The possibility that the haemoglobin was functioning by giving up a proportion of its oxygen at pressures above 58 mm. is therefore not ruled out. In the case of *Chironomus* Leitch examined the whole animal and found that at 7 mm. oxygen pressure the blood was little if at all reduced, while at 5 mm. it was only 60 % saturated. She concluded that the pigment is used in oxygen transport at pressures below 6 mm., but not above 7 mm. Here again, however, the technique used is not suitable for establishing the fact that the pigment is not used at higher pressures.

Barcroft & Barcroft (1924) found that the haemoglobin of *Arenicola* has a very high affinity for oxygen, its unloading pressure (pressure of oxygen at which haemoglobin is 50 % saturated) being about 2 mm., and they suggested that its function was not to carry oxygen but to act as an oxygen store when the worm was subjected to oxygen deficiency. They found that the oxygen content of the blood was enough to last the worm about an hour—this period being less than, but of the same order as, the period during which the worms are closed in their tubes at low water. Borden (1931) confirmed this. She also found that in *Planorbis* the blood would hold enough oxygen to last the animal 25 min.; she considered that the oxygenated pigments were not unimportant as reserves of oxygen. From the low pressure at which the pigments dissociate (*Planorbis* 1–10 mm. oxygen, Barcroft, 1928; *Arenicola* 1–3 mm., Barcroft & Barcroft, 1924), she argued that the haemoglobin acts as an oxygen carrier at low pressures of oxygen, but this was not demonstrated experimentally.

Redfield & Florkin (1931) found that the unloading pressure of the haemoglobin of the gephyrean worm *Urechis* is 12 mm. They decided that the oxygen bound to haemoglobin is not utilized under ordinary conditions. This conclusion is difficult to reconcile with their finding that the haemoglobin is not completely saturated in vivo, though it is saturated when in equilibrium with air. They concluded that haemoglobin acts as an oxygen transporter when the oxygen content of the worm's burrow is reduced during low tide and as an oxygen store when respiratory movements are suspended.

The possibility that the pigments serve a respiratory function at atmospheric pressures of oxygen is therefore not excluded by the results of any of this work. In the case of *Chironomus* both indirect and direct methods have been used to determine the oxygen pressure at which haemoglobin became effective as an oxygen carrier. It is significant that the indirect method (Leitch) gave a much lower result than the direct (Harnisch, Ewer, see below).

Evidence of a direct nature as to the respiratory function of blood pigments is obtained by comparing the oxygen uptake of normal animals with those whose haemoglobin is rendered functionless as an oxygen transporter by saturation with carbon monoxide. The work of Jordan & Schwarz (1920), Dolk & van der Paauw (1929) and Thomas (1935) on the earthworm has been discussed above; the results

indicate that the haemoglobin does not carry oxygen at atmospheric partial pressures of oxygen. Harnisch (1936) found the same in *Tubifex*, but his work is open to criticism and his results do not agree with those of Dausend (1931). Harnisch also worked on *Chironomus* larvae and found that their oxygen consumption is not affected by carbon monoxide in air-saturated water but is lowered at oxygen pressures of 84 mm. and below. This work also is open to serious criticism, but Mrs R. F. Ewer (1941) in this Department, using better methods, has obtained a similar result.

Other work of this direct nature has shown that invertebrate respiratory pigments function as oxygen transporters even at atmospheric pressures of oxygen. Jürgens (1935) claimed that this was so in *Nereis*, but his experimental evidence is weak, for he worked with narcotized animals subjected to pure carbon monoxide. Dausend (1931) found that in *Tubifex* the oxygen uptake of worms treated with carbon monoxide is lower than that of normal worms even in air-saturated water. Ewer & Fox (1940) found the same in *Sabella*, whose blood contains chlorocruorin. Krüger (1938), as discussed above, found, as I did, that the oxygen consumption of worms treated with carbon monoxide is lower than that of normal worms even at atmospheric partial pressure of oxygen.

In the case of *Sabella* (Ewer & Fox, 1940), the fact that chlorocruorin functions at air pressure was to be anticipated from our knowledge of its relatively high loading pressure (Fox, 1932). The prevalent opinion that haemoglobin in the invertebrates cannot be effective in oxygen transport at anything but very low oxygen pressures may be attributed to the low loading and unloading pressures which have been found for the pigments (see Redfield, 1933). It should be pointed out, however, that for a pigment to be effective it must be loaded with oxygen at the respiratory surface and unloaded at the respiring surface; whatever its loading pressure, the pigment will act as an oxygen transporter if the respiring tissues are at an oxygen pressure lower than the loading pressure, so that the oxygenated pigment becomes, at least partially, unloaded. That the oxygen pressures inside the body of invertebrates may be quite low is shown by the work of Adler (1918), who found that the coelomic fluid of an earthworm in air had an oxygen pressure of 14 mm. The most efficient pigment for an animal which is subjected to oxygen deficiency of the environment will be one whose loading pressure is low, so that, unlike a pigment with a high loading pressure, it will be loaded at the respiratory surface even when the oxygen pressure of the medium is low.

In conclusion is may be said that, except in *Chironomus*, there is no firm evidence that invertebrate haemoglobin does not transport oxygen at air partial pressure of oxygen. Further investigations of the oxygen consumption of animals treated with carbon monoxide may show that their respiratory pigments are of value not only at low oxygen pressures but also at atmospheric partial pressure, as in *Tubifex*, *Sabella* and *Lumbricus*.

SUMMARY

The oxygen consumption of earthworms (*Lumbricus herculeus* Savigny) has been measured at 10° C., in the dark, in atmospheres containing 20, 10, 5, 2·5 and 1 % of oxygen (i.e. at partial pressures of oxygen of about 152, 76, 38, 19 and 8 mm.

mercury), with and without the addition of enough carbon monoxide to saturate the haemoglobin of the blood.

In the absence of carbon monoxide the rate of oxygen consumption was significantly the same at 152 and 76 mm.; below 76 mm. it fell sharply.

The rate of oxygen consumption of carbon monoxide-treated worms was significantly lower than that of normal worms at oxygen pressures of 152, 76, 38 and 19 mm. but not at 8 mm.

The respiration of slices of earthworm has been measured in atmospheres containing 20 % of oxygen, and 20 % of oxygen together with 20 % of carbon monoxide. The rate of respiration in the presence of carbon monoxide was 110 % of that in its absence. It is concluded that the lowering of the rate of respiration of whole worms caused by carbon monoxide was not due to inhibition of respiratory enzymes, but to its effect on haemoglobin. Haemoglobin therefore transports oxygen at atmospheric as well as at lower partial pressures of oxygen.

Less oxygen was carried by haemoglobin at 19 mm. than at 38 mm. It is deduced that the loading pressure of earthworm haemoglobin is higher than 19 mm.

The haemoglobin of the blood was responsible for supplying about 23 % of the respired oxygen when the oxygen pressure was at 152 mm., 35 % at 76 mm., 40 % at 38 mm., and 22 % at 19 mm. of oxygen.

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