CIRCULATION IN A GIANT EARTHWORM, *GLOSSOSCOLEX GIGANTEUS*

II. RESPIRATORY PROPERTIES OF THE BLOOD AND SOME PATTERNS OF GAS EXCHANGE*

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INTRODUCTION

The giant earthworms represent the largest living terrestrial animals without specialized respiratory organs known to us. Their exclusive dependence on the skin for gas exchange suggests that work on gas transport mechanisms will yield interesting results.

In a previous paper (Johansen & Martin, 1965), the dynamics of circulation in the larger blood vessels and lateral hearts of *Glossoscolex giganteus* was described. The present paper is concerned with respiratory properties of the blood, combined with blood-gas analysis of anaerobically obtained blood samples from freely moving animals. Vasomotor responses of the cutaneous vessels to some drugs and to different external media have been analysed for the effects on gas transport.

MATERIAL

The earthworm, *Glossoscolex giganteus*, is one of the largest of all living earthworms, attaining lengths in excess of 1 m. and weights above 500 g. The species inhabits tropical South America along the eastern part of Brazil from Minas Gerais in the North to Rio Grande do Sul in the South. The animals used in the present study were collected in the vicinity of São Paulo. They were kept in moist soil at 15–20° C. for not longer than 2 weeks before use. A total of forty specimens was used in the experimental work.

METHODS

An essential feature of the experiments was to draw blood samples and study intravascular pressures in non-anaesthetized animals under conditions as close to normal as possible. The experimental procedure included chronic cannulation of blood vessels with indwelling polyethylene catheters. The methods used in inducing anaesthesia and implanting catheters have been described earlier (Johansen & Martin, 1965). The catheters allowed intravascular pressures to be monitored continuously as well as

providing access for anaerobic blood sampling and injection of drugs. The animals were returned to moist soil or studied on a moist surface in contact with open air for periods of up to 6 days. At the end of an experiment the animal was exsanguinated and the blood analysed for haemoglobin content and oxygen capacity. Oxyhaemoglobin dissociation curves were obtained from some animals which yielded an abundant supply of blood. The blood-gas analyses were carried out according to the methods of Roughton & Scholander (1943) and of Scholander & van Dam (1956). Points on the dissociation curve were obtained after equilibrating the blood for at least 20 min. in a tonometer. Oxygen tensions were analysed on a Beckman Spinco 160 polarographic oxygen analyser. Haemoglobin content was measured according to the acid haematin method (Hawk, Oser & Summerson, 1947) and by the direct analysis of iron content after Wong (1928). The measurements of blood pressure used to evaluate vasomotor phenomena were made with Statham pressure transducers and an Offner dynograph. The drugs were injected in a small volume using earthworm Ringer solution for dilution (Lockwood, 1961).

<div align="center">RESULTS</div>

<div align="center">*Oxyhaemoglobin content and dissociation*</div>

The oxygen capacity of fifteen animals averaged 14·0 vol. %, ranging from 10·2 to 19·8 vol. %. Analyses of iron content of the bloods yielded values ranging from 23 to 41 mg. % with a mean of 30 mg. %. In Fig. 1 there is demonstrated the relationship of haemoglobin content, expressed as mg. % iron, to the oxygen capacity in volume %.

An oxyhaemoglobin dissociation curve for undiluted earthworm blood is presented in Fig. 2. The values were determined at 20° C. and at pH values between 7·50 and 7·58. The t_{50} value is close to 7·0 mm. Hg and the t_{100} between 12 and 14 mm. Hg. The curve shows a conspicuously sigmoid shape. Lowering of the pH to 6·5 had only minor influence on the t_{50} value.

<div align="center">*Oxygen content of circulating blood*</div>

Oxyhaemoglobin dissociation curves have only limited application in the evaluation of gas exchange unless information about blood gas levels in normally circulating blood is available. The blood in the anterior portions of the dorsal and in the ventral blood vessels represents that being provided to the metabolizing tissues in the earthworm. Negligible differences in oxygen content between these vessels are to be expected since no respiratory exchange vessels or metabolizing tissues are interposed between them. Because cannulating procedures were much easier in the dorsal vessel all anaerobic blood samples were obtained from this vessel. The oxygen content in blood samples from the dorsal vessel varied greatly, ranging from 0·7 to 9·8 vol. % in animals completely recovered from anaesthesia and left in air on a moist surface. The corresponding values for saturation with oxygen ranged from 5 to 73%, with an average of 41% (Table 1).

A number of stimuli exerted a profound influence on the oxygen content of the blood in the dorsal vessel. Most notable was a dramatic increase when an animal was buried in shallow moist soil. In one animal the oxygen saturation increased from 46% up to 77% after the body established contact with the soil. More

control over conditions could be gained by varying the composition of the air to which the animals were exposed. An elevated external carbon dioxide level (two experiments only) was followed by a drop in the oxygen content. Exposure to low oxygen pressures or to pure nitrogen caused a rapid but reversible drop in oxygen content. One specimen which yielded an oxygen content of 6·5 vol. % while
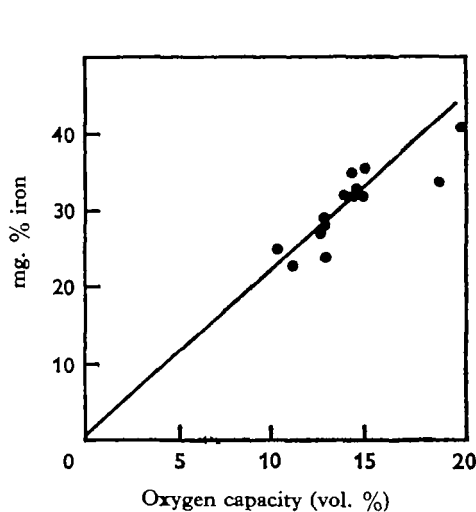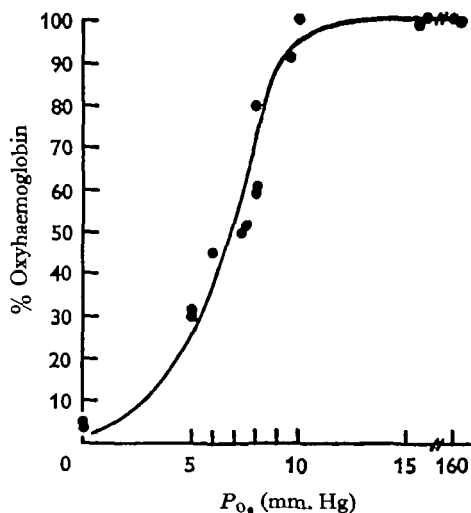

Fig. 1.   Fig. 2.

Fig. 1. Relationship of haemoglobin content expressed as mg % iron to the oxygen capacity in vol. % for *Glossoscolex giganteus*.

Fig. 2. Oxyhaemoglobin dissociation curve at 20° and pH 7·8, for *Glossoscolex giganteus*.

Table 1. *Oxygen content and saturation in dorsal vessel blood from free-moving earthworms when resting in moist air, lightly buried in moist soil or following drug injections*

	Resting in most air		In moist soil		Adrenalin injection		Acetylcholine injection	
Animal	O_2 cont. (vol. %)	% O_2 sat.	O_2 cont. (vol. %)	% O_2 sat.	O_2 cont. (vol. %)	% O_2 sat.	O_2 cont. (vol. %)	% O_2 sat.
1	3·0	28	—	—	—	—	—	—
2	6·5	48	9·8	73	—	—	—	—
3	9·8	73	—	—	—	—	—	—
4	5·2	35	—	—	—	—	—	—
5	7·1	52	—	—	3·4	25	—	—
6	8·8	57	—	—	2·9	19	10·2	6
7	1·9	10	—	—	—	—	—	—
8	3·9	25	—	—	—	—	—	—
9	0·7	5	—	—	0·7	5	—	—
10	5·0	37	—	—	1·1	8	—	—
11	5·8	46	9·8	77	3·9	30	—	—
12	5·8	41	9·1	65	—	—	—	—
13	4·0	40	8·2	82	—	—	—	—
14	6·5	51	—	—	3·1	24	—	—
15	6·5	46	—	—	0·6	4	—	—
16	8·1	57	—	—	2·7	19	5·7	40
Mean	5·5	41	9·2	74	2·3	17	7·9	23

resting on a moist surface in air was transferred to an environment of pure nitrogen. Within 10 min. the oxygen content dropped to 1·7 vol. %. Transferred back to air the blood reached the original level again within 10 min.

Intravascular injections of 2–5 µg. of adrenalin resulted in a marked decrease in the circulating oxygen levels. Visual observation showed that not only did the cutaneous vessels constrict in response to the drug but even the dorsal blood vessel reduced its diameter. Fig. 3 illustrates the increase in blood pressure in the dorsal blood vessel that follows such an adrenalin injection. Acetylcholine gave no consistent response when injected in small doses (less than 5 µg.) into the dorsal vessel.
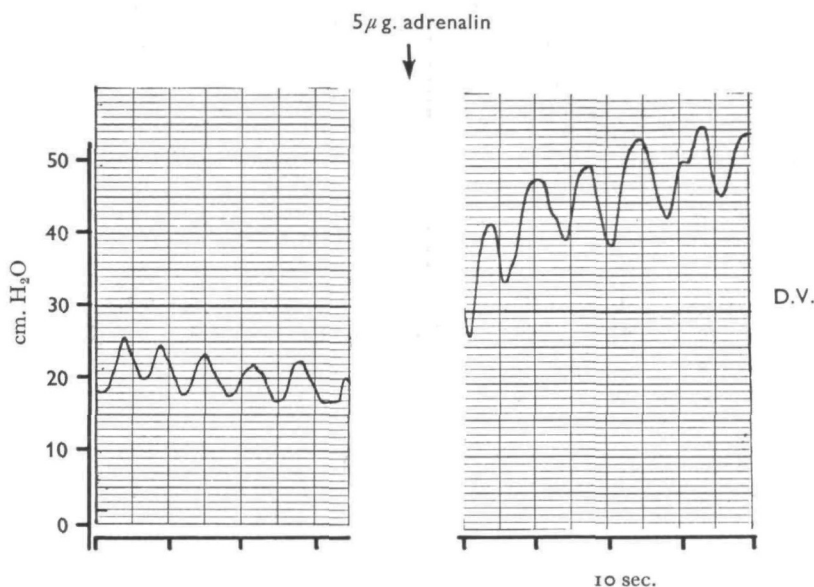

Fig. 3. Blood pressure increase in the dorsal vessel of *Glossoscolex* in response to adrenalin injection.

DISCUSSION

The haemoglobin levels, and consequently the oxygen capacities, that have been reported for invertebrate animals have been uniformly low, in general less than ten times the capacity of the blood without pigment (Prosser, 1962). The generally low oxygen capacities of invertebrate bloods have been related to low metabolic oxygen requirements and to physico-chemical limitations of the freely dispersed haemoglobin. The latter feature has led to some generalizations which are severely challenged by the present observations. The high molecular weight of a haemoglobin in solution will obviate the danger of too rapid an excretion by filtration of the pigment (Jones, 1963), but a high pigment concentration will result in a high blood viscosity. The low levels previously reported have therefore been thought to represent physiological limits. The average of 14 vol. % for oxygen capacity encountered in the present work considerably exceeds the previous reports.

A haemoglobin of low molecular weight in free solution would have the disadvantage of elevating the colloid osmotic pressure of the blood in high concentrations and

perhaps be incompatible with adequate water balance (Florkin, 1949). The commonly accepted solution to the problem of obtaining proper water balance and optimal buffering ability has been the development of red blood corpuscles, particularly in the vertebrate animals. The present results indicate that oxygen capacities exceeding those of most poikilothermic vertebrates may be attained with a haemoglobin of high molecular weight in solution in the blood.

The dissociation curve of the oxyhaemoglobin of *Glossoscolex giganteus* is similar to those reported for other earthworms. Haughton, Kerkut & Munday (1958) found a t_{50} value of 8 mm. Hg for *Lumbricus terrestris* at 20° C. and pH 7·3; and a t_{50} of only 6 mm. at 20° C. for *Allolobophora terrestris*. The curves were shifted significantly to the right with increasing temperature. Manwell (1959) obtained similar values for *Lumbricus terrestris*, and also demonstrated a moderate Bohr effect. The unusually high oxygen affinities of invertebrate haemoglobins have no parallels among the vertebrates.

Dissociation curves represent only part of the information necessary to evaluate gas exchange and the role of a respiratory pigment. Of equal importance is experimental evidence of the blood-gas content in the perfusion paths to and from the respiratory organs and the metabolizing tissues. Such measurements are strikingly few for invertebrate animals excepting for the larger haemocyanin-containing molluscs. Nevertheless attempts to explain the role of haemoglobin in actual gas transport are still based mainly on the *in vitro* dissociation curves and have affected the teaching of the subject. The present work provides directly and repetitively obtained *in vivo* blood-gas measurements for a large annelid possessing a high-affinity pigment.

Considerable discussion has centred around the role of high-affinity invertebrate haemoglobins in gas transport. A time-honoured view has held that the bound oxygen is present primarily as a reserve becoming useful only when full loading has ceased or in times of severe shortage of O_2 in the environment (Jordan & Schwarz, 1920; Dolk & van der Paauw, 1929; Fox, 1940). Another view is that large diffusion barriers exist between the external medium and the vascular channels for gas exchange, particularly in the cases where specialized respiratory organs are lacking. A further implication based on this presumption is that a selection pressure, against high internal oxygen tensions and development of specialized respiratory organs, is linked with a susceptibility to oxygen poisoning (Manwell, 1959). It has been suggested that such conditions may in some cases have favoured the development of high-affinity pigments (Jones, 1963).

The role of high-affinity respiratory pigments in invertebrates has often been evaluated by inactivation of the pigment with carbon monoxide, while following the oxygen consumption. Results from such experiments differ widely. The papers of Jordan and Schwarz (1920), Dolk & van der Paauw (1929) and Thomas (1935) on earthworms conclude that haemoglobin does not contribute to gas transport at atmospheric partial pressure of oxygen, though it might in varying degrees at lower partial pressures. Johnson (1942), however, maintains that haemoglobin does contribute to oxygen transport in *Lumbricus* all the way from 150 mm. Hg to 8 mm. Hg in oxygen pressure.

The data reported here, although more complete in an analytical sense than most earlier work, need to be confirmed and extended before a complete evaluation of

respiratory pigment function in haemoglobin-containing earthworms can be attempted. But the data make it possible to point out some simple facts which have been neglected or disregarded entirely in earlier treatments. In earthworms the blood returning from the regions of gas exchange with the atmosphere is largely mixed with blood returning from vascular circuits serving strictly nutritive purposes before it is redistributed to the metabolizing tissues via the ventral vessel. The important consequence is that the arterialized blood returning from the skin is brought down in oxygen
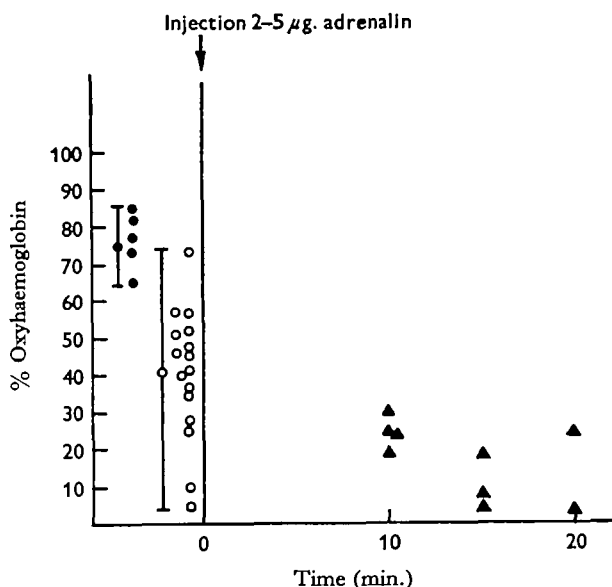

Fig. 4. Levels of oxygen saturation in dorsal vessel blood of *Glossoscolex* when resting in moist air (O), buried in shallow moist soil (●) or subsequent to adrenalin injections.

tension and content before it is offered to the respiring tissues. In the case of *Glossoscolex* the blood in the dorsal vessel, later to become tissue afferent blood, has an oxygen content of about 75% of saturation under experimental conditions as close to normal as possible. An almost complete turnover of oxygen can now be accomplished within an extremely narrow range of P_{O_2}. The high oxygen turnover is further promoted by the unusually sharp sigmoid shape of the oxygen dissociation curve; a feature that seems essential to retain enough driving pressure for the exchange of oxygen with the tissues in a high-affinity system.

A closely similar situation seems to exist in the polychaete *Eunice sebastiensis* (Johansen & Petersen, 1966) and is likely to be present in many other invertebrates. The speculation seems warranted that the apparent structural deficiency in the vascular arrangement of annelids, allowing for extensive mixing of arterialized and oxygen-depleted blood, is compensated for in the transport function of the haemoglobin. The exceptionally high oxygen affinities displayed by invertebrate haemoglobins may have developed in response to this condition.

Gas exchange based on the skin acting as the respiratory surface will inevitably depend on the underlying circulation. The present study offers suggestive evidence of

a very delicate control of blood flow through the skin. Figs. 3 and 4 and Table 1 attest to a striking effect of adrenaline on the skin circulation. A reduction in the oxygen saturation of the blood in the dorsal vessel from 74 to 17% following a small dose of adrenalin very probably reflects a general vasoconstriction of the cutaneous vessels which greatly diminishes blood flow. Mendes & Nonato (1957) applied adrenalin (10^{-1} g./l.) to the skin of *Pontoscolex*, *Pheretima* and *Glossoscolex* and noted an increase in the rate of oxygen consumption. The possibility was advanced that the response was due to dilatation of the skin capillaries. The present data does not seem to support such a view, and it may be suggested that adrenalin may have a direct stimulatory effect on tissue metabolism in the earthworm similar to that described for other animals (Cottle & Carlson, 1956).

A most striking indication of a vasoactive adjustment in these experiments was the marked increase in the oxygen saturation of the blood when the animal made contact with moist soil. If this is evoked by tactile stimuli it would seem to be a most advantageous regulatory device in earthworms. From the observations reported here it is not possible to evaluate separately possible effects of increased or decreased metabolic rate in comparison to cutaneous vasodilatation and light conditions, and these aspects of respiratory physiology remain to be explored.

SUMMARY

1. Oxyhaemoglobin dissociation curves have been obtained for *Glossoscolex giganteus*. The t_{50} value was 7 mm. Hg. at 20° C. and pH 7·58. There was no significant Bohr effect.

2. Oxygen capacity averaged 14·0 vol. % for fifteen animals. The corresponding value for haemoglobin expressed as iron content was 30 mg. %.

3. Blood was sampled anaerobically from the dorsal vessel on non-anaesthetized freely moving specimens and analysed for O_2 and CO_2 content. Blood-gas levels were studied in animals placed on moist surfaces or buried in shallow moist soil. The effects of drug injections and changes in the composition of the external gases were measured.

4. Patterns of gas exchange and the role of haemoglobin in gas transport in the earthworm are discussed.

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