# ON THE FUNCTION OF HAEMOGLOBIN IN LIMNIC OLIGOCHAETA

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## 1. INTRODUCTION

There is no agreement so far as to the function of haemoglobins in Invertebrates. According to one view, at normal or high oxygen pressures, they would merely store oxygen, being a task of the blood plasma the transport of this respiratory gas to the tissues in a state of physical solution. Only at low oxygen tensions would haemoglobins take a real part in the respiratory gas exchanges. This belief originated from the fact that Invertebrate haemoglobins possess, as a rule, low unloading tensions. It is also based on experiments in which, by physiological suppression of haemoglobin, no respiratory depression was observed unless the animals were placed in a medium possessing a very low oxygen tension. Nevertheless, the litterature on the subject is relatively rich in reports intending to prove that haemoglobins in Invertebrates act exactly as in Vertebrates, being responsible for the transport of oxygen to the tissues even at the atmospheric oxygen pressure. Respiration, according to these reports, is significantly depressed whenever, by a proper technique, haemoglobins are put out of action. Compreensive reviews being numerous (BARCROFT 1925; DAUSEND 1931; REDFIELD 1933; HARNISCH 1935; EWER 1942; JOHNSON 1942; FLORKIN 1948 and so on), we shall here only summarize the state of the question in the Oligochetes, since we chose specimens of *Limnodrilus* for an attempt of solution of this controverted problem in the Annelids.

DAVY (1892, apud JOHNSON 1. c., p. 266) claimed that haemoglobin in the blood of the earthworm (*Lumbricus*) has a respiratory function. However, this claim was not based on experiments in which, taking advantage of the fact that haemoglobin has a greater affinity for carbon monoxide than for oxygen, the oxygen uptake of normal individuals was compared with that of animals whose haemoglobin had been put out of action by saturation with carbon monoxide. JORDAN and SCHWARZ (1920), using this technique, verified that, in *Lumbricus*, haemoglobin acts as an oxygen carrier only at low $O_2$ tensions (23-30 mm Hg). These results, however, were criticized by DOLK and VAN DER PAAUW (1929) on the grounds that the pure CO to which the worms were subjected for some hours before the experiment have affected

the respiratory enzymes as well as haemaglobin. Besides, JORDAN and SCHWARZ drew conclusions from experiments in which the $O_2$ consumption of normal animals was compared at the same time with that of CO-treated ones and this could not have been done on account of individual variations. DOLK and VAN DER PAAUW, using the same lot animals, compared the respiratory rates in mixtures of $N_2/O_2$ and $N_2/CO/O_2$ of the same $O_2$ percentage, but, even so, they did not obtain essentially different results: only at an $O_2$ pressure less than 57 mm Hg respiration depressed in CO-treated animals. THOMAS (1935) criticizes these results because they were obtained with narcotized animals. Moreover, DOLK and VAN DER PAAUW seem to have draw conclusions which are not justified by their experimental data. His reinvestigation of the matter, however, did not shed more light on the problem. According to his results, haemoglobin in *Lumbricus* has no respiratory function at pressures lower than 114 mm. since the oxygen consumption determined for animals treated by CO or not was the same in such cases. Above 114 mm. the $O_2$ uptake of normal animals increased sharply, while that of CO-treated ones remained constant. KRÜGER (1938, apud JOHNSON 1. c., p. 267) seems to be the first to give an experimental support to DAVY's claim. He determined that in *Lumbricus* haemoglobin transports oxygen even at atmospheric $O_2$ pressure, since in his experiments the $O_2$ uptake of CO-treated worms was lower than that of normal at pressures of 152, 114, 76 and 38 mm. JOHNSON (1. c.), however, complains of the fact that KRÜGER did not add to his paper details of the technique used. For this reason, JOHNSON re-investigated the matter once more, obtaining results very similar to those of KRÜGER. The rate of oxygen consumption of CO-treated worms was significantly inferior to that of normals at $O_2$ pressures of 152, 76, 38 and 19 mm., but not at 8 mm.

Concerning the studies with limnic Oligochetes, the present state of the question is more or less the following. DAUSEND (1. c.) determined that the oxygen consumption of *Tubifex* treated by CO is less than that of normal worms even in water saturated with air. HARNISH (1. c.), on the contrary, verified that *Tubifex* haemoglobin does not carry oxygen at atmospheric $O_2$ pressure. JOHNSON (1. c.), among others, argues that DAUSEND's results are right and HARNISCH's not, because the latter's technique was not good. However, although recognizing that JOHNSON's criticism to HARNISCH's technique is partially right, we consider that DAUSEND's work is not less free of this kind of criticism. Therefore, we decided to reinvestigate the problem, using individuals of *Limnodrilus*. Besides we thought it worth while determining the behaviour of haemoglobin in an Oligochete living under tropical conditions.

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## 2. MATERIAL AND METHODS

The Oligochetes were obtained from a ditch in Pirituba, in the surroundings of S. Paulo. With Mrs. EVELINE du BOIS-REYMOND MARCUS' indispensable help, they were identified as belonging to the genus *Limnodrilus*, very possibly *Limnodrilus hoffmeisteri* (cf. MARCUS 1942, p. 167). In the laboratory the animals were kept in large Petri dishes containing well aerated

water and fed with boiled lettuce leaves (cf. HYMAN 1938, p. 143). We never used animals which stayed in the laboratory for more than ten days. For the experiments, the worms were carefully removed from the dishes by means of Watchmaker's forceps. Any kind of narcotics was thus avoided in the operation. Extreme care was taken in order of not injuring the animals selected for the experiments. From the large dishes the animals were taken to small ones of the so-called "stender-dish" type and there, in clean water, they stayed over-night. As a rule, 30 worms were put in each stenderdish and a period of time of at least 16 hours and not exceeding 20 elapsed before these worms were finally placed in the respirometer flasks. The respiromter was a BARCROFT-WARBURG apparatus. 30 worms *plus* clean tapwater (pH = 7.9–8.4), measuring exactly 2 cc., were placed in each of the WARBURG flasks. The average capacity of the flasks was 15 cc. (20° C). For the $CO_2$ absorption, 0.3 cc. of 10% NaOH and the filter roll recommended by DIXON and ELLIOT (1930) were added to the center-well of the flasks. After fastened to their respective manometers, the flasks were placed in a bath at 20 $\pm$ 0.2° C and were shaken at 60 complete oscillations per minute. After allowing 10 minutes for temperature and pressure equilibration, the manometer taps were closed and at the end of a 15 minute interval the zero readings were taken. Readings of all manometers were posteriorly taken at 15 minute-intervals, during one hour. This first part of the experiment intended to furnish data on the normal $O_2$ uptake of the worms at temperature given. In preparation for the second part of the experiment, half of the experimental flasks were perfused with a mixture of $N_2$ and $O_2$ of the desired percentage of both components. The other half with a mixture of $N_2$, CO and $O_2$ in which the $O_2$ percentage was the same as in the former mixture. After the perfusions were over, a new hourly $O_2$ uptake was determined for the animals in each flask, using the same procedure of the first part. In these circunstances, it was possible to determine, by comparing the respiration of the same lot of animals, (a) the influence of $O_2$ tension variation upon the $O_2$ uptake of *Limnodrilus*, (b) the influence of CO upon the $O_2$ consumption at various $O_2$ tensions and, therefore, the role of haemoglobin in *Limnodrilus*. More details about the technique used will be found in the following chapters reporting the results obtained.

## 3. EXPERIMENTAL PART

a. The Relation between the $O_2$ consumption and the $O_2$ tension in *Limnodrilus*.

The relation between oxygen consumption and oxygen tension in animals is still a controverted matter. We can't help considering it here, because in any study concerned with the role of haemoglobins in respiration, a first necessary step in the investigation is to determine in what degree does the respiration of the animal studied depend upon the oxygen tension of the surroundings. Since, however, there is no place here for a review of the present state of the question, we can only refer the reader to DAUSEND (1. c.), HARNISCH (1. c.) and HYMAN (1929) and briefly summarize only what is known in the case of the Oligochetes.

KONOPAKI (1907), as THUNBERG (1905), found out that in *Lumbricus* the $O_2$ consumption depends upon the $O_2$ concentration : when the latter decreases from 20 to 10%, the former also decreases, first slowly then sharply. DOLK and VAN DER PAAUW (1. c.), on the contrary, using a better method (KROGH'S respirometer), reported that *Lumbricus* can regulate respiration down to 2.5%. This result, however, has been criticized for not being in agreement with the $O_2$ pressure determined in the *Lumbricus* tissues : 1.9% at ordinary tensions. Nevertheless, THOMAS (1. c.), in a way, confirmed DOLK and VAN DER PAAUW'S results, when he found out a constant consumption of oxygen in *Lumbricus* between 15 and 5%. In 1942, JOHNSON determined again, in *Lumbricus*, the relation of $O_2$ consumption to $O_2$ concentration. He found out that, at 10° C, and in the dark, the rate of $O_2$ consumption was significantly the same at 152 (20%) and 76 (10%) mm. Hg ; below 76 it fell sharply. For the limnic Oligochetes, DAUSEND (1. c.), in *Tubifex*, determined that the $O_2$ uptake always depends upon the $O_2$ tension of the water. It decreases with the latter : first slowly ; between 1.5–1.0%, however, it falls sharply. HARNISCH (1936), on the contrary, reported that in his experiments with *Tubifex* in good initial respiratory conditions (hence, in "primary oxybiosis") the $O_2$ uptake was essentialy the same between 100% and 3% $O_2$. Below 3% the $O_2$ consumption was about one third of that observed at 7%.

*Experiments*

In our experiments with *Limnodrilus*, we first determined the hourly $O_2$ consumption in common air of animals which had been kept previously in clean and well aerated water for 16–20 hours. Then, the manometer taps and the gas vents of the side-bulbs were opened and the flasks perfused during 30 minutes with a mixture of nitrogen and oxygen of the desired percentages of both components. The nitrogen used in the mixture, before entering the gasometer, was twice washed in bottles containing an alkaline solution of pyrogallol in order to remove as much as possible traces of oxygen. During the perfusion the flasks were gently shaked. This *plus* the fact that inside the flasks there was only a very shallow amount of water assured the necessary physical conditions for the attainment of an equilibrium between the water and the new atmosphere introduced by the perfusion. A new hourly $O_2$ consumption was, then, determined and at the end of the experiment the animals were carefully removed from the flasks, dried in a oven at 110° C and finally weighed.

The results are exposed in table 1 and in the curve of fig. 1. The mean oxygen consumption of *Limnodrilus* in the second hour of the experiments is less than in the first even when the water inside the flask continued to be in equilibrium with common air (ca. 89.7% of that of first hour). When the experimental water was equilibrated with 15% oxygen, the decrease in respiration was essentially the same (89.8%). After the perfusion of the flasks with atmospheres containing 10% oxygen, the mean respiratory rate expressed in percents of respiration in normal conditions, was reduced to 76.8.

In order to determine how far *Limnodrilus* could sustain a relatively high respiratory rate under very low oxygen concentration, we did a series of experiments, in which the animals, in the second hour, were submitted

## TABLE 1

Mean rates of oxygen consumption (at 25° C) and deviation from normal (in %) of *Limnodrilus* submitted or not to 20% CO at various oxygen pressures.

| Experimental Series | N.° of experiments | Partial pressures of oxygen (in percents) | | Rates of oxygen uptake in cu.mm./mg(dry)/hr. | | | | Mean rate in 2nd hour as % of 1st hour |
|---|---|---|---|---|---|---|---|---|
| | | | | 1st hour | | 2nd hour | | |
| | | 1st hour | 2nd hour | mean | Std. dev. | mean | Std. dev. | |
| 1 ..... | 8 | 21 | 21 | 1,01 | 0,14 | 0,90 | 0,13 | 89,7 |
| | 8 | 21 | 21+20% CO | 1,05 | 0,11 | 0,64 | 0,06 | 60,7 |
| 2 ..... | 10 | 21 | 15 | 1,08 | 0,15 | 0,97 | 0,16 | 89,8 |
| | 10 | 21 | 15+20% CO | 1,08 | 0,16 | 0,71 | 0,19 | 65,2 |
| 3 ..... | 8 | 21 | 10 | 0,95 | 0,18 | 0,92 | 0,17 | 98,1 |
| | 7 | 21 | 10+20% CO | 0,99 | 0,24 | 0,77 | 0,17 | 77,7 |
| 4 ..... | 5 | 21 | 5 | 0,79 | 0,33 | 0,68 | 0,36 | 90,4 |
| | 6 | 21 | 5+20% CO | 1,03 | 0,34 | 0,66 | 0,08 | 64,5 |
| 5 ..... | 5 | 21 | 2,5 | 0,97 | 0,15 | 0,82 | 0,19 | 84,2 |
| | 6 | 21 | 2,5+20% CO | 1,03 | 0,14 | 0,65 | 0,18 | 61,6 |
| 6 ..... | 6 | 21 | 1 | 1,13 | 0,08 | 0,87 | 0,08 | 76,8 |
| | 6 | 21 | 1+20% CO | 1,14 | 0,09 | 0,66 | 0,03 | 60,1 |

to $N_2/O_2$ = 99/1 and to nitrogen only (twice washed in alkaline pyrogallol) after more prolonged perfusions of the flasks with such gas mixtures. Table 2 summarizes the results.

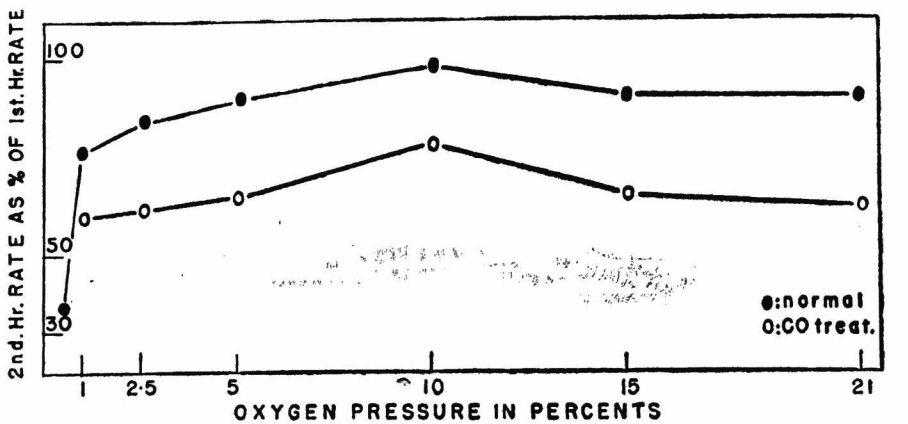


Fig. 1 — Rates of oxygen consumption of normal and CO-treated *Limnodrilus*, in the 2nd. hour of the experiments, expressed as % of rate in 1st. hour.

**TABLE 2**

| N.° of Expers. | Atmosph. in 2nd. h. | Duration of. perf. | Mean respiratory rate in 2nd. h. as % of lst. | Standard deviation |
|---|---|---|---|---|
| 2 | $N_2/O_2=99/1$ | 45' | 73 | — |
| 6 | $N_2/O_2=99/1$ | 60' | 63 | 0.08 |
| 6 | $N_2$ | 60' | 36 | 0.10 |

It can be seen, that even after doubling the duration of the perfusion, *Limnodrilus* continued to show a relatively high respiratory rate at 1% oxygen. In the nitrogen used, respiration went down to 36%, but no complete depression was ever observed (lowest value : 18%).

b. The action of carbon monoxide on the respiration of *Limnodrilus* at various oxygen tensions.

In order to test the influence of carbon monoxide on the oxygen consumption of *Limnodrilus*, the flasks, in which the animals had been kept during the first part of each experiment, were perfused with appropriate mixtures of CO, $N_2$ and $O_2$. These mixtures were prepared as follows. Carbon monoxide was obtained by adding sulphuric acid to boiling formic acid. The gas, before entering the gasometer, was twice washed in concentrated KOH. The $N_2$ was purified with alkaline pyrogallol. The flasks were perfused during 30 minutes, in the dark, with gentle shaking. Then the gas vents and the manometer taps were quickly closed and after 15 minutes a new series of 15 minutes interval readings took place, the flaks being kept in the dark all the time. The results are exposed in table 1 and in the curve of fig. 1. They clearly show that when *Limnodrilus* is exposed to 20% CO, respiration is significantly depressed even at the atmospheric partial pressure of oxygen. At 15%, 5%, 2.5% and 1% a value approximately the same was found for depression. In agreement however with the rise observed at 10% in the $O_2$ uptake of normal *Limnodrilus*, the depression observed at this tension in the CO-treated individuals was also smaller (77,7% of 1st. hour). Anyway, it seems reasonable to say that, according to our results, *Limnodrilus*, when submitted to 20% CO, shows a significant depression in the respiratory rate at 21, 15, 10, 5, 2.5 and 1% oxygen.

## 4. DISCUSSION

a. *Limnodrilus* maintains a relatively high respiratory rate down to extremely low oxygen tensions of the surroundings. This agrees with the type of *habitat* of the oligochete. In fact, *Limnodrilus*, as *Tubifex* (v. BRAND 1946, p. 63), lives in the bottom of polluted lakes and streams and is hence supposed to endure, at least temporarily, an anaerobic or nearly anaerobic surrounding. RICHARDSON (1925, p. 346) found *Limnodrilus* in one situation where the dissolved oxygen was under one part per million.

Our results (table 1) indicate that the mean oxygen uptake in the second hour of the experiment is lower than that of the first hour even when the water in the WARBURG flasks continued to be in equilibrium with an atmosphere containing 21% oxygen. The depression observed, however, can only reflect a diminished muscular activity in the second hour due a recovery from the disturbance caused by setting up the experiment. Since, on the other hand, the depressions observed in the second hour, when the water was put in equilibrium with 15, 10 and 5% $O_2$, do not essentially differ from that at 21%, they are not to be interpreted as due to the oxygen impoverishment of the water. They also reflect a diminished activity of the worms. From 2.5% downwards stronger depressions were observed, but they are far from indicating a complete loss of *Limnodrilus* ability of regulating respiration. At 1% oxygen, the respiratory rate was still as high as 76.8% of that observed in normal conditions. We tried to find out whether with longer perfusion significantly stronger depressions could be observed in the second hour at 1% (Table 2). The results indicate that the duration of the perfusions has a certain importance, but not an essential one. As to the result obtained with supposedly pure nitrogen (table 2), that is, a mean depression of 36% in six cases, they can only be explained by admitting that the nitrogen used still contained traces of oxygen. Therefore, this result can be used, as it was in the curve of fig. 1, as representative of the depression at an oxigen tension between zero and 1%.

The results obtained with *Limnodrilus* agree with those of HARNISCH (1. c.) for *Tubifex* and are in opposition with those reported by DAUSEND (1. c.) also for *Tubifex*. In attempting to find out the reason why *Tubifex*, in DAUSEND'S experiments, was unable of regulating respiration, it is first necessary to compare his technique with that used by HARNISCH and in the experiments with *Limnodrilus*. In DAUSEND'S experiments, the renewal of the oxygen content of the experimental water followed the procedure commonly used when the WINKLER method is employed for the oxygen measurements. First, the respiratory rate of animals kept in large containers filled with well aerated water was measured. Then, this water was gradually replaced by another one containing the desired oxygen content. This process took place in half an hour and was followed by a new determination of the respiratory rate. HARNISCH used a procedure which is fundamentally the same one adopted in the experiments with *Limnodrilus*: he measured the oxygen consumption in a WARBURG apparatus, but during 2–3 hours, readings being taken each half an hour. Oxygen tensions lower than in the air were obtained by mixing nitrogen with air and the manometers were twice perfused with such mixture, shaken at five minute intervals. No precise indications, however, are given as to the shaking rate during the experiments or to the duration of the perfusions.

DAUSEND reported that the oxygen uptake in *Tubifex* is widely dependent upon the oxygen concentration and explained it by admitting that there is a permanent oxygen defficit in *Tubifex* tissues due to the small permeability rate through the respiratory sufaces. In favor of this hypothesis, he recalls that in *Tubifex* there are always anoxybiontic processes going on, event at high oxygen tensions. HARNISCH, however, objects against this view. The oxygen uptake in animals, he points out, independs of the oxygen concentration as long as the oxygen can reach the deepest tissues,

i. e, as long as there is in them a positive oxygen pressure. Or, as PFLÜGER (1872) put it, as long as the cell can have enough oxygen, it regulates its own needs, the limiting factor of metabolism being not the oxygen from the outside but the amount of stuff to burn. Only in those animals with inadequate respiratory and circulatory mechanisms, such as *Actinia, Anemonia, Sipunculus* and others, the oxygen uptake depends of the oxygen tension for the simple reason that, in such cases, there must be tissues where the oxygen pressure is constantly tending to zero (HENZE 1910, p. 271). *Tubifex*, says HARNISCH, is provided with a well developed circulatory apparatus and an oxygen carrying compound and is, accordingly, expected to be highly independent of the oxygen concentration. He prefers to explain DAUSEND'S results by the admission that, in his experiments, DAUSEND employed individuals which had an oxygen debt and, in that case, as HARNISCH himself demonstrated, the respiratory rate depends upon the oxygen concentration : the higher the oxygen tension, the quicker the oxygen debt is paid off. Now, it is not resonable to admit that with DAUSEND'S procedure his animals contracted oxygen debt at the beginning of each experiment. On the contrary, DAUSEND seems to have taken good care in order to prevent that to happen (see his page 364). It is therefore hard to explain why *Tubifex*, in DAUSEND'S experiments, showed such a wide dependence upon the oxygen concentration. The only possible criticism to DAUSEND'S way of investigating is against his procedure of measuring the oxygen uptake, which involves the well known restrictions of the WINKLER method.

In our experiments with *Limnodrilus* there is reason to believe that, with the perfusion procedure adopted, the water in the WARBURG flasks, at the beginning of the second part of the experiment, was really in equilibrium with the new atmospheres introduced inside the flasks. We also believe that our animals, at the beginning of the first hour or of the second, had no oxygen debts, since they were previously kept over-night in a shallow amount of well aerated water and stayed in the vessels, before the perfusions, only a short period of time (one hour). We cannot think of a better way of interpreting our results than by accepting HARNISCH'S view on the subject. *Limnodrilus*, as *Tubifex*, is a slender animal in which probably respiration is carried on through the whole body surface. Furthermore, this oligochete possesses a well developed circulatory system. It is, therefore, in position to sustain a relatively high respiratory rate even at extremely low oxygen tensions.

b. *Limnodrilus*, when exposed to 20% CO, shows a respiratory depression at 21, 15, 10, 5, 2.5 and 1% oxygen. This depression is more or less the same at each of the $O_2$ tensions tested, the hourly $O_2$ consumption of the CO–treated animals being around 60% of that of the same individuals determined, just prior to the CO-exposure, in normal conditions.

These results are in accordance with those reported by DAUSEND (1. c.) for *Tubifex* and disagree with those of HARNISCH also for *Tubifex*. DAUSEND accepts the view that Invertebrate haemoglobins possess, as a rule, very low unloading tensions. Accordingly, they enable the animals to endure very poor oxygen conditions. He, therefore, admits that only at very low oxygen tensions, the Invertebrate haemoglobins become an important agent in the oxygen transport to the tissues, while at high oxygen tensions the oxygen physically dissolved in the blood is enough for the tissues needs.

In *Tubifex*, however, he states, since, for particular reasons, the oxygen consumption depends of the oxygen tensions, things would be entirely different. He compared the oxygen curves he drew for normal and CO-treated worms and observed that : 1. the amount of oxygen that is given up to the tissues through haemoglobin is over a wide range independent of the oxygen concentration ; 2. haemoglobin in *Tubifex*, down to 1 c.c. oxygen per litre, can be completely saturated with oxygen, hence, it must have a loading tensions less than 25 mm. Hg. Thus, when *Tubifex* is exposed to oxygen tensions lower than normal, the respiratory depression observed would not be a consequence of haemoglobin incapacity of fixing the amount of oxygen required by the tissues, but of the relatively small respiratory surfaces and little capacity of the blood plasma to transport physically dissolved oxygen. On the contrary, haemoglobin with such a low loading tensions is of great help in these circunstances especially when there is a permanent oxygen defficit in the tissues, as DAUSEND admitts for *Tubifex*. DAUSEND also believes that *Tubifex* respiratory dependence of the oxygen concentration is related to the fact that this Oligochete possesses a very small quantity of haemoglobin in its blood.

HARNISCH was only concerned with the respiratory rate of *Tubifex*, with and without secondary oxybiosis, in a atmosphere containing 10% CO and a partial oxygen pressure approximately equal to the atmospheric. Animals without any oxygen debt (hence, in primary oxybiosis) showed no respiratory depression, but those with oxygen debt (hence, in secondary oxybiosis) did show, according to HARNISCH, a significant depression. He concluded from these results that haemoglobin in primary oxybiosis does not carry oxygen to the tissues, but merely store it. However, in secondary oxybiosis, it really takes part in the process of paying off the oxygen debt. In commenting DAUSEND's results, HARNISCH again suspects that DAUSEND's animals were rather what the calls "$N_2$ — animals" (in secondary oxybiosis) than "$O_2$ — animals" (in primary oxybiosis).

Now, it is hard to compare our results with DAUSEND's and HARNISCH's, since we believe that both authors did not use the proper technique when they submitted *Tubifex* to the CO-treatment. DAUSEND gives no indications as to the maintainance of the CO-treated animals in the dark, during the experiments. Besides, the CO percentage of the gas mixtures which perfused the experimental water was too low (1.5%). Thus, we cannot be sure that in DAUSEND's measurements haemoglobin was really inhibited by CO. The question of the proper percentage of carbon monoxide to be used in this kind of experiment was particularly focused by both EWER (1. c.) and JOHNSON (1. c.) They criticized HARNISCH (1936) because he used 20% CO in his experiments with *Chironomus larvae*, not taking into account the possible direct effect of CO on cellular oxidations. They argued that, since in HARNISCH's experiments the amount of oxygen varied from 80 to 0.8%, the relative pressure of CO increased from 0.25 to 25 as the oxygen concentration decreased. A direct effect of CO, becoming more marked as its relative pressure increased, would then be responsible for the results rather than the elimination of haemoglobin. EWER and JOHNSON, therefore, in their experiments, decreased the CO percentage in accordance with the oxygen tension used. For instances, when JOHNSON, in the earthworm, used 8 mm. Hg of oxygen (ca. 1%), only 19 mm. Hg. (ca. 2%) CO was employed. There-

fore, JOHNSON never went so low as using 1.5% as DAUSEND did. Besides, JOHNSON controled spectroscopically in all cases the conversion of haemoglobin into the carboxy-compound. DAUSEND did not add to his paper this valuable information. HARNISCH also omitted to tell whether he placed his CO-treated animals, in the dark or not. Besides, with his technique of poisoning haemoglobin with CO, putting his animals first in an atmosphere of pure CO, one can never be sure that no oxygen debt was produced. HARNISCH himself admits that *Chironomus* larvae (*Tubifex* too ? He does not mention it.), after 10 minutes perfusion with pure CO, shows a definitely increased oxygen uptake. He, accordingly, reduced the duration of the perfusion to 5-7 minutes.

In our experiments, care was taken in order to have, just before the CO--treatment, animals which with great probability had no oxygen debts. The gas mixtures containing 20% CO perfused the WARBURG vessels slowly, while they were gently shaked and the whole bath was covered with a double layer of thick black cloth. During all the experiment this dark condition prevailed. EWER (1. c.) and JOHNSON (1. c.), as already mentioned, criticized HARNISCH because he used 20% CO in all his experiments without considering of a possible direct action of CO upon the tissues. Our work is susceptible of being criticized on the same grounds, because we also used 20% CO throughout our experiments. However, we do not believe that EWER and JOHNSON'S criticism is correct. First of all, with such small oligochetes like *Tubifex* or *Limnodrilus* it is hard to make use of spectroscopical methods in order to control the conversion of haemoglobin into carboxyhaemoglobin when low CO tensions are employed. Consequently, one is obliged to use a high CO percentage all the time if he wants to be sure that this conversion really took place. Secondly, it is necessáry to examine whether 20% CO, acting concomitantly with oxygen tensions varying from 21% to 1%, might be responsible for the effects observed or not. From what we know about the action of CO upon the tissues, we are not entitled to believe in a respiratory depression due to CO action. In fact, according to FENN & COBB (1932 a and b), SCHMITT & SCOTT (1934) and STANNARD (1940), frog's skeletal and cardiac muscle react to CO by converting it to $CO_2$, at the expense of atmospheric $O_2$, as reflected by the considerable increase of the oxygen uptake. TOBIAS, LAWRENCE, ROUGHTON, ROOT and GREGERSEN (1945) proved that man is able to convert radio-active CO into radio-active $CO_2$. MENDES (1948) found out that *Rana pipiens* embryos, at stages before the appearence of circulation, greatly increase their respiratory rate in presence of CO. Finally, as JOHNSON himself reported, the respiration of earthworm slices is greatly increased in atmospheres containing 20% CO and 20% oxygen. A depression of tissues respiratory rate due to CO is then to be excluded. On the other hand, it is hard to admit that HARNISCH'S results (no significant changes induced by CO) are due to a possible stimulation of tissue respiration by carbon monoxide. This stimulation has been observed only in cases were CO had a direct access to the tissues (fragment of tissues, embryos at early stages). HARNISCH worked with intact animals and, in this circunstance, CO could reach the tissues only when carried by the blood fluid. However, CO is extremely little soluble (at 40° C, its solubility in water by volume is 0.0178, according to HENDERSON & HAGGARD 1943, p. 159) and this practically invalidates the hypothesis of CO reaching

the tissues in sufficient amount to cause sensible increase of the respiratory rate. It is better, therefore, to interpret HARNISCH's results by admitting that, due to his imperfect technique of poisoning haemoglobin with CO, his so called CO-trated animals were in reality normal animals which, furthermore, as already mentioned, probably possessed oxygen debts as consequence of having been kept in pure CO for some time. As to DAUSEND, it is probable that, although using an unsatisfying CO-treatment, he did get some haemoglobin inhibition.

Returning to our own result, it is important to emphasize that when haemoglobin was made unuseful for the oxygen transport, the respiratory rate of CO-treated animals was in essence equally depressed at 21, 15, 10, 5, 2.5 and 1% oxygen. By comparing the two curves of fig. 1, the following conslusions can be drawn : 1. *Limnodrilus* haemoglobin does take part in the process of carrying oxygen to the tissues even at the atmospheric oxygen pressure ; 2. the amount of oxygen that through haemoglobin is given up to the tissues independs of the oxygen concentration ; 3. great part of the oxygen used up in the metabolic processes reaches the tissues in a state of physical solution, as show by the relatively high respiratory rate (ca. 60% of normal) after prolonged exposure to CO at any of the oxygen tensions tested. This last conclusion also suggests that in *Limnodrilus* the respiratory surfaces and the $O_2$ physically transported through the blood fluid are sufficient to provide a good aeration of the tissues.

## 5. SUMMARY

a. The oxygen consumption of *Limnodrilus hoffmeisteri* has been measured at 25° C in water in equilibrium with atmospheres containing 21, 15, 10, 5, 2.5 and 1% oxygen, with (in the dark) and without addition of carbon monoxide in sufficient amount to saturate the haemoglobin of the blood.

b. The mean normal oxygen consumption of *Limnodrilus*, at 25° C, is 1.02 cu.mm. oxygen/mg. (dry)/h.

c. In the absence of carbon monoxide the respiratory rate of *Limnodrilus* did not essentially differ in water in equilibrium with 21, 15, 10, 5 and 2.5% oxygen. In water in equilibrium with 1% oxygen a slight respitory depression was observed. Even in water in equilibrium with atmospheres containing commercial nitrogen purified with alkaline pyrogallol, *Limnodrilus* still exhibited a significant $CO_2$ output. This indicates that *Limnodrilus* is able to take advantage of minimal traces of oxygen.

d. The respiratory rate of CO-treated animals was significantly lower than that of normals at all oxygen tensions tested. Even in water in equilibrium with 1% oxygen, normal *Limnodrilus* showed a respiratory rate higher than CO-treated ones.

e. Comparing the respiratory rates of normal and CO-treated *Limnodrilus* at the various oxygen tensions tested, the following conclusions can be drawn : 1. haemoglobin does take part in the processes of carrying oxygen to the tissues even in water in equilibrium with common air ; 2. the amount of oxygen it gives up to the tissues independs of the oxygen concentration ; 3. in *Limnodrilus*, large respiratory surfaces and a good blood fluid's capacity

of transporting oxygen are probably responsible for the fact that, at low oxygen tensions, even after haemoglobin inhibition, the respiratory rate is still great as compared with the normal rate.

## 6. RESUMO

a. O consumo de oxigênio de *Limnodrilus hoffmeisteri* foi medido a 25° C, em água em equilíbrio com atmosferas contendo 21, 15, 10, 5, 2.5 e 1% de oxigênio, com (no escuro) e sem adição de monóxido de carbono em quantidade suficiente para saturar a hemoglobina do sangue.

b. O consumo normal médio de oxigênio de *Limnodrilus*, a 25° C, é 1,02 $mm^3$/mg (seco)/hora.

c. Na ausência do monóxido de carbono a taxa respiratória de *Limnodrilus* na água em equilíbrio com 21, 15, 10, 5 e 2.5 de oxigênio não varía essencialmente. Na água em equilíbrio com 1% de oxigênio observa-se uma ligeira depressão na respiração. Mesmo na água em equilíbrio com atmosfera de nitrogênio comercial, purificado com pirogalol alcalino, *Limnodrilus* mostra uma produção significativa de $CO_2$, o que indica capacidade de aproveitar os menores traços de oxigênio.

d. A taxa respiratória dos animais submetidos ao CO é significativamente menor que a dos normais, em tôdas as tensões de oxigênio experimentadas. Mesmo em água em equilíbrio com 1% de oxigênio, *Limnodrilus* normais mostram uma taxa respiratõria maior que a dos submetidos ao CO.

e. Comparando-se as taxas respiratórias dos *Limnodrilus* normais com as dos tratados com CO, nas diversas tensões de oxigênio usadas, pode-se tirar as seguintes conclusões : 1. A hemoglobina toma parte no transporte de oxigênio aos tecidos, mesmo na água em equilíbrio com o ar ; 2. A quantidade de oxigênio cedida aos tecidos independe da concentração do oxigênio do meio ; 3. Em *Limnodrilus*, superfícies respiratórias relativamente grandes e o fluído sanguíneo dotado de bôa capacidade de transporte de oxigênio são responsaveis pelo fato de que em baixas tensões de oxigênio, mesmo depois da inhibição da hemoglobina com CO, a taxa respiratória ainda é grande em comparação com a taxa respiratória normal.

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