THE RESPIRATORY METABOLISM OF TROPICAL EARTHWORMS

II. Studies on the cutaneous respiration.

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INTRODUCTION

Respiratory exchange in the Oligochetes takes place generally through the body wall (STEPHENSON 1930, p. 181). This respiration may consist simply in a direct exchange through the parietes with an entire absence of parietal blood vessels (*Chaetogaster* and *Aeolosoma*) or the exchange may be facilitated by the presence of blood vessels. These may occur in a copious network on the inner face of the body wall, the vessels being applied to or branching on the inner face of the body wall without penetrating the parietes (many Limicolae) or parietal vessels may penetrate the substance of the body wall, sometimes sending capillaries loops into the epidermis. The last situation is found, as a rule, in all Oligochetes where the body wall is of any considerable thickness, but can also occur in thinwalled small Tubificidae.

The penetration of the epidermis by capillaries in Oligochotes has been known for a long time. CLAPAREDE (1869) in *Lumbricus* described that in the clitellar region the terminal branches of the vascular system push their way in among the cells, forming loops, which, however, stop short some little way below the surface. As to the vascularity of the rest of the epidermis, BEDDARD (1895, p. 75) claims to be the first to have pointed out that this was the case with the Megascolecidae *Pleurochaeta Moseley* (*Megascolex coeruleus*), some species of *Perichaeta* (*mostly Pheretima*) and with the Tubificidae *Limnodrilus*. In 1887, howe-

ver, ROSA (apud STEPHENSON l. c.) described blood capillaries in the epidermis of an aquatic Glossoscolecida (*Criodrilus lacuum*). Intraepidermal capillaries in Oligochetes have since been described in the Tubificidae again by NOMURA (1913, p. 25) and MARCUS (1942, p. 180), in species of Moniligastridae of the genus *Drawida* (apud STEPHENSON l. c., p. 183), in aquatic Glossoscolecidae such as *Alma nilotica* (GRESSON 1927) and in the terrestrial Megascolecida *Dichogaster budgetti* (BEDDARD 1900, apud STEPHENSON l. c., p. 183). As to the Lumbricidae, LENHOSSEK (1895, apud STEPHENSON l. c., p. 183) described intraepidermal vessels in *Lumbricus*, at some distance behind the clitellum, whereas SZÜTS (apud STEPHENSON l. c., p. 184) found an abundant system of epidermal capillary loops in *Allolobophora dubiosa*. No reference could be found in the literature with regard to the forms which are commonly found in Brazil, such as the Glossoscolecidae *Glossoscolex* and *Pontoscolex* and the Megascolecida *Pheretima*. As to the latter, although BEDDARD (l. c., p. 75) mentioned the occurrence of intraepidermal vessels in "Perichaeta", which might include some species of *Pheretima*, not even BAHL (1936, p. 35) in his Monograph on *Pheretima posthuma* is explicit enough about the morphological features of these blood vessels.

In the course of a series of studies on the respiratory metabolism of tropical earthworms, after a previous investigation of the normal respiratory rate, of the role of haemoglobin and of the relation between body size and respiratory metabolism (MENDES AND VALENTE 1953), the question of the cutaneous respiration assisted by epidermal capillaries emerged naturally. This paper reports the results from both morphological and physiological researches on the cutaneous respiration of our common earthworms. Morphological studies were performed in *Glossoscolex sp.*, *Pontoscolex sp.* and *Pheretima hawayana*. *Pontoscolex* and *Pheretima* were used in the experiments. Details of the techniques employed will be given below.

EXPERIMENTS AND RESULTS

a. *The intraepidermal capillaries of Glossoscolex, Pontoscolex and Pheretima*. In order to find out whether or not the ear-

thworms under investigation possessed intraepidermal capillaries, the animals were fixed in Bouin, after previous cleaning of the intestinal tract, and sectioned transversally. The sections were stained with hematoxilin Regaud. This histological procedure was good enough to make clear the existence of intraepidermal capillaries in the worms. Therefore, we did not make use of the so-called double method of Cajal employed by LENHOSSEK (l. c.).

Fig. 1 of the table I shows a transverse section from a region in the neighborhood of the clitellum of *Glossoscolex,* where the intraepidermal capillaries can be clearly seen in a relatively dense network. Figs. 2 and 3 of the same table show enlargements of another sections from the same region, where the shape of the epidermal loops can be better followed. The loops are double and the ascending limb divides about two thirds of the height of the epidermis in the manner of the letter Y, each branch of the Y forming a separate loop. Although it is not clear in the figures, the descending limbs of the loops unite again at the same level where they originated. These double loops were also found by LENHOSSEK (l. c.) in *Lumbricus*. However, he also found single loops which could not be detected in *Glossoscolex*.

Fig. 4 of table I shows a transverse section of *Pontoscolex* also from a region in the vicinity of the clitellum. It can be seen that the epidermal loops in this earthworm are also double and resemble in shape those of *Glossoscolex*. No single loops were also found here.

Fig. 5 of table I shows, finally, a transverse section through a region near the clitellum of *Pheretima*. The loops here are single and can be followed clearly from the basis of the epidermis. No double loops could be found.

In connection with the shape of the epidermal capillaries, it is interesting to note that in *Phere ima,* a member of the family Megascolecidae, the loops are single; in *Glossoscolex* and *Pontoscolex,* members of the fam. Glossocolecidae, the loops are double; and, finally, in *Lumbricus*, of the family Lumbricidae, the loops are single and double, according to LENHOSSEK.

b. *The action of external use of adrenalin and acetylcholine on the respiratory rate of Pontoscolex and Pheretima.* The fact that

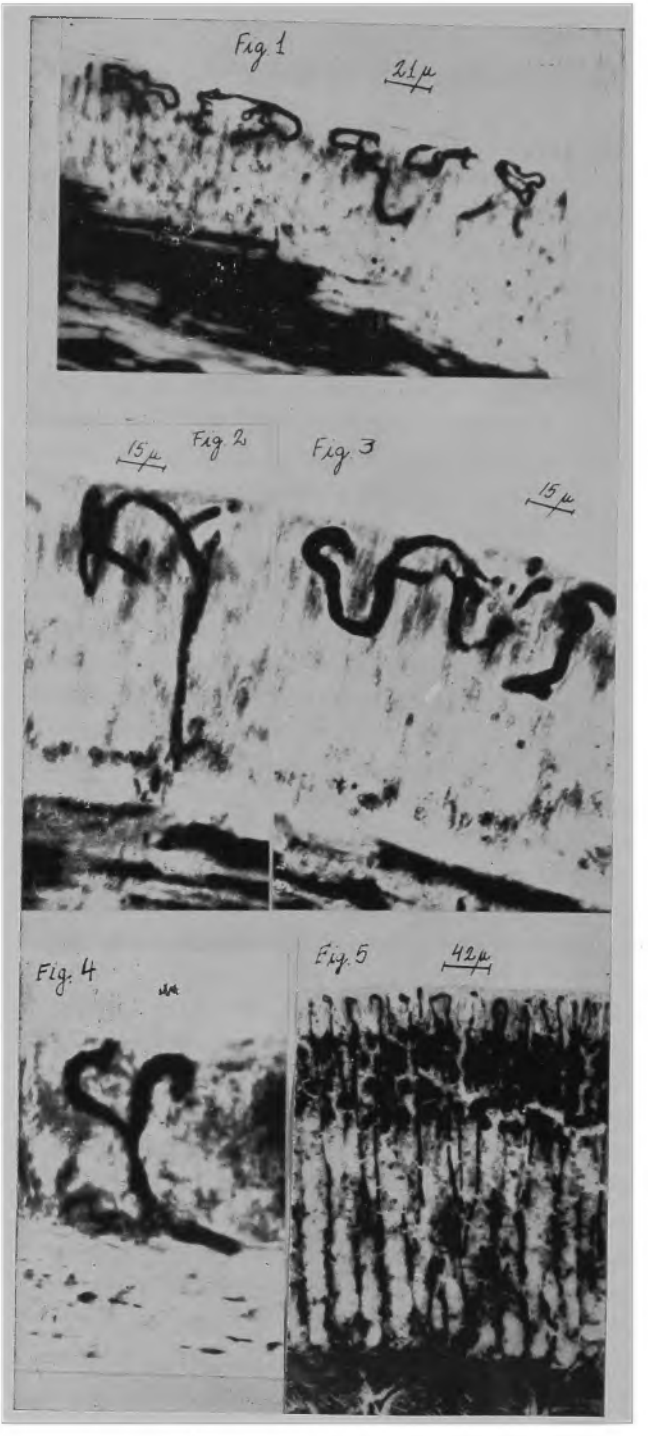

Fig. 1
Glossoscolex sp.
(near clitellum)

Figs. 2 & 3
Glossoscolex sp.
(near clitellum)
(Enlargements)

Fig. 4
Pontoscolex sp.
(near clitellum)

Fig. 5
Pheretima hawayana
(near clitellum)

TABLE I. Intraepidermal capillaries in Brazilian earthworms.

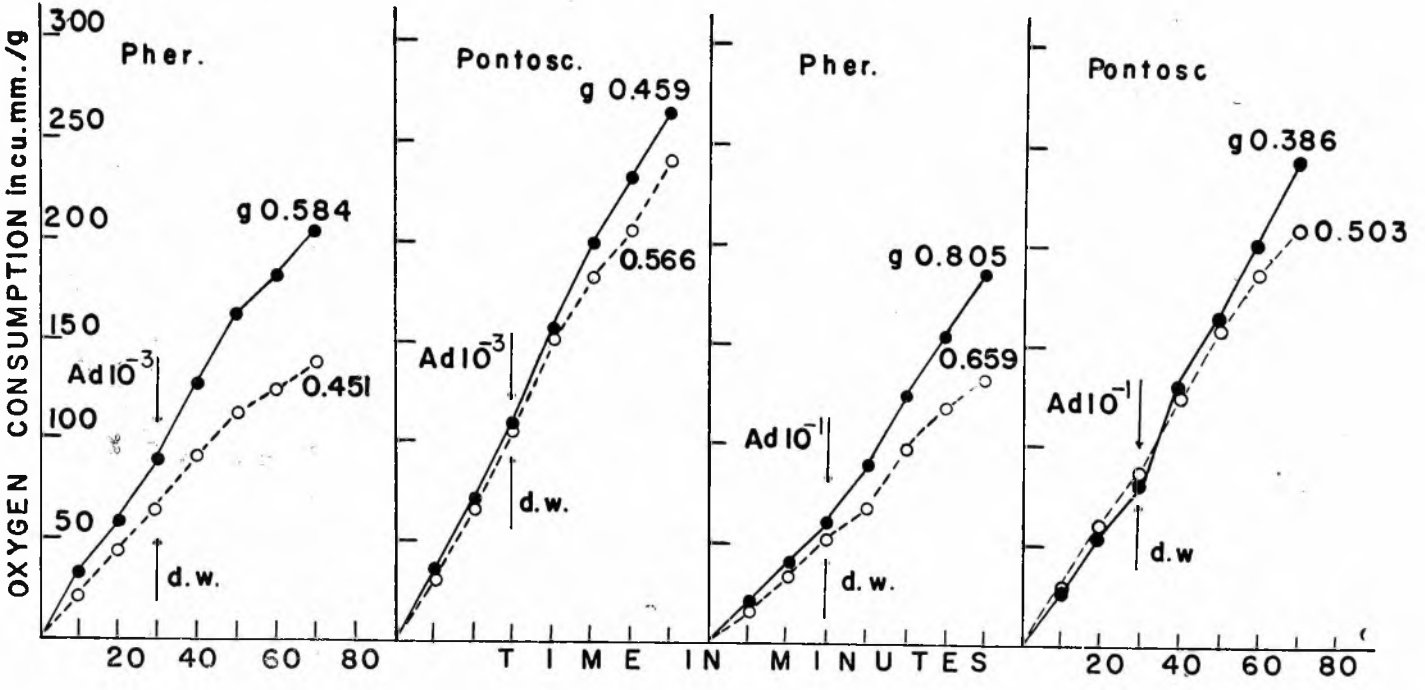

Fig. 6: The action of adrenal'n on the intraepidermal bloodvessels of of the earthworms *Pheretima* and *Pontoscolex*, as judged from the modification of the respiratory rate. The arrows indicate when the drug was tipped on the animals.

the Oligochetes studied exhibited intraepidermal capillaries suggested that there might be a peripheral control of respiration through variations in the caliber of these blood vessels and invited us to submit the animals to the external action of drugs having a known effect on capillaries.

The technique used to measure the respiration of the worms was in general similar to that of the previous work of the series (MENDES & VALENTE l. c.). The only modification was that a certain amount of the drug solution was added to the side bulbs of the Warburg vessels and care was taken to keep the animals from entering them by obstructing their entrance with a piece of filter paper. After placing the animals in the chamber, 12% KOH was added to the center well and 0.5 cc. of distiled water were pipetted into the side-bulb of half of the vessels (blanks) and 0.5 cc. of drug solution added to the bulb of the other half (experimental). The temperature of the bath was $25 \pm 0.5°C$, the vessels were gently and slowly shaken and the whole apparatus covered with a thick black cloth. After measuring the respiration during 15 minutes with 5 minute interval readings, the contents of the side-bulbs were tipped upon the worms and the eventual changes in the oxygen uptake were followed during the course of one hour.

Adrenalin (Parke and Davis Co.) and Acetylcholine (Roche Products Co.) were used as the substances to act upon the capillaries. Acetylcholine solutions were made up from the solid substance. Adrenaline solutions from the 10^{-3} solution contained in the ampules. When 10^{-1} Ad was prepared, solid substance also was employed. After the experiments the animals were dried and weighed. As a rule they endured the drug treatment and even when concentrations as strong as 10^{-1} were used they were at the end of the experiment apparently quite well.

Adrenaline was tested in the following concentrations: 10^{-5}, 10^{-3} and 10^{-1}. With 10^{-5} no changes in the respiratory rate were observed. With 10^{-3} (Fig. 6 graphs a and b) it is difficult to speak of any marked change in the oxygen uptake. With 10^{-1} very definitely, however, an increase of the respiratory rate took place both in *Pheretima and Pontoscolex* (Fig. 6, graphs c and d).

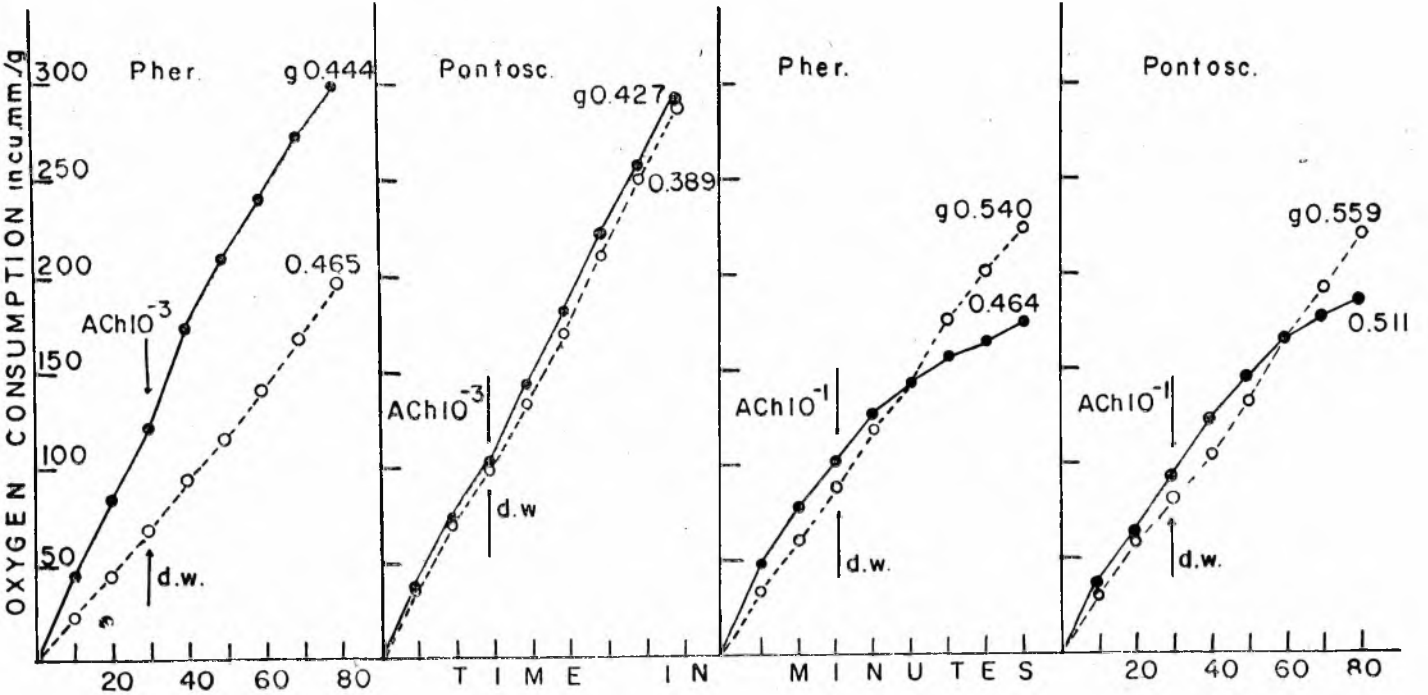

Fig. 7: The action of acetylcholine on the intraepidermal bloodvessels of the earthworms *Pheretima* and *Pontoscolex*, as judged from the modification of the respiratory rate. The arrows indicate when the drug was tipped on the animals.

Acetylcholine was also tested as 10^{-5}, 10^{-3} and 10^{-1} solutions. The graphs exposed in Fig. 7 are representative of the results generally obtained during the experiments with the two last concentrations. With 10^{-5} no changes were also observed in the respiratory rate. With 10^{-3}, as in the case of 10^{-3} Ad, it is hard to tell of any particular action of acetylcholine. With 10^{-1} a definite decrease in the oxygen consumption was observed, both in *Pheretima* and *Pontoscolex*.

The possibility of an acetylcholine breakdown by the mucous expelled by the animals was also checked, using a technique similart to that of AMMON (1934). In a first series of experiments, intact living animals were put in the vessels in atmosphere of 5% CO_2 in nitrogen and from the sidebulbs a 10^{-3} acetylcholine solution in bicarbonated saline (PROSSER & ZIMMMERMANN 1943, p. 78) was added to the main chamber. The results, although irregular, were never in the sense of a definite CO_2 production inside the vessels. Rather, gas absorption took place in some cases. In a second series of experiments, mucous was extracted from the animals with ether and dissolved in the main chamber. No CO_2 production was also observed in this series.

DISCUSSION

a. The penetration of an epithelial tissue by blood vessels, according to STEPHENSON (l. c.), is extremely rare. Besides the mentioned occurrences in Oligochetes, capillaries are also found in the epidermis of leeches and apparently in the ear of alligators and less clearly in the ear of rabbit and of man and in the olfactory mucous membrane of the guinea-pig (LENHOSSEK l. c.). From a list of SOUZA & SAWAYA (1928), however, it seems that the occurrence of intraepithelial capillaries is not so rare, since it was observed in many regions of the human body (in the male urethra by SOUZA & SAWAYA l. c.), in many other mammals, in birds, reptiles, amphibians and fishes. A great number of these alleged cases of penetration os blood vessels in epithelial tissues, however, are critized on the ground that due to poor histological technique the subcutaneous vessels were shifted into the epithelial layer and taken as intraepithelial. On the other hand, it is true that for ma-

ny of the reported occurrences of intraepithelial blood vessels it is hard to find a physiological meaning. In those cases, however, where it is possible to correlate the presence of intraepithelial capillaries with the fact that the regions where they occur serve to respiration, this peculiar position of blood vessels acquires an obvious physiological meaning. This situation is found in many fishes, such as *Cobitis* (LORENT 1878, apud WINTERSTEIN 1921, p. 145) and amphibians, such as Gymnophiona (SARASIN 1887, FURHMANN 1912, MENDES 1941 and GRINKRAUT 1949), Urodela (especially BETHGE 1897) and Anura (LEYDIG 1872, apud WINTERSTEIN l. c., p. 192, NOBLE 1925), where respiration through an epithelial layer facilitated by the presence of sub-or-intraepithelial capillaries is considered as an accessory or even, as in the case of the lungless Amphibians, the main respiratory process. The Oligochetes, in this respect, surely resemble the lungless Amphibians. Their lack of special respiratory organs is compensated for by the utilization of the entire skin as a sort of branchial organ and the efficiency of the skin as a respiratory organ obviously must be largely increased by the penetration of the capillaries into the outermost layers, as BEDDARD (l. c., p. 76) already recognized. In tropical earthworms such as *Glossoscolex sp.*, *Pontoscolex sp.* and *Pheretima hawayana,* which often are exposed to low tension of oxygen in the air of their burrows, the intraepidermal disposition of capillaries is certainly a great help in reckoning with such a difficulty.

The fact that in the Megascolecida *Pheretima hawayana* the intraepidermal loops are single, in the two genera of Glossoscolecidae (*Glossoscolex* and *Pontoscolex*) they are double and, finally, that in the Lumbricida *Lumbricus* the two types of loops are found, kept us wondering whether or not a correlation exists between the shape of these vessels and the position in the system, or, even, the mode of living. Unfortunately, due to the lack of information as to the shape of the intraepidermal capillaries in the majority of the reported occurrences, little can be advanced here with regard to this question. Capillaries ending blindly were mentioned in the epidermis of a *Microdrilum,* the Tubificida *Limnodrilus hoffmeisteri* by STEPHENSON (l. c., p. 183). MARCUS (l. c., p. 180),

however, could not confirm their existence. Besides *L. hoffmeisteri* was in 1935 identified with *L. socialis* (*gotoi*) by MICHAELSEN, where NOMURA (l. c., p. 25) described the vessels as forming loops. On the other end of the system, we find in the Lumbricida *Allolobophora dubiosa* a high degree of complexity of the intraepidermal capillaries. According to SZÜTS (l. c.) in this Oligochete the loops form complicating branchings of which "baskets" of capillaries are produced. These capillaries, as SZÜTS expresses it, strive to spread themselves out over as large a surface as possible, a disposition which is correlated by the author with the aquatic habit of the worm.

b) To our knowledge no experiments exist performed with substances affecting the capillaries in order to find out whether or not, by altering the caliber of sub-or-intraepithelial vessels in presumably respiratory surfaces, a modification of the respiratory rate would occur.

In dealing, however, with the external application of drugs, the question arises of whether the substances really permeated through the skin and reached the capillaries. In Crustaceans (PROSSER 1941, p. 1146) it was possible to make acetylcholine act upon the heart of the crayfish from a 10^{-4} solution, which entered the body by the gills alone. In our earthworms, from the position occupied by the capillaries in the epidermis, we think that the chances of the drugs having reached them were good, especially when strong concentrations were used. The only objection against this view derives from a possible barrier which the mucous expelled by the animals, as a reaction to the sudden contact with the fluids tipped from the sidebulbs, would oppose to the penetration of the drugs. This mucous may have acted as a physical barrier or, even, by neutralizing the action of the substances. This would also explain why only with very strong concentrations, such as 10^{-1}, definite effects of acetylcholine and adrenalin were observed. The possibility of an acetylcholine breakdown, however, by the mucous alone was dismissed by the absence of any cholinesterase powers revealed by the tests.

Adrenalin and acetylcholine, when used in concentrations strong enough (10^{-1}) to overcome the mucous barrier, caused respective-

ly and increase and a decrease of the respiratory rate. The observed effects canot be atrributed to modifications of the muscular activity of the animals. After the first contact with the sidebulb contents, whether distiled water or drug solution in any of the concentrations used, the animals would become a little agitated and then stay quiet in the vessels with occasional crawlings. We, therefore, are inclined to admit that the effects observed were possibly due to drug action upon the capillaries, from which resulted modifications of the blood flow and ultimately of the oxygen uptake. From the observed increase of the respiratory rate obtained with 10^{-1} Ad, the drug might be considered as producing vasodilatation in earthworms. From the decrease obtained with 10^{-1} ACh, constriction power might be attributed to this drug. This is the reverse of what is observed in the Vertebrates. This reverse action of adrenaline and acetylcholine upon the Oligochetes effectors was, in a way, also observed in the case of the heart, where PROSSER & ZIMMERMANN (l. c.) found that acetylcholine can stop in systole the hearts of *Arenicola* and *Lumbricus* (it blocks the frog heart in diastole) whereas, at least in *Arenicola,* adrenaline arrests the heart in diastole (it stops the frog heart in systole).

SUMMARY

1. Studies on the cutaneous respiration of one Megascolecida (*Pheretima*) and two Glossoscolecidae (*Glossoscolex* and *Pontoscolex*) were performed in order to find out whether, in these earthworms, intraepidermal capillaries occurred or not and, in the case of a positive answer whether or not these capillaries might be influenced by drugs having a known effect in other capillaries.

2. Intraepidermal capillaries exist in the three earthworms studied. In *Pheretima* they have the shape of single loops (table I, fig. 5), whereas in *Glossoscolex* and *Pontoscolex,* the intraepidermal loops are double (Table I, figs. 1, 2, 3 and 4).

3. Attention is called to the fact the shape of the capillaries seems to be a constant of the families: the Megascolecida *Pheretima* with single intraepidermal loops, the Glossoscolecidae *Glossoscolex* and *Pontoscolex* with double loops and the Lumbricida *Lum-*

bricus (LENHOSSEK l. c.) with single and double loops. The necessity of more detailed information on the shape of the intra-epidermal capillaries in Oligochetes is stressed in order to establish possible phylogenetical and ecological correlations.

4. When used in concentrations strong enough to overcome the mucous barrier opposed by the animals (10^{-1} solutions) adrenalin and acetylcholine caused respectively an increase and a decrease of the respiratory rate.

5. No cholinesterase activity was detected in the mucous extracts.

6. From the increase of the respiration obtained with adrenalin, the assumption is made that under the action of the drug a larger flow of blood went through the capillaries, as a consequence of their dilatation. From the decrease of respiration, on the other hand, observed with acetylcholine, a constriction of the capillaries is suggested under the action of the drug, which resulted in a smaller blood flow.

7. These action of adrenalin and acetylcholine upon capillaries is the inverse of what is observed in vertebrates, for instances, in the frog. The fact that acetylcholine causes arrest in systole of the hearts of *Arenicola* and *Lumbricus* and, in *Arenicola* at least, adrenalin causes heart blocks in diastole, is recalled.

SUMÁRIO

1. Foram estudados alguns aspectos da respiração cutânea de um Megascolecideo (*Pheretima*) e dois Glossoscolecideos (*Glossoscolex* e *Pontoscolex*), a saber, a existência nesses 3 oligoquetos terrestres tropicais de capilares intraepidérmicos tais como foram descritos em *Lumbricus* por LENHOSSEK (1895) e, no caso de uma resposta positiva, se êsses capilares poderiam ser afetados por drogas que têm uma ação conhecida sôbre outros capilares.

2. Capilares intraepidérmicos existem nos 3 vermes estudados. Em *Pheretima*, têm êles a forma de uma alça simples (Fig. 5 da tab. I), enquanto que em *Glossoscolex* e *Pontoscolex* as alças intraepidérmicas são duplas (Figs. 1, 2, 3 e 4 da tab. 1).

3. Chama-se a atenção para o fato de que a forma dos capilares parece ser uma constante das famílias: O Megascolecideo

Pheretima com alças simples, os Glossoscolecideos *Glossoscolex* e *Pontoscolex* com alças duplas e o Lumbricideo *Lumbricus* (LENHOSSEK l. c.) com alças duplas e simples. Acentua-se a necessidade de estudos mais detalhados sôbre a forma dos capilares intraepidérmicos nos Oligoquetos para as correlações filogenéticas e ecológicas.

4. Quando empregadas em concentração suficientemente forte para vencer a barreira de muco expelida pelos animais (10^{-1}), adrenalina e acetilcolina causaram respectivamente um aumento e uma diminuição da taxa respiratória em *Pheretima* e *Pontoscolex*.

5. Não foi observada atividade colinesterásica em extratos de muco.

6. Do aumento respiratório obtido com adrenalina, pressupõe-se que sob a ação da droga ocorreu um maior fluxo de sangue pelos capilares intraepidérmicos, como conseqüência de sua dilatação. Da diminuição observada com a acetilcolina, sugere-se uma vaso-constrição dos elementos situados intraepidermicamente, de que resultou menor afluxo de sangue.

7. Essas ações da adrenalina e da acetilcolina sôbre capilares é o inverso do que se observa nos vertebrados, p. ex., na rã. De um certo modo, essa inversão de ação das duas drogas também se observa em outro efetuador dos Oligoquetos, a saber, o coração. Aqui, PROSSER & ZIMMERMANN (1943) demonstraram que acetilcolina pode parar em sistole o coração de *Arenicola* e *Lumbricus* (ela bloqueia o coração da rã em diastole), enquanto que a adrenalina, pelo menos em *Arenicola*, para o coração em diastole (na rã o coração é detido em sistole).

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