

THE RESPIRATORY METABOLISM OF TROPICAL EARTHWORMS

I. The respiratory rate and the action of carbon monoxide at normal oxygen pressure

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(1 figure)

Introduction

This is the first report of a series of studies on the respiration and metabolism of Brazilian (or tropical) earthworms. These studies seemed to us interesting from both the ecological and the physiological points of view, since (a) there is little information about the respiratory metabolism of tropical earthworms, especially with regard to the influences of environmental factors; (b) an analysis of the comparative respiratory rates of our common earthworms (the so-called "minhocas", *Pheretima* and *Pontoscolex*) and the much larger forms (the so-called "minhocussus", *Glossoscolex*) might prove valuable to the question of the size factor in the respiration of Invertebrates; (c) a study of the function of hæmoglobin in terrestrial Oligochetes is still wanted due to contradictory results (JOHNSON 1942; MENDES, PÉREZ GONZÁLEZ & COUTINHO 1951).

The present work deals with the determination of the normal oxygen uptake of individuals just removed from the earth or after a 24 hour stay in moist chamber, in the absence or in the presence of carbon monoxide in sufficient quantity to saturate the blood hæmoglobin.

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Material and methods

The specimens of *Pheretima* and *Pontoscolex* were collected in the Faculty garden, those of *Glossoscolex* came from the banks of the Piracicaba river, near Piracicaba. In the laboratory, *Pheretima* and *Pontoscolex* were immediately used or stayed before use 24 hours in the dark, in moist chambers made of Petri dishes lined with moistened filter paper. *Glossoscolex* specimens were kept in a large terrarium. From such an environ-

ment came the individuals marked in the tables I and II a "just collected". Other individuals were submitted, as *Pheretima* and *Pontoscolex*, to a 24 hour stay in the moist chambers. Unfortunately, due to the fact that until now we could not obtain individuals in a sexually mature condition, the specimens of *Pontoscolex* and *Glossoscolex* are here referred to as simply *Pontoscolex sp.* and *Glossoscolex sp.* As to *Pheretima*, previous classification assured us the species to be *Pheretima hawayana*. This is the commonest species found in our gardens and used in our classes of physiology. *Pontoscolex* is smaller, darker and quite distinct in color from *Pheretima*. Adult *Pheretima* has an average length of 10 cm; adult *Pontoscolex* about the half of that. As to *Glossoscolex* it is hard to determine its average length, since it is an animal which has an amazing ability of altering the body length. Approximately the length of the "contracted" stage is 20 cm and that of the "distended" one about 40 cm.

In the *Pheretima* and *Pontoscolex* series, in each experiment the animals were first cleaned and dried and their volume determined as follows. They were put in a graduated cylinder and distilled water was added from a burette up to a mark high enough to make the animal completely submerged. By subtracting the number of c.c. of water added from the value of the level reached in the cylinder the volume of the animal was then roughly determined. If, during the operation, the animals defecated (what often happened as a consequence of the stimulus of submersion), a new determination of the volume had to be made. The loss of volume due to the ejection of mucus provoked by manipulation or contact with water, however, could not be calculated. This unfortunately rough determination of the volume of the animal was indispensable in order to calculate the k_{O_2} of each experimental flask, since the respirometer used was a WARBURG-BAR-CROFT apparatus. For each animal employed it was necessary to calculate a new constant. In the calculation of the k_{O_2} the amount of oxygen present in the fluid phase, which was solely formed by the 0.3 c.c. of 10% KOH added to the center well, was neglected. No filter-roll also was added to the center well for fear that, after imbibition with KOH, it might injury the animal, which occasionally during the experiment would move around in the flask. But we never had accidents due to the animals, attempts to get into the center-well. In the *Pontoscolex* and *Pheretima* series flasks with single side-arms provided with venting plugs, with volumes approximately equal to 15 ml were adopted. In the *Glossoscolex* series the procedure was more or less the same, the alterations being due to the much greater size of the animals. Flasks for large objects, with internal volumes varying from 127 to 143 ml were used. These flasks possess one side-arm provided with a non-venting plug, but no center well. They are composed by two connectable parts so that in order to put the animal inside them, one does not need to struggle with the narrow upper aperture. In the absence of a center-well, the KOH solution (1.0 c.c.) was added to the side-arm and a strip of filter paper was immersed in it so that one of its extremities laid freely in the space of the main chamber. The animal was placed in the bottom of the flask and in order to prevent it to run into the side-arm, a baffle made of a perforated zinc disc was interposed between the animal and the rest of the main chamber. In the calculation of the k_{O_2} the volume of this zinc disc of course was taken into consideration.

Experiments and results

In each experiment an equal number of control and experimental flasks were employed. The flasks were placed in the water bath at $25 \pm 0.05^\circ\text{C}$, in the dark and shaken throughout the experiment at 36 complete oscillation per minute. A preliminar test made with flasks out of the water-bath and in presence of the light, assured us that at the shaking rate used the animals remained practically motionless inside the vessels as though the tigmotaxis exerted by the glass walls of the flasks upon the animal was stronger than any disturbance eventually induced by the shaking. On the other hand we could not dispense the gentle shaking used, since the lack of filter roll in the center well was already a much too great hinderance to an efficient absorption of the eliminated CO_2 to be endured by performing the experiment with no shaking at all. At the beginning of the experiment 15 minutes were allowed for temperature and pressure equilibration. After the zero readings, new readings were taken at 15 minute intervals during one hour and 15 minutes, the manometric excursion during the first 15 minutes being discarded in the calculation of the Q_0^2 . Next step was the perfusion of the "control" flasks with air and of the "experimental" ones with a mixture of $\text{N}_2/\text{O}_2/\text{CO} = 59/21/20$ during 15 minutes. A new hourly oxygen consumption followed. At the end of the experiment the animals were taken out of the flasks, cleaned and dried with filter paper and finally weighed.

The procedure adopted allowed (a) a comparison of the respiratory rates of animals just removed from the earth and of animals submitted to a 24 hour period of lack of ingestion of natural food ; (b) an analysis of the size factor in respiration since we used animals whose body sizes varied over a large range ; (c) a study of the effects of hæmoglobin inhibition with CO upon the respiratory rate at normal oxygen pressure, hence the role of the pigment when the animals are exposed to common air.

The results are exposed in the tables I and II and in the graph of fig. 1. Table I reports a correlation between the volume (determined at the beginning of the experiment), the wet weight (determined more than two hours later, at the end of the experiment) and the respiratory rate. Earthworms are, as well known, animals with their intestinal duct normally filled with a relatively great amount of earth. This makes difficult to determine, as it can be done for other animals with a negligible intestinal content, the weight of the animal's actually amount of respiring tissues. That is why in order to reduce such an important source of error, we made two series of experiment, one with animals just collected in the earth and another with animals deprived of earth ingestion for 24 hours and, in both cases, we weighed the animals at the end of the experiment. We tried to run a third series of experiments with animals kept in the moist chamber for two or more days, but we did not succeed in keeping the animals in good conditions for the experiment. Both *Pontoscolex* and *Pheretima*, as well as *Glossoscolex*, did not show good adaptation to life in moist chamber as often reported for *Lumbricus*. Anyway, keeping the animals in the moist chamber for 24 hours left them some time to clean at least partially their intestinal duct from the ingested earth and thus afforded us a better chance to come to a nearer weighing of their respiring tissues. The series with

T A B L E I

Correlation between volume, wet weight and respiratory rate
(cu.mm. 02 / g(w.) / h.) in tropical earthworms, at 25°C.
(Animals disposed according to weight)

SPECIES	Just Collected			24 h. starving		
	Vol. (c.c.)	Wet weight (g)	Respir. rate	Vol. (c.c.)	Wet weight (g)	Respir. rate
<i>Pontoscolex</i> sp.	0.4	0.360	284	0.5	0.381	254
	0.6	0.456	227	0.5	0.400	258
	0.6	0.457	257	0.5	0.452	250
	0.6	0.503	222	0.5	0.464	272
	0.7	0.534	246	0.5	0.506	200
	0.7	0.543	260	0.6	0.510	230
	0.6	0.554	268	0.8	0.543	272
	0.7	0.573	259	0.7	0.546	224
	0.8	0.602	222	0.8	0.555	214
	0.7	0.666	255	0.7	0.575	198
	0.9	0.688	242	0.6	0.586	193
	0.8	0.691	208	0.8	0.596	231
	0.7	0.754	169	0.8	0.680	206
	0.8	0.778	194	0.8	0.680	178
	0.9	0.787	268	0.6	0.726	185
	0.9	0.801	209	0.8	0.750	145
	1.0	0.876	236	0.8	0.829	201
1.2	0.955	130	0.9	0.854	178	
1.1	1.016	138	1.0	0.952	177	
1.5	1.056	180	1.0	0.900	184	
<i>Pheretima hawayana</i>	0.8	0.567	209	0.6	0.651	271
	0.8	0.670	186	0.8	0.738	180
	0.8	0.672	203	0.8	0.794	248
	1.0	0.709	180	1.0	0.809	143
	1.0	0.740	159	0.9	0.912	174
	1.0	0.787	180	1.0	1.008	106
	1.0	0.848	199	0.8	1.096	188
	1.0	0.886	205	1.4	1.138	150
	1.1	0.908	230	1.8	1.161	211
	1.3	0.979	185	1.6	1.211	159
	1.8	1.230	182	1.2	1.265	204
	1.4	1.240	135	1.4	1.292	131
	1.4	1.308	149	1.5	1.510	134
	1.6	1.308	164	1.7	1.540	113
	1.4	1.329	110	1.8	1.698	140
	1.6	1.496	158	2.0	1.808	150
	1.5	1.512	112	2.0	2.002	181
	1.9	1.936	132	2.3	2.066	111
1.8	2.001	126	2.3	2.249	88	
2.0	2.017	111	2.8	2.766	60	
<i>Glossoscolex</i> sp.	9.3	6.672	59	3.3	2.514	50
	9.2	8.606	54	2.0	3.350	83
	11.3	8.617	57	6.4	5.521	61
	10.6	10.078	62	8.0	6.296	109
	11.4	10.665	55	8.8	6.708	70
	18.3	11.144	73	5.0	6.750	56
	14.4	12.570	60	7.0	6.934	92
	16.9	13.661	100	6.3	7.200	50

animals just collected served as a control of the effect of this partial cleaning of the intestinal duct upon the calculation of the respiratory rate as well as a rough indication of the consequences of a 24 hour period of starvation. An analysis of table I reveals that at least for *Pontoscolex* and *Pheretima* there is a good correlation between size and respiratory rate, the smaller individuals respiring relatively more. This can also be seen in the graph of fig. 1. This correlation is not only intraspecific but also interspecific, since *Pontoscolex*, being smaller than *Pheretima*, exhibited a relatively higher respiratory rate. (See also table II.) As to *Glossoscolex*, although in comparison with *Pontoscolex* and *Pheretima* they showed a much smaller oxygen consumption, the intraspecific correlation between size and respiration was not clear, in spite of the much greater variation in volume and weight. Table I also shows that the wet weight determined at the end of each experiment only in few instances correspond to the volume determined at the beginning, the reason for it being the fact that during the experiments the animals, as a rule, emitted large amounts of intestinal material. The data of table II for maximum, minimum and mean oxygen uptake in the first hour, however, show that in spite of the fact that the 24 hour starving animals were allowed to evacuate at least a good portion of their intestinal content, the respiratory rates of both just collected and 24 hour starving individuals are not significantly different.

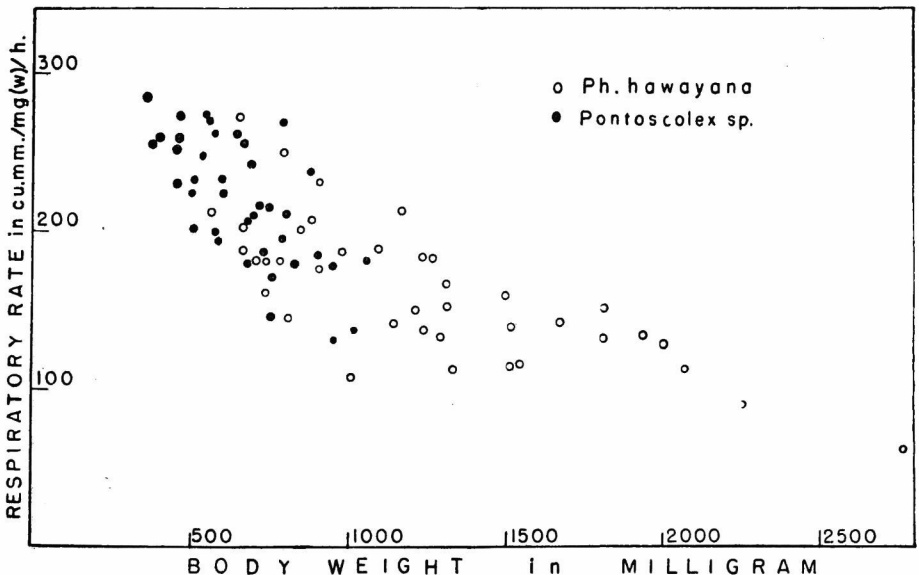


Fig. 1 The relation between body weight and respiratory rate in *P. hawayana* and *Pontoscolex sp.*

As to the consequences of the CO-poisoning, table II shows clearly that in an atmosphere formed by 59% N₂, 21% O₂ and 20% CO there occurred a significant depression in the respiration of *Pontoscolex*, *Pheretima* and *Glossoscolex*.³ The oxygen consumption of the CO-treated animals

T A B L E I I

Rates of oxygen consumption of *Pontoscolex sp.*, *Pheretima hawayana* and *Glossoscolex sp.*, at 25°C, just after collecting or after 24 hours of starvation, in pure air or under the action of 20% CO₂ at the atmospheric oxygen pressure.

SPECIES	Nutritive state	Num-ber of cases	Partial oxygen pressure in %		Rates of oxygen uptake in cu.mm./g(wet)/hour						2nd. hour mean rate as % of 1st. hour
			1st. hour	2nd. hour	First hour			Second hour			
					max.	min.	mean	max.	min.	mean	
<i>Pontoscolex sp.</i>	Just. coll.	10	21	21	268	130	221±41	257	113	202±44	91±4
		10	21	21+20%CO	284	138	227±45	158	76	124±27	55±8
<i>Pheretima hawayana.</i>	24 h. starv.	10	21	21	272	145	223±36	260	140	197±35	88±9
		10	21	21+20%CO	272	177	202±33	163	85	114±25	56±6
<i>Pheretima hawayana.</i>	Just coll.	10	21	21	230	110	172±36	218	93	154±36	89±8
		10	21	21+20%CO	205	111	160±39	131	48	91±30	56±7
<i>Pheretima hawayana.</i>	24 h. starv.	10	21	21	271	111	168±52	211	95	139±37	84±17
		10	21	21+20%CO	211	60	146±53	117	43	84±25	60±9
<i>Glossoscolex sp.</i>	Just coll.	8	21	21	100	54	67±12	89	41	59±13	88±8
		7	21	21+20%CO	69	39	50±10	32	20	27±3	56±6
<i>Glossoscolex sp.</i>	24 h. starv.	9	21	21	95	49	68±12	90	43	62±10	92±9
		11	21	21+20%CO	109	38	64±17	66	24	38±9	59±8

in comparison with that of animals which in the second hour of the experiment continued to be exposed to common air, was 55-60% of that of the first hour, whereas in the controls the variation amounted to only 84-92%. The results thus indicate that the suppression of hæmoglobin led to a decrease of oxygen transport to the tissues and hence the pigment in *Pontoscolex* as well as in *Pheretima* and *Glossoscolex* acts as respiratory pigment at the normal oxygen pressure.

Discussion

JOHNSON'S experiment (1.c.) are to our knowledge the most recent and best controlled work concerned with the determination of the respiratory rate in earthworms. JOHNSON used *Lumbricus herculeus* weighing 2.5-5.0 g after a previous stay of at least 3 days in damp soil and darkness. The experimental procedure adopted was in many ways similar to ours, except that the temperature used was 10°C. JOHNSON found in the first hour of the experiments at normal oxygen pressure that for two lots of animals the mean oxygen uptakes were 45.2 and 43.5 cu.mm./g(wet)/h. However, he did not add to his paper a table relating the animals weights to the respiratory rates and this renders difficult to compare his results with ours. Anyhow, assuming that *L. herculeus* weighing 2.5-5.0 g showed respiratory rates between 45.2-43.5 at 10°C and normal oxygen pressure, an inspection of table I of this paper reveals that, although our experiments were run at a much higher temperature, our individuals of comparable weights showed corresponding oxygen consumption values. In fact, and this is especially clear in the case of the 24 hour starving animals, whereas *P. hawayana* weighing 2.066, 2.249 and 2.766 g consumed respectively 111, 88 and 60 cu.mm. O₂ per g body and hour, *Glossoscolex* weighing 2.514, 3.335 and 5.521 g consumed respectively 50, 83 and 61. Within the weight range given (2.5 — 5.0), however, *L. herculeus* showed at 10°C an oxygen consumption lower than that exhibited by our earthworms at 25°C. Now, taking for granted that 25°C and 10°C are temperatures to which *Pheretima* and *Glossoscolex*, on one side, *L. herculeus* on the other, can be often exposed throughout the year in their respectively tropical and temperate climates, the question arises of whether this comparison between our results and JOHNSON'S agrees with what is known about the relation between temperature and metabolism in poikilothermal animals. More than ten year ago, this subject was taken by MUNRO FOX and WINGFIELD in a series of papers (FOX 1936, FOX & WINGFIELD 1937, FOX 1938, WINGFIELD 1939, and FOX 1939) with especial reference to the question of latitude. In the last paper of the series an exhaustive review of the data was given. It was then established that within a single species or in closely related ones, the forms naturally exposed to a colder environment (Arctic, English and Mediterranean individuals were studied) exhibited as a rule an acclimatization of the metabolism in the sense that their different physiological activities were as intensive as those of the forms of warmer regions. At a given temperature, an inversion of the so-called size-rule was observed, since the individuals of colder regions, although usually bigger in size, showed a higher metabolism. In the cases of *Lumbricus*, *Pontoscolex*, *Pheretima* and *Glossoscolex* we are not, of course, dealing with animals belonging to

the same species or even genus, but to different sub-families or families (STEPHENSON 1930). Yet one might expect that the above relationship would hold somewhat. This however did not happen since the tropical species of comparable weights showed at 25°C a higher metabolism than the temperate one at 10°C.

The data of table II show that in spite of allowing the animals 24 hours for a partial cleaning of their intestinal tract, the mean respiratory rate of the 24 hour starving earthworms did not significantly differ from that of earthworms just collected. One might conclude from this that keeping the animals starving for 24 hours was then an useless procedure. We must bear in mind, however, that especially in the cases of animals so filled with ingested earth as earthworms, this is an indispensable precaution if one desires to obtain a as close as possible determination of the real respiratory rate or to establish a relationship between respiration and the actual amount of respiring tissues. Earthworms are in this respect difficult animals to work with since the investigator has to find a way of getting rid of as much earth as possible from the animal's intestine at the risk of making it too limp for the experiment due to prolonged maintenance out of the earth. We do not think the authors have ever worried about this. JOHNSON (l.c.), for instances, just kept the animals in damp soil and darkness for some time before the experiments and warned against leaving the animals for many hours in the apparatus because they can become limp. Now, the fact that our 24 hour starving animals exhibited a respiratory rate not significantly different from that of the just collected could find an explanation by admitting that in the calculation of the oxygen consumption per g body and hour, the decline in oxygen uptake consequent from lack of ingestion of food was in practice compensated by the loss of weight due to ejection of intestinal material during the time the animals stayed in the Petri dishes. On the other hand, the admitted decline in oxygen consumption due to the 24 hour period of starvation can reciprocally be inferred from the insignificantly different respiratory rates of the just collected and starving animals, in spite of the much larger amount of inert intestinal material in the formers, which was unavoidably weighed in the determination of the wet weight. If the assumption is true, we must admit the lack of ingestion of earth during 24 hours had a great influence on the oxygen consumption of the earthworms studied. We must, however, not discard the possibility that the 24 hour stay in the moist chamber might have affected in itself the animals general conditions thus leading to a decrease of activity.

Table I shows that at least for *Pheretima* and *Pontoscolex* a good correlation was observed between size and oxygen consumption, as generally reported for animals, in that the smaller forms exhibited a relatively higher oxygen consumption. With regard to size factor in metabolism, comprehensive review like KLEIBER'S (1947) for homeotherms or WINGFIELD'S (1939) for poikilotherms make almost unnecessary to summarize again the state of the question. We will only recall that in some tropical Mammalians, as for instances the dog and the man (GALVÃO 1947, 1948 a&b, 1950 and 1951), the metabolism is rather proportional to the body weight than to the body surface and, as already mentioned, in many poikilotherms, within a single species or in closely related ones, the forms of colder regions, usually bigger in size, have a higher metabolism. In what

concerns the terrestrial Oligochetes, only two references could be found of experiments dealing with size factor in metabolism. HINO (1929, apud WINGFIELD 1939, p. 107) reported that in *Pheretima communissima* the gaseous metabolism bears a direct relation to the body surface. RAFFY (1930) studied the oxygen consumption of *Lumbricus* in water and in air, obtaining also oxygen consumption values which were inversely proportional to the weights of the animals. When we first thought of investigating the relationship between size (surface or weight) and metabolism in earthworms, what we had in mind was that earthworms being animals which respire by diffusion through the general indifferntiated surface assisted by intraepidermal capillaries (LENHOESSEK 1895, apud STEPHENSON l.c., p. 184; NONATO & MENDES, in the press) they would be a nice material to study this problem in poikilotherms. In practice, however, it was not so due to the mentioned difficulties in accurately measuring the actual amount of respiring tissues. Anyway, the results confirmed in general the theoretical predictions, especially in what concerned the interspecific comparison between size and respiration. The fact that intraspecifically *Glossoscolex* failed to exhibit a clear relation between wet weight and respiratory rate is difficult to explain, especially because, due to the much larger differences in size, here a more extensive observation of the size rule was expected. Maybe the animals most of the times were not in satisfactory conditions. In fact they came from Piracicaba in tin cans and stayed too often during the trip exposed to excessive heat and bad aeration.

Our results (table II) unanimously indicate that in *Pontoscolex* and *Pheretima*, as well as in *Glossoscolex*, just collected or after a 24 hour stay in moist chambers, a significant depression in respiration occurs at the atmospheric oxygen pressure in the presence of 20% CO. Thus, the pigment plays an important part in the oxygen transport to the tissues even when the animals are exposed to common air. These results are in agreement with those obtained by KRÜGER (1938, apud JOHNSON l.c., p. 267) and JOHNSON (l.c.) in *Lumbricus*. On the contrary, JORDAN & SCHWARZ (1920), DOLK & VAN DER PAAUW (1929) and, in a way, THOMAS (1935) reported that carbon monoxide only induces a significant depression in the respiration of *Lumbricus* at oxygen tensions far below the atmospheric. The controversy on the role of hæmoglobin in Invertebrates in general and in earthworms in particular is already a too debated matter to be recapitulated here (JOHNSON l.c.; MENDES, PÉREZ-GONZÁLEZ & COUTINHO l.c.). We, therefore, merely want to emphasize that JOHNSON'S work has over previous ones the advantage of having been done on a far improved technical ground, the results consequently being more reliable. Our results, obtained with a similar technique, in three species of three different genera of earthworms living under tropical conditions are a new support to the admission that hæmoglobin acts in terrestrial Oligochetes as a true respiratory pigment.

Summary

1. The respiratory rate of 3 species of 3 different genera of terrestrial Oligochetes from Brazil, namely, *Pontoscolex* sp., *Pheretima hawayana* and *Glossoscolex* sp. has been measured at 25°C, in the dark, in a Warburg-Barcroft apparatus, with gentle shaking, in the absence and in the presence

of 20% CO, in animals just removed from earth or kept in moist chamber during 24 hours.

2. In the absence of carbon monoxide, the respiratory rate (expressed in cu.mm. O₂/g(wet)/h.) of just collected *Pontoscolex* weighing 0.360-1.056 g, was between 284 and 130, that of 24 hour starving, weighing 0.381-0.900 g, between 272 and 145. Just collected *Pheretima*, weighing 0.567-2.017, exhibited an oxygen consumption between 230-110, that of the 24 hour starving specimens, weighing 0.651-2.766 g, being between 271 and 60. *Glossoscolex* just removed from earth, weighing 6.672 — 18.684 g showed respiratory rates between 100 and 39; those kept in moist chambers for 24 hours, weighing 2.514-18.455 g, between 109 and 38.

3. As an explanation of why the respiratory rate of the 24 hour starving specimens, in the 3 series, was not significantly different from that of the just collected the following assumption is made. In the calculation of the Q₀₂ of the 24 h. starving earthworms a compensation must have occurred between a possible decline in oxygen consumption due to lack of ingestion of food during 24 and the loss of weight due to emission of intestinal material (earth), thus leading to a Q₀₂ similar to that of the just collected. The danger of error in determining the oxygen consumption per g body and hour in animals, like earthworms, possessing large amounts of inert material in the intestine, is emphasized.

4. A good correlation, both intra-and-interspecific, was observed between size (expressed in wet weight) and metabolism (expressed as the respiratory rate) in *Pontoscolex* and *Pheretima*, the smaller individuals respiring comparatively more (table I, fig. 1). In *Glossoscolex*, where due to the greater variation in size of the animals employed, this correlation was expected to prove still clearer, it did not (table I). No explanation could be found for the fact, except that perhaps the animals employed were most of the times in unsatisfactory conditions consequent from the fatiguing trip to S. Paulo, when they were exposed to excessive heat and bad aeration. Anyway, in comparison to the much smaller *Pontoscolex* and *Pheretima*, *Glossoscolex* showed a relatively lower respiratory rate.

5. In the presence of 20% CO and at the atmospheric oxygen pressure, there occurred a significant depression in the respiratory rate of *Pontoscolex*, *Pheretima* and *Glossoscolex*, which was reduced to more or less the half of that observed in the absence of CO (table II). Thus, the inhibition of hæmogoblin by CO led to a decrease of oxygen transport to the tissues and, therefore, the pigment has a respiratory function even when the animals are exposed to common air.

Sumário

1. A taxa respiratória de 3 espécies de 3 diferentes gêneros de oligoquetos terrestres do Brasil, a saber, *Pontoscolex* sp., *Pheretima hawayana* e *Glossoscolex* sp., foi medida a 25°C, no escuro, num aparelho de Warburg-Barcroft, com fraca agitação, na ausência e na presença de 20% de monóxido de carbono, em animais recém retirados da terra ou mantidos previamente em câmara úmida por 24 horas.

2. Na ausência de monóxido de carbono, a taxa respiratória (expressa em mm³ de O₂/g (fresco)/h.) de *Pontoscolex* recém retirado da terra, pesando entre 0,360-1,056 grs., foi entre 284 e 130 ; a de animais submetidos a jejum por 24 horas, pesando 0,381-0,900 grs., entre 272 e 145. *Pheretima* recém-capturada, pesando 0,567-2,017 grs., mostrou um consumo de oxigênio entre 230 e 110 ; as submetidas a 24 horas de jejum, pesando 0,651-2,766 grs., entre 271 a 60. *Glossoscolex* recém retirado da terra, pesando 6,672-18,684 grs., exibiu taxas respiratórias entre 100 e 39 ; os mantidos nas câmaras úmidas, pesando, 2,514-18,455 grs., entre 109 e 38.

3. Como explicação de porque a taxa respiratória dos indivíduos submetidos a jejum por 24 horas não foi, nas 3 séries, significativamente diferente da dos recém-capturados, admite-se o seguinte : No cálculo do QO₂ dos animais jejunos deve ter havido uma compensação entre o possível declínio no consumo de oxigênio devido à falta de ingestão de alimento durante 24 horas e a perda de peso conseqüente à emissão de material intestinal (terra), de que resultou um QO₂ semelhante ao dos recém-capturados. O perigo de erro na determinação do consumo de oxigênio por grama de corpo e hora em animais, como os oligoquetos terrestres, contendo grande quantidade de terra nos intestinos, é salientado.

4. Uma boa correlação intra-e-interespecífica foi observada entre o tamanho (expresso em peso fresco) e o metabolismo (expresso pela taxa respiratória) em *Pontoscolex* e *Pheretima*, respirando os indivíduos menores relativamente mais (tab. I e fig. 1). Em *Glossoscolex*, onde em virtude da maior variação de tamanho dos animais empregados, seria de se esperar uma melhor correlação, tal não se deu. Não se achou explicação satisfatória para o fato. Talvez, os animais não se achassem na maioria das vezes em condições satisfatórias devido ao extenuante transporte a S. Paulo, quando se expunham a calor excessivo e mau arejamento. Todavia, em comparação com *Pontoscolex* e *Pheretima*, de porte muito menor, *Glossoscolex* apresentou uma taxa respiratória relativamente menor (tab. I).

5. Em presença de monóxido de carbono (20%) e à pressão atmosférica de oxigênio, houve significativa depressão na taxa respiratória de *Pontoscolex*, *Pheretima* e *Glossoscolex*, que se reduziu a cerca de metade da observada em ar isento de CO (tab. II). Assim, a inibição da hemoglobina pelo monóxido de carbono redundou num decréscimo de transporte de oxigênio aos tecidos e, portanto, o pigmento nos 3 oligoquetos possui uma função respiratória mesmo quando os animais estão expostos ao ar comum. Esses resultados condizem com os observados em *Lumbricus* por KRÜGER (1938) e JOHNSON (1942) e se opõem aos de outros autores, segundo os quais a hemoglobina nos oligoquetos, como em muitos outros Invertebrados, é mera armazenadora de oxigênio à pressão atmosférica do referido gas.

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