THE RESPIRATORY METABOLISM OF TROPICAL EARTHWORMS

III. *The influence of oxygen tension and temperature*

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In the two previous paper of this series we have focused (a) the normal respiratory rate, the role of haemoglobin and the relation between size and respiratory rate (MENDES & VALENTE, 1953) and (b) the intraepidermal capillaries and the action of externally applied adrenaline and acetylcholine on the respiratory rate (MENDES & NONATO, 1957) of some Brazilian earthworms (*Pheretima hawayana, Pontoscolex sp.* and *Glossoscolex sp.*). We now present the results of experiments performed to determine in what extent the variations of oxygen tension and temperature affect respiration.

In the present work only *Pheretima hawayana* has been used because its wide geographical distribution makes it the ittest earthworm in Brazil for a research aiming at the detection of climatic adaptations.

In fact, *Pontoscolex* and *Glossoscolex* can be regarded as typical genera of Equatorial and Tropical America, whereas *Pheretima* has been transported all over the world. In Japan it forms the largest number of endemic worms, it is characteristic of Burma and of the Indo-Malayan division, it occurs in Australia and Tasmania, being almost the only genus in the intervening islands between Australia and India. This "mighty genus", according to STEPHENSON (1930), relatively young and highly specialized, is by far the largest genus of Terricolae and appears to have power of conquering large territories and holding them itself alone, crushing all competitors. STEPHENSON (l. c.) also states that a number of species, including *P. hawaya-*

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na, are highly peregrine and have established themselves widely in the warmer regions of the globe, but he did not mention species of the genus when he discussed the Neotropical region nor included South America in its geographical distribution.

Pheretima, however, is known to occur in South America at least since the publication of the treatise of *MICHAELSEN* (1900), in which *P. barbadensis* is mentioned to exist in Chile (Santiago) and Brazil (Porto Alegre and Manaus) and *P. hawayana* in Brazil (Porto Alegre, Santos and S. Paulo).

STEPHENSON (l. c.) also informs that attempts to introduce *Pheretima* in temperate and cold climates have failed and that in England it has been maintained only in botanical gardens, not spreading outside.

Now, the fact that members of the genus can reach in the Southern hemisphere as far as Santiago (winter temperatures down to -4°C, occasional snowfalls) and in the Northern hemisphere up to Hakodate (*P. hilgendorfi*), Japan, where winters can be severe, does raise questions as to the inability of species of *Pheretima* to endure colder climates and fully justifies the choice of *P. hawayana* for our study. São Paulo, on the eastern edge (Serra do Mar) of the great Brazilian plateau, has a subtropical climate, with winter temperatures occasionally going within a day from almost zero (before sunrise) to 20°C (ca. 2 PM). This is partly due to the relatively high altitude of the city (ca. 800 m.). A study, therefore, of the respiratory responses to temperature variation of such a cosmopolitan earthworm exposed to such a climate may prove valuable for intra-and-interspecific comparisons with members of definitely temperate (such as Santiago) or warm (such as India) regions. As a matter of fact, in what concerns the relationship between respiration and temperature in earthworms, the literature is very scarse, as we shall see later in the discussion of this paper.

The respiratory responses of *P. hawayana* to varying oxygen tensions were studied because, to our knowledge, no such a type of work exists concerning tropical earthworms. Besides, the question of the interrelation between oxygen consumption and oxygen tension in earthworms is still open to discussion, since despite of recent evi-

dence (JOHNSON 1942, for instances) pointing to at least a relative dependence of the consumption on tension, this oligochete continues to be considered as good regulator of respiration (see BISHOP 1953) on basis of less accurate work.

METHODS

The animals came from the Faculty garden and stayed, before use, 24 hours in the dark, in moist chambers made of Petri dishes lined with moistened filter paper. The experimental procedure adopted was already described in detail in the first work of this series (MENDES & VALENTE, l. c.).

In the "oxygen tension" section of the present work, the oxygen uptake was measured at 25°C and 36 complete oscilations of the Warburg flasks per minute, during one hour and then compared with that of a second hour run at air tension or at a certain lowered oxygen tension. Gas mixtures were prepared using oxygen and nitrogen (twice washed in alkaline pyrogallol) in the desired percentages. The perfusion of the flasks took place usually during 10 minutes, while they were under shaking.

In the "temperature" section, different temperatures were obtained either heating the bath up to 45°C or cooling it with cooling unit, whose coil was kept completely immersed in the water. In either case it was possible to keep a chosen temperature within a maximum of 0.5°C variation, even when extremes temperatures were used. Before "zero readings", the flasks containing the animals stayed in the bath at least 15 minutes in order to get equilibration of the temperatures inside and outside.

One animal per flask was used throughout the experiments, which, of course, were performed in the dark. More details of the methods will be given below.

EXPERIMENTS

Oxygen consumption and oxygen tension in Pheretima. As stated above, the effects of varying oxygen uptake of *Pheretima* were studied by measuring a first hour uptake for each animal and then a second hour rate at air or at a certain lowered oxygen tension for

the same animal. This procedure allowed (a) to check the effect of the second hour stay in the flasks, by simply renewing the air inside the flasks instead of perfusing with a gas mixture of a lesser oxygen content: (b) to compare, for a same animal, the immediate effect of lowering the oxygen tension.

Table I and graph 1 show the results obtained. Whether considered *per se* or in percents of the 1st. hour rates, the values of the

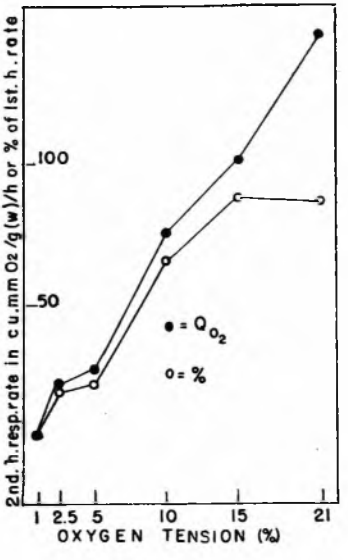

Fig. 1 — The relation between oxygen consumption and oxygen tension in *Pheretima hawayana*

2nd. hour rates clearly show that *P. hawayana* cannot regulate respiration from 10% O_2 downwards. At 15% the respiratory rate is still insignificantly lower than at air, but from 10% downwards the decrease in the oxygen consumption is highly significant. The data also show that at air tension, a 2nd. hour stay in the flasks did not significantly alter the respiratory rate. The same holds for the 2nd. hour rate at 15% O_2, which, as a matter of fact, in percents of the 1st. hour rate is higher than at air tension, although not significantly. In terms of QO_2, in the graph of fig. 1, this cannot be seen on account of the higher values obtained both at 1st. and 2nd. hour (lighter animals were used) in the first series as compared with corresponding ones

in the second. The fact also explains why the curves in figure 1 do not agree between 21 and 10% 0_2 and emphasizes the advantage of expressing the rate at the different oxygen tensions in percents of the first hour rate rather than in absolute values measured.

TABLE I

The respiratory rates of the earthworm *Pheretima hawayana* at different oxygen tensions. 24 hour starving animals, in the dark, at 25°C and 36 strokes per minute. Mean local atmospheric pressure = 702 mm. Hg.

Exp. Ser.	N.° of Exps.	Gas phase in the flasks		Rates of oxygen consumption cu.mm. at N.T.P. per g per h. Means & std. devs.		P_1	2nd. hour mean rate as % of 1st. hour	P_2
		1st. h.	2nd. h.	1st. h.	2nd. h.			
1	10	air	air	169 49	139 35	0.8	85 05	
2	10	air	15%0_2	118 20	101 14	0.8	87 12	0.7
3	10	air	10%0_2	116 07	75 07	0.01	65 07	0.01
4	10	air	5%0_2	112 34	46 24	0.01	42 17	0.01
5	10	air	2.5%0_2	98 14	37 10	0.01	38 10	0.01
6	10	air	1%0_2	105 13	24 07	0.01	24 09	0.01

At the end of the experiments, in all series, the animals crawled out of the flasks by themselves, indicating that even the extremely low oxygen tensions used did not affect them.

Oxygen consumption and temperature. Table II and the graphs of figures 2 and 3 show the results obtained when the respiratory rate of *P. hawayana* was measured at different temperatures. In all experiments the animals passed directly from room temperature (ca. 25°C) to the temperature of the water bath and time was only given to equilibrate the temperatures in and outside the flasks. The results therefore express the immediate effects of transferring the earthworms from room to experimental temperatures.

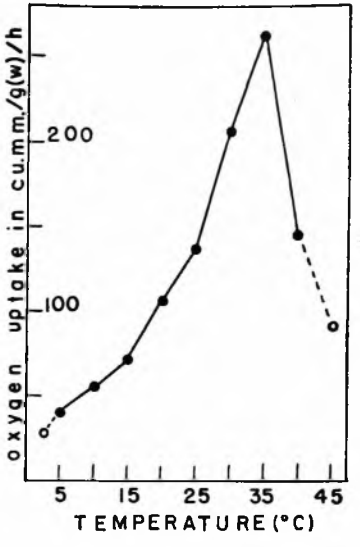

Fig. 2 — Graph relating the oxygen consumption to temperature in *Pheretima hawayana*.

In table II, for each temperature, the weight range is given in order to show that, due its relative narrowness, size effect little interfered with the results. We considered dangerous, in this section, to

TABLE II

The effects of temperature variation on the respiration of the earthworm *Pheretima hawayana*. Animals taken from room temperature (ca. 25°C). In the dark. Shaking rate = 36/min. Gas phase = air. $Q0_2$ = cu.mm.02/g(w.)/h.

Exper. Series	Temper. (°C)	N.° of cases	Weight range in g.	Mean $Q0_2$ & std. dev.	P
1	40	10	0.623-0.863	142 34	0,7
2	35	10	0.622-0.882	261 47	0,01
3	30	10	0.627-0.905	206 50	0,01
4	25	10	0.693-0.863	137 30	—
5	20	10	0.627-0.900	106 28	0,05
6	15	10	0.604-0.839	70 11	0,01
7	10	10	0.606-0.892	54 10	0,01
8	5	10	0.622-0.891	40 08	0,01
9	45	6	0.242-0.445	90 26	0,01
10	2	5	0.732-1.118	28 03	0,01

measure the respiratory rate of a same animal first at room temperature and then, in a second hour at a higher temperature. For the sake of uniformity, the same criterion was used when temperatures below 25°C were used. We think, however, that the ten experiments of each series afforded enough data to face the inconveniences of individual variations.

Taking 25°C as a starting point, a rise in temperature significantly increased the oxygen consumption up to 35°C. A Q_{10} of almost 2 is obtained when the rates at 25°C and 35°C are related. At 40°C the respiratory rate almost returned to the 25°C level and at 45°C the six data of table II show that respiration, expressed in $Q0_2$, is significantly reduced as compared with the 25°C rate. These results, however, do not exactly express the situation of the animals above 35°C. In fact, as shown in the graph of figure 3, at

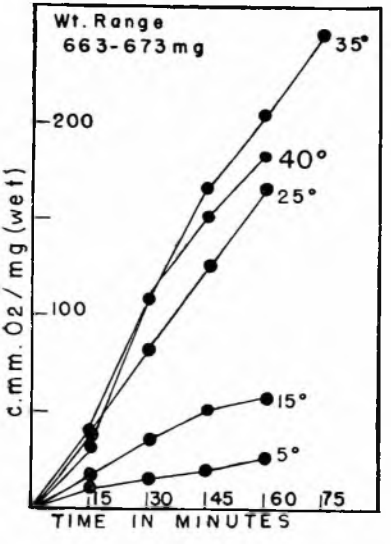

Fig. 3 — Time course of the oxygen consumption of *Pheretima hawayana* at different temperatures (°C.

40°C, respiration is at first greatly increased, to decrease after 30 minutes, leading to an hourly rate nearer the 25°C level. This decrease, after an initial increase, is still stronger and earlier at 45°C. Fig. 3 also shows that, at 35°C, the respiratory increase tends to decline after 45 minutes, when a slight inflection in the curve is ob-

served. Thus, the hourly rates determined at 40°C and 45°C really reflect a sudden increase in metabolism due to temperature rise, followed by a decrease due to temperature damage. In fact, from 35°C upwards, at the end of the experiments the animals were taken out of the flasks motionless and hemorrhagic. After one hour stay at 45°C, the animals were apparently dead, disrupt and dit not recover. At 40°C, body disruption was not observed, but the animals seldom recovered. Recovery was often observed after the hourly stay at 35°C.

A decrease in temperature also affected respiration since, from 20°C downwards, the respiratory rate was significantly lower as compared with the 25°C level. A Q_{10} of almost 2 is obtained when the rates at 25° and 20° are related respectively with those at 15° and 10°C. The graph of fig. 3 shows that the decrease can be detected after 15 minutes. The animals, however, were not apparently affected by the lowered temperatures used, since even after a 75 minutes stay at temperatures as low as 2°C, they emerged from the flasks in good condition. At 5°C they got out by themselves after a while at room temperature; at 2°C they seemed in a state of cold narcosis from which they generally recovered after a few hours. No external injury was observed in the animals submited to temperatures down to 2°C.

P. hawayana and Rapid Compensation for Temperature — Although it was not the purpose of this work to investigate *P. hawayana*'s ability to restore the normal respiratory rate after a prolonged stay at low temperatures, thus compensating for different temperatures, a preliminary study was made as to rapid compensation.

In this series, the respiratory rates of two lots of animals were measured in a first hour at 25°C and, then, one lot remained at this temperature and the other was transferred, within the Warburg flasks, to a bath at 5°C. Afterwards, the respiration of both lots was measured again for a certain length of time, with periodical renewal of the air inside the flasks to prevent oxygen debts. Unfortunately, due to the experimental conditions used, both lots, after ca. 24 hours, showed signs of being limp (they had been previously submitted to a 24 hour period of starvation in Petri dishes) and the experiment could not go on. Nevertheless, it provided at least some evidence which suggest

that in *P. hawayana* there is no rapid compensation for temperature. In fact, as shown in table III, after being transferred to 5°C the earthworms respired significantly less than those maintained at 25°C during all the duration of the experiment. A slight increase in oxygen consumption is observed when the rates at 9-10 PM (April 26) and those of the following day are compared. This increase, however, is not significant in terms of the rate at 25°C. As a matter of fact, the values determined in this series for 5°C never attained the level of those determined immediately after placing the animals at 5°C (see table II). The extremely low values registered after ca. 5 hours at 5°C (April 26, 9-10 PM) and the slightly higher ones of the following day indicate that, at least for this low temperature, the decrease in oxygen consumption immediately observed in the animals transferred from 25°C (table II) is not followed by a rapid compensatory increase.

It is interesting to mention that in this series, during the experiments, occasionally the animals put at 5°C failed to exhibit any

TABLE III

A test for rapid temperature compensation in *P. hawayana*
Gas phase: air $Q0_2$ = cu.mm.0_2/mg.(w.)/h. 36 strokes/min.

		LOT I				LOT II				
Day	Hours	N.° of animals	Temp. (°C)	Mean $Q0_2$	Std. dev.	N.° of animals	Temp. (°C)	Mean $Q0_2$	Std. dev.	P
April 26	4-5 PM	3	25	128	19	4	25	131	11	—
" "	6 PM		(lot kept at 25°)				(Lot transferred to 5°C)			
" "	9-10 PM	3	25	98	42	4	5	2	—	0.01
April 27	10-11 PM	3	25	110	34	4	5	18	4	0.01
" "	12AM-1 PM	2+	25	105	—	4	5	13++	9	0.01

+ one animal died ++ one animal did not practically respire.

oxygen consumption between two readings and in some cases gas was produced inside the flasks instead of being absorbed. Although in the control lot one of the animals died, the results show that no respiratory depression was due to the prolonged stay in the flasks.

DISCUSSION

1. Modern work on the *interrelation between oxygen consumption and the oxygen tension* in earthworms starts with JORDAN & SCHWARZ (1920), who, using specimens of *Lumbricus terrestris* narcotized with 10% alcohol, reported that, at 25°C, above 7% 0_2 the consumption independs of the tension. From these results and also from those obtained when hemoglobin was put out of action by carbon monoxide, they concluded that in the earthworm the oxygen normally used is that dissolved in the plasma and that the pigment serves for the subterranean life where the oxygen tension is low. The AA. used the gas pipette to measure respiration and drew conclusions from the respiratory rates of different animals submitted to different oxygen tensions.

DOLK & VAN DE PAAUW (1929) repeated JORDAN & SCHWARZ' work because they thought it was precarious to compare the rates of different animals on account of the unequal earth content of the gut, which might have influenced on the weight used as basis to express the oxygen consumption. They used single animals in a Krogh's microrespirometer, anesthetized with 8-9% alcohol, but made no reference as to whether the experiments were performed in the dark or not. In order to know the effect of the brief (6-8 minutes) immersion of the animals in alcohol, a procedure which aimed at obtaining "standard conditions", they previously followed the respiration of worms so narcotized for 48 hours and reported that after 20 hours the animals were quietly respiring at a constant rate, with the dorsal blood vessel normally pulsating. Consequently, they used in the experiments worms which were put in the respirometer chamber soon after a brief immersion in alcohol and there stayed overnight, with periodical renewal of the internal air, to keep the oxygen tension normal till just before the initial readings. Oxygen tensions below normal were obtained by letting the animals exhaust the gas inside the chamber and they were roughly calculated from the initial air volume at the animal's disposal and the subsequent oxygen consumption. The 0_2 uptake was not expressed in function of body weight, but in cu.mm./30 min. or in % of the first reading. With this procedure the

AA. believed to have avoided the inconveniences of individual weight variations.

Despite of using such a different procedure, DOLK & VAN DE PAAUW did not essentially obtain results different from JORDAN & SCHWARZ'. In fact, down to 2,5% 0_2 the oxygen consumption of the worms independed of the tension. This led the AA. to admit that earthworms are animals in which the metabolic rate is governed by the tissues over a wide range of oxygen tensions. Above 2,5% 0_2, a positive oxygen pressure would exist in the tissues, the combustion process being regulated by other limiting factor than oxygen transport. Recalling the conflicting views of Pflüger & Pfeffer and Thunberg on the subject, they went on to state that only in animals with tissue oxygen pressure constantly tending to zero the metabolic rate increases with the external 0_2 tension. In those with a positive pressure, even when the external tension is very low, the limiting factor is not tension, but enzymes and foodstuf. Earthworms, however, are not to be compared with Vertebrates, where oxygen uptake also does not very with oxygen tension over a wide range. In redblooded Invertebrates such as the earthworms, according to DOLK & VAN DER PAAUW'S results, it is the plasma not the blood pigment that is in charge of the oxygen transport down to very low external 0_2 tension; down to 7.5% 0_2 their curves for normal and CO-treated animals are the same. Thus, despite the fact that with increasing oxygen tensions more oxygen can dissolve in the plasma, the animals do not necessarily make use of it.

THOMAS (1935) criticized both JORDAN & SCHWARZ' and DOLK & VAN DER PAAU's works on the grounds that the alcohol concentrations used for narcosis were too strong. This *plus* individual variation towards alcoholic anaesthesia were likely to lead to false results. He therefore reinvestigated the matter, using more or less the same technical procedure as DOLK & VAN DER PAAUW and also not taking into account the body weight to express the oxygen consumption. He observed a great variation in the respiratory responses of the worms submitted to a brief immersion in 8-9% alcohol and reported that the respiration of worms treated with 6% alcohol dit not differ from that of normal ones kept quiet inside a long glass tube.

In either case, letting the animals exhaust the environmental oxygen to get low 0_2 tensions, THOMAS observed that from 20.9% to 15% 0_2 the oxygen uptake decreased and thereafter remained constant down to 3%, to decline again. The fact that above 15% 0_2, the consumption increasead with tension was considered by THOMAS as indicating that DOLK & VAN DER PAAUW's view regarding the interrelation between consumption and tension in earthworms is incorrect. He suggested that by some unknown mechanism (acting probably on the capillaries) more oxygen is admitted to the tissues above 15%, leading to a higher 0_2 uptake. Finally, he did not attribute to hemoglobin the constancy observed between 15 and 3%, because it occurred also in the CO-treated animals.

In 1942, JOHNSON, in a study of the function of hemoglobin in *Lumbricus herculeus,* measured parallelly the oxygen consumption of normal and CO-treated animals at different oxygen tensions. This work was undertaken on account of the faulty technique of the previous studies, which included (a) the narcosis of the animals, (b) the lack of reference to the light condition during the experiments, (c) a poor process of obtaining low 0_2 tensions by letting the animals exhaust the gas during a too long stay in the respiratory chamber and, finally (d) conclusions based on too little (sometimes only 2 cases!) number of experiments. JOHNSON's technique is in many respects comparable to that of the present work. The animals were kept at least 3 days in damp soil (earth or filter paper?) and darkness before the experiments. The oxygen consumption was measured in a Barcroft differential respirometer, in the dark and at 10°C, and expressed in function of the body weight. Oxygen tensions lower than normal were obtained by perfusing the flasks with proper $N_2/0_2$ mixtures. For each oxygen tension tested a large number of animals was employed and the data were treated statistically. All this procedure intended to minimize the effects of individual variation and made undoubtly JOHNSON's work the first sound experimental study dealing with respiration in earthworms. It is worth while therefore, to compare its results with ours.

JOHNSON's *Lumbricus,* at 10°C, regulated respiration down to 76 mm. Hg (ca. 10% 0_2), below this value respiration fell sharply.

Pheretima, at 25°C, already respired significantly less at 10% 0_2 than at air tension; thereafter respiration continued to regularly decline with decreasing tension. JOHNSON's results differ from all previous studies essentially in that already after 10% 0_2 there occurred (at 10°C) a loss in regulation, whereas in JORDAN & SCHWARZ; DOLK & VAN DER PAAUW's and THOMAS' works this happened at respectively 7, 2.5 and 3% 0_2 (at 25°C).

An attempt to explain why in our experiments the loss of regulation occurred already at 10% 0_2 involves necessarily the consideration of the temperatures used in JOHNSON's and our works. At air saturation, *Pheretima* exhibited a $Q0_2$ of 139 (table I) at 25°C and 54 (table II) at 10°C; JOHNSON's *Lumbricus* showed at 10°C a $Q0_2$ of about 38 (heavier animals were used!). It is probable then that the higher temperature used was responsible for the lesser ability of *Pheretima* to regulate respiration. In fact, one would expect a higher metabolism at 25°C than at 10°C, hence a greater dependence on the oxygen tension (BISHOP, l. c.).

This kind of reasoning leads to the consideration of whether DOLK & VAN DER PAAUW's view on earthworm respiration is correct or not; namely, that metabolism in this oligochete is solely regulated by enzymes and food-stuff and not by oxygen transport, the 0_2 pressure in the tissues being positive even when the external 0_2 tension is extremely low. BISHOP (l. c.), reviewing the question of the interrelations between oxygen consumption and oxygen tension in animals, emphasized the fact the different responses to decreased 0_2 tensions, in some cases, can be attributed to "different activity adjustments", sluggish animals being far more independent on tension than active ones. Lack of dependence of the oxygen consumption on tension, on the other hand, can be due to "sheer efficiency of respiratory mechanisms at low tensions, such as plasma hemoglobin which enables many aquatic animals to extract oxygen at very low 0_2 tensions".

Now, earthworms (at least *Pheretima* at 25°C!), although moving when undisturbed by slow peristaltic waves, can hardly be considered as sluggish animals. Their prompt reaction to peripheral stimulation by the "Zuckreflex" or quickly jumping, indicate a high

muscular tonus, which can only be maintained at the expense of a considerable degree of metabolic activity. It is difficult, therefore, to admit that in earthworms the tissular 0_2 pressure is kept positive, especially at extremely low 0_2 tensions. Neither can the presence of hemoglobin in earthworms blood be used as indicating independence of the 0_2 uptake on tension, in the sense of BISHOP's words. Whether one considers earthworms' haemoglobin as an oxygen storer (JORDAN & SCHWARZ, DOLK & VAN DER PAAUW) or an oxygen carrier (JOHNSON; MENDES & VALENTE) it is always difficult to understand how the pigment would serve to help regulation at the intermediate 0_2 tensions between air saturation and the tensions at which respiration begins to depend on the available oxygen. In this respect, it is important to emphasize the parallelism of the curves for normal and CO-treated animals observed either in the works of DOLK & VAN DER PAAUW and THOMAS or JOHNSON's, despite the diverging results regarding the function of hemoglobin. It is a pity that BISHOP, although knowing about JOHNSON's work (the critical tension, 76 mm. $Hg0_2$, obtained for *L. herculeus* at 10°C is mentioned at table 43 of his review), preferred to base his admission of earthworms as good regulators entirely on the less accurate work of DOLK & VAN DER PAAUW (pages 246-248).

Using the degree of activity as a criterion for, at least partially, explaining the interrelations between 0_2 consumption and 0_2 tension, one is tempted to analyse why earthworms under alcoholic anaesthesia should independ on tension over such a wide range. This could be understood in terms of a general metabolic depression due to narcosis, so that one would get, even at air saturation, a low respiratory rate for comparison with the rates at the decreased 0_2 tensions. Besides, the low degree of activity of the narcotized animals would really contribute to lessen the 0_2 depletion in the tissues, enabling them to apparently regulate respiration.

It is difficult, however, to compare JOHNSON's or our data with those obtained with narcotized animals, since neither DOLK & VAN DER PAAUW nor THOMAS expressed their results in terms of body weight, reporting only the volumes of the animals. Nevertheless, admitting roughly a volume / weight ratio ca. 1, probable

respiratory rates ($Q0_2$) can be calculated from their data expressed in cu.mm. 0_2/30 min., obtained at the beginning of the experiments (air saturation). Table IV includes all DOLK & VAN DER PAAUW's data and 3 of the 4 results presented by THOMAS, for a comparison with the $Q0_2$ of *Pheretima* of comparable volumes obtained by MENDES & VALENTE.

This rough calculation of $Q0_2$ for *Lumbricus* does not indicate that in DOLK & VAN DER PAAUW's experiments there was a respiratory depression (one out of 3 data is lower than those of *Pheretima* of comparable volumes); from THOMAS's calculated $Q0_2$, however,

TABLE IV

A comparison of the respiratory rates of *Lumbricus terrestris* and *Pheretima hawayana* to show the possible effects of alcoholic anaesthesia. Temperature = 25°C. Air saturation.

Experimental conditions	Light conditions	Volume (ml)	$Q0_2$	AUTHOR
narcotized	?	1.43	160	DOLK & VAN DER PAAUW
normal	dark	1.40	131	MENDES & VALENTE
narcotized	?	1.30	99	DOLK & VAN DER PAAUW
narcotized	?	1.08	156	DOLK & VAN DER PAAUW
normal	dark	1.00	143	MENDES & VALENTE
normal	dark	0.90	174	MENDES & VALENTE
normal	dark	0.60	271	MENDES & VALENTE
narcotized	?	0.43	168	THOMAS
narcotized	?	0.42	162	THOMAS
narcotized	?	0.18	208	THOMAS

there seems to be reason to admit depression since, despite the decreasing volumes, the rates do not proportionally increase as expected on basis of the value obtained for the 0.60 ml. *Pheretima*.

Narcosis was used in order to get "standard conditions", that is, quietness inside the respiratory chamber. THOMAS himself demonstrated that this is an useless procedure. Earthworms, due to thigmotaxis, can stay quiet and motionless inside glass flasks, especially in the dark.

2. Taking room temperature (25°C) as a starting point, a rise or a decrease in temperature by 5° intervals significantly altered the

respiratory rate of *Pheretima*. Up to 35°C, there occurred a respiratory increase. At 40°C, however, temperature definitely injured the animals, leading to a mean $Q0_2$ near the 25°C level, which only express an initial increase in respiration followed by a decrease due to temperature injury. Below 25°C respiration regularly decreased with temperature, but the animals were not apparently affected even by temperatures as low as 2°C.

Except for the work of KIRBERGER (1953), we do not know of any other particular paper dealing with the effect of temperature on the respiration of earthworms. The previous information on the subject seems to amount to the following: VERNON (1897, apud BULLOCK 1955), in his classical work, mentioned that in *Lumbricus* there exists a plateau extending over ca. 12°C when carbon dioxide output is plotted against temperature.

KIRBERGER is a member of the Kiel group led by PRECHT (see, for instances, PRECHT 1949) to which we owe in the last ten years important works on the relation between vital processes and temperature both at the organism and the cellular level. In 1953, she studied the problem in some Invertebrates, including the Oligochetes *Lumbriculus variegatus* (limnic) and *Eisenia foetida* (terrestrial). As to the earthworms, she previously found that a sudden increase of temperature from 15 to 25°C remarkably enhanced the respiratory rate, that is, the rate at 25°C in animals adapted to this temperature is lower than the rate at the same temperature of animals freshly transferred from 15°C. This temperature shock can be avoided by slowly (degree by degree) transferring the animals from 15°C to 25°C. The oxygen consumption of starving animals was about 70% of the fed ones. Whether starving or fed, *Eisenia* adapted to 15°C and 25°C exhibited at the experimental temperatures used (15, 20 and 25°C) the same respiratory rates. That is, the rates at 15, 20 and 25°C regularly increased whether the animals were transferred from a 15° or a 25° environment. No seasonal influence was also observed. The return point ("Umkehrpunkt") of the respiratory curve with increasing temperatures was located between 36°C and was found to be independent of the adaptation temperature. *Lumbriculus* behaved differently, in the sense that it showed respiratory adaptation;

the 0_2 uptake at the same experimental temperatures diminished with increasing adaptation temperatures. This different behaviour was attributed to the different biotopes of the two worms, terrestrial *Eisenia* being in nature less exposed to temperature variations than aquatic *Lumbriculus*. The succinodehydrogenase activity of the earthworm *Eisenia* was also found to be independent of the adaptation temperatures. Finally, KIRBERGER exposed *Eisenia* to a 30 minute immersion in bathes at different high temperatures in order to determine its heat resistance expressed as percents of survival. The resistance rose with increasing adaptation temperatures, seasonal influences being observed. No clear adaptation, however, was observed in the cellular level as juged by the succinodehydrogenase activity. Trying to explain why the "Umkehrpunkt" of the heat resistence depends of the adaptation temperature and that of the respiratory curve not, KIRBERGER emphasized that in the former case one has to consider the irreversible injury of the proteins because here the "Umkehrpunkt" coincides with the extreme limit temperature of life. This would also explain the lack of influence of the adaptation temperature on the "Umkehrpunkt" of the respiratory curve.

The data of table IV of this paper indicate that *Lumbricus* and *Pheretima* of comparable sizes have more or less equivalent respiratory rates when our results are confronted with DOLK & VAN DER PAAW'S, but that THOMAS' *Lumbricus,* although smaller, respired less than *Pheretima*. Had these authors not worked with narcotized animals, their results would serve to compare at a same temperature (25°C), the respiratory rates of a temperate and a tropical earthworm. JOHNSON's results interest in the sense that they were obtained with an accurate technique. Unfortunately, however JOHNSON neither added to the paper a table relating the animals weights to the respiratory rates nor had in mind determining a $Q0_2$ x temperature curve. Nevertheless, we can assume that 2.5-5.0 g. *L. herculeus* showed a mean respiratory rate of 38.8 at 10°C and air 0_2 tension. At the same conditions, 0.606-0.892 g. *Pheretima* exhibited an average $Q0_2$ of 54. Size effects, of course, render render difficult the comparison of both results. Based on the size rule, however, one might infer from JOHNSON's data that 0.6-0.8 g. *L. herculeus* would respire at 10°C more

than *Pheretima*. This could be taken as an indication of compensation for low temperature by the temperate form as opposed to the tropical form, provided that the latter would not return to the 25°C level (137) after acclimation at 10°C (see below). Reciprocally, tropical forms comparable in size to *L. herculeus* might be expected to exhibit at 25°C more or less the same respiratory rates as those of the temperate form at 10°C. Table V, which includes data of the first paper of this series (MENDES & VALENTE), shows that this may not be the case, since *Glossoscolex* with a similar weight range respired significantly more at 25°C than *L. herculeus* at 10°C.

TABLE V

A comparison of the respiratory rates of *L. herculeus, P. hawayana* and *Glossoscolex sp.* to check possible latitude effects and compensation for temperature. $Q0_2$ = cu.mm.0_2/g(w.)/h.

Animal	Wt. range (g)	Temper. (°C)	N.° of cases	Mean $Q0_2$	AUTHOR
P. hawayana	2.0-2.7	25°C	3	84.6	MENDES & VALENTE
L. herculeus	2.5-5.0	10°C	9	38.7	JOHNSON
Glossoscolex	2.5-5.5	25°C	3	64.6	MENDES & VALENTE

KIRBERGER's starving earthworms adapted to 25°C respired at 25, 20 and 15°C respectively 137, 87 and 68 cu. mm. 0_2/g/h. Our 0.6-0.9 g. *Pheretima* in practically the same conditions respired 137, 106 and 70. Here again the lack of information about the weights of the animals used in KIRBERGER's experiments ("Exemplare von annähernd gleicher mittlerer Grösse") renders difficult the detection of possible latitude effects. In both cases, however, the decline in temperature significantly lowered the $Q0_2$ and in neither case plateaus have been found to suggest compensation for temperature.

All these speculations, of course, do not make unnecessary a more detailed study of the interrelations between respiration and temperature in temperate earthworms for comparison with our data. Temperate forms, exposed and adjusted to large seasonal variations of temperature must be able to react to sudden changes in a manner different from that of tropical species. This aspect of the problem leads to the consideration of the question of a probable rapid compensation form temperature in earthworms.

3. It is generally admitted that poikilotherms operate at lower rates in colder habitats and seasons. In an exhausting review of the subject, BULLOCK (l. c.) reminds that the evidences that many coldblooded animals, on the contrary, are relatively independent of temperature, go back to at least 1899, when KREHL & SOETBEER concluded that in respect to temperature poikilotherms are not simply "die Spielbälle der Umgebung", but show metabolic adaptation of their protoplasm. That is, given time or even rapidly, "these species tend to maintain a certain level of metabolism or other characters measured as rates, compensating for different temperatures by homoeostatic mechanisms of various kinds".

Compensation for temperature has been studied mainly in the cases of aquatic animals, the bulk of information concerning temperate forms. According to EDWARDS & NUTTING (1950), SCHOLANDER, FLAGG, WALTERS & IRVING (1953) and others, a relative poverty of compensatory adaptation may be the rule for insects and possible other terrestrial groups.

VERNON's studies included earthworms, at least the temperate forms, among those terrestrial animals able to compensate (and rapidly) for temperature. KIRBERGER, however, working on a better base, did not confirm this finding. In her experiments, *Eisenia,* whether adapted to 25 or to 15°C, was unable to restore the normal respiratory rate, regularly following rise and decline in temperature with corresponding increase and decrease of the 0_2 uptake. At the cellular level, however, catalase activity and dehydrogenase activity showed some dependence on the adaptation temperature.

The results obtained with *Pheretima* also do not extend to this tropical earthworm the ability to rapidly compensate for temperature. Neither did *Pheretima* remain temperature insentive over the wide temperature range used (2-45°C), nor was it able to restore the 25°C respiratory level after a 24 hour stay at 5°C. Besides, *Pheretima* and KIRBERGER's *Eisenia* exhibited more or less the same "Umkehrpunkt", the former's situated between 35 and 40°C and the latter's located between 36-38°C (independent of the adaptation temperature).

Whether or not *Pheretima,* given time, would be able to compensate for temperature will be investigated in the near future in a

general study of the behaviour of terrestrial tropical poikilotherms towards temperature variation.

CONCLUSIONS

1. The oxygen consumption of 24 hours starving specimens of the earthworm *Pheretima hawayana* has been measured in the dark, in a Warburg apparatus, at 36 strokes per minute, under varying conditions of 0_2 tension and temperature.

2. Down to 15% 0_2, *P. hawayana* regulates respiration. At 10% 0_2, its respiratory rate is already significantly lower than at air tension. Thereafter, it continues to decline with decreasing 0_2 tensions.

3. The data of the literature on the interrelations between 0_2 uptake and 0_2 tension in earthworms are critically reviewed, especially those obtained with narcotized animals.

4. *P. hawayana* increases its respiratory rate when temperature rises up to 35°C. This temperature can be considered as critical on the upper side of the temperature range. At 40°C, the $Q0_2$ almost returns to the 25° level, but this only expresses a sudden great increase due to temperature rise, followed by a strong decrease due to temperature damage. At 45°C, this sudden increase is followed by an earlier and stronger decrease, leading to a $Q0_2$ lower than normal. At the end of the experiments at 40 and 45°C, the animals were motionless, hemorrhagic and disrupt.

5. *P. hawayana* regularly diminishes its 0_2 uptake, when temperature decreases from 25°C down to 2°C. The animals, however, are not apparently affected even by the extremely low temperatures used; after a 90 minute stay at 2°C they seemed to be in a state of cold narcosis, from which they emerged after a few hours at room temperature.

6. The scarcity accurate studies of the relations between temperature and respiration for temperate earthworms renders difficult the analysis of the behaviour of tropical *Pheretima* towards temperature variation. An attempt, however, was made to correlate the results with the few data of the literature in order to check probable latitude effects and compensation for temperature. A slight indica-

tion of compensation in the European *Lumbricus herculeus* is suggested in view of the results obtained with *Pheretima,* although this findings is not supported by data obtained with *Glossoscolex*.

7. Old evidence (VERNON 1895, apud BULLOCK 1955) points to *Lumbricu's* ability to rapidly compensate for temperature variations. The work of KIRBERGER (1953) with *Eisenia* did not confirm this finding. *Pheretima* neither remained temperature-insensitive over the wide temperature range used (2°C-45°C) nor was able to restore the 25°C level after a 24 hour stay at 5°C. Thus, it does not seem to rapidly compensate for temperature variation. Whether or not it slowly compensates will be the object of a future research, which will include the responses of other tropical terrestrial Invertebrates to temperature variation.

CONCLUSÕES

1. O consumo de oxigênio de espécimes, jejunas de 24 horas, da minhoca *Pheretima hawayana* foi medido no escuro, em um aparelho de Warburg, a 36 agitações por minuto, sob condições variadas de tensão de 0_2 e temperatura.

2. Até 15% de 0_2, *P. hawayana* regula a respiração. A 10% de 0_2, sua taxa respiratória já é significativamente inferior à do ar. Depois, ela continua a declinar com a tensão de 0_2 decrescente.

3. Os dados da literatura acêrca das interrelações entre o consumo de oxigênio e a tensão de 0_2 nos oligoquetos terrestres foram revistos crìticamente, especialmente os obtidos com animais narcotizados, ressaltando-se o perigo dessa técnica.

4. *P. hawayana* aumenta sua taxa respiratória com o aumento de temperatura até 35°C. Essa temperatura pode ser considerada crítica no lado superior da gama de temperatura. A 40°C, o $Q0_2$ quase retorna ao nível de 25°C, mas isso apenas traduz um súbito grande aumento de metabolismo devido ao aumento de temperatura, seguido de um forte decréscimo devido ao dano térmico. A 45°C, êsse súbito aumento é seguido por um mais precoce e mais forte decréscimo, levando a um $Q0_2$ inferior ao normal. No fim dos experimentos a 40 e 45°C, os animais ficaram imóveis, hemorrágicos e superficialmente rompidos.

5. *P. hawayana* diminui regularmente o consumo de oxigênio quando a temperatura decresce de 25°C a 2°C. Os animais, porém, não pareceram afetados mesmo pelas temperaturas extremamente baixas usadas; após 90 minutos a 2°C, os animais pareciam em um estado de "narcose pelo frio" (cold narcosis), do qual emergiram ao cabo de algumas horas à temperatura ambiente.

6. A escassez de estudos cuidadosos das relações entre temperatura e respiração para as minhocas de clima temperado torna difícil uma análise do comportamento de *Pheretima* em face da variação de temperatura. Tentativa, todavia, foi feita no sentido de se correlacionar os resultados com os poucos dados da literatura, a fim de descobrir prováveis efeitos de latitude ou compensações. Leve indicação de compensação, isto é, retôrno respiratório ao normal ao cabo de prolongada permanência a baixa temperatura, foi sugerida para a espécie européia *Lumbricus herculeus,* em face dos dados obtidos para *Pheretima*.

7. Velho indício (VERNON 1895, apud BULLOCK 1955) sugere que *Lumbricus* ràpidamente compensa o metabolismo quando a temperatura varia. O trabalho de KIRBERGER (1953) não confirmou em *Eisenia* êsse resultado. *Pheretima* nem permanece insensível às variações de temperatura na ampla gama usada (2°-45°C), nem restaura o nível de 25°C respiratório após 24 horas a 5°C. Assim, não parece capaz de compensação rápida. Se compensa, porém, lentamente, será objeto de uma pesquisa futura, que incluirá também as respostas de outros invertebrados terrestres tropicais à variação de temperatura.

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