# CERTAIN FACTORS INFLUENCING THE RESPIRATORY METABOLISM OF THE LEECH (*Hirudo medicinalis*)

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While considerable data are available concerning the respiratory activity of invertebrates much is still found lacking regarding the annelids. In this group of animals reports have been limited to *Lumbricus*, *Nereis*, and *Hirudo*.

Thunberg (1905) reports an increase in oxygen consumption from 20 to 97 per cent atmospheric oxygen for *Lumbricus*. Konopacki (1907) finds no additional increase in oxygen consumption above 50 per cent oxygen, in earthworms. Dolk and Paauw (1929) record a constant rate of respiration down to ⅛th of the oxygen concentration of air, for earthworms. Amberson, Mayerson, and Scott (1924) find a dependence of oxygen consumption upon oxygen tension in *Nereis*. Hyman (1932) reports that no respiratory regulation exists in *Nereis* when the animals are placed in tubes resembling their natural habitat. Lindeman (1932) records an independence of oxygen consumption for *Hirudo* from normal saturation of water with air to about 20 per cent saturation.

Among the groups closely allied to the annelids should be mentioned the work of Henze (1910) which indicates a dependence of *Sipunculus* upon oxygen concentrations; and that of Hall (1931) on *Urechis*, an echiuroid worm, which states that oxygen consumption is independent of the oxygen pressure down to a value of 70 mm. Hg. (or approximately 50 per cent saturation). The latter author reports also that the oxygen pressure may drop from 115 to 4 mm. of Hg. in jars covered with oil.

Thus we see a uniformity apparently does not exist in the results of various workers with annelids and related forms.

## MATERIALS AND METHODS

The method of measuring the dissolved oxygen and the chamber in which the animals were kept were the same as used by Hiestand (1931) with the exception that the leeches were not confined to a cage within the jar but allowed complete freedom. The experiments included from four to nine animals.

For several tests the water was stirred by an electrically propelled paddle which was inserted through a hole in the rubber stopper. The speed of revolution, controlled by a system of reduction pulleys, was never great enough to molest the annelids, being about 75 turns per minute.

City tap water, aerated by bubbling air through it for several hours, was the respiratory medium. This aeration not only expelled the free carbon dioxide with which the water was well saturated, but also brought the oxygen content to equilibrium with atmospheric air. Since there was a possibility that a certain amount of the oxygen consumption attributed to the leech might have been caused by microorganisms present, tests, varying in length from three to forty-four hours, were made to establish certainty on this subject. In all cases there was no appreciable decrease in oxygen tension. This not only dismissed the possibility of tension reduction by bacteria but also established the efficiency of the method of sealing the respiration chamber.

The animals were permitted to remain undisturbed in an open jar for a week, a time sufficient for laboratory acclimatization. They were not fed until before the last series of experiments. During starvation the animals were found to lose weight at a comparatively rapid rate. Over a period of 39 days the weight loss was approximately 4.3 grams compared to a previous total weight of 13.610 grams. This diminution in weight was due to catabolic changes within the leeches and hence did not have any abnormal effects on the respiration rate.

The effect of food on the rate was reserved as the last experiment. The leeches were placed on the inner surface of a rabbit's ear and permitted to engorge themselves until satisfied.

Throughout the experiment uniform standard conditions were closely adhered to. The tests were carried out in an isolated room where the temperature was 23° C. with variations less than 1°. A small electric light bulb supplied a constant light during the experiments and in intermediate periods. Approximately 11 ml. samples of water were withdrawn at each sampling. Of this 10 ml. were used for oxygen determination.

It was found that tap water formed a natural environment for the leeches. Distilled water could not be used since prolonged confinement in it caused several leeches to die. Also a considerable quantity of mucous secretion was exuded by the animals in distilled water. Possibly this was due to the osmotic pressure difference between the leech and the water.

## Results

**Normal Respiration Rate.** In the first series of experiments 6 leeches having a total weight of 13.672 grams were used. A series of tests was carried out to determine the average respiration rate in water, the oxygen tension of which was reduced by the respiration of the animals themselves. At the start of the experiment the jar contained approximately 950 ml. of water. Determinations were made usually at two hour intervals until 10 hours had elapsed. An average result is shown in Table I. It was noted in these first and later experiments that when successive determinations were made on successive days, the *rate of oxygen consumption diminished slightly during the successive trials* (see Tables II and III) ; in other words the leeches responded by "acclimatization" and their metabolism diminished somewhat. If a few days elapsed in which time the animals were not subjected to low oxygen tensions, their respiration rate returned to normal.

Table I (normal)

| Hrs. | Mgr. $O_2$ per liter |
|---|---|
| 0 | 9.10 |
| 3 | 5.88 |
| 6 | 3.76 |
| 8 | 2.84 |
| 10 | 2.04 |

Table II

| Hrs. | Mgr. $O_2$ per liter |
|---|---|
| 0 | 8.90 |
| 2 | 7.00 |
| 4 | 5.20 |
| 6 | 4.76 |
| 8 | 3.84 |
| 10 | 3.24 |

Table III

| Hrs. | Mgr. $O_2$ per liter |
|---|---|
| 0 | 8.50 |
| 2 | 7.04 |
| 4 | 5.90 |
| 7 | 4.68 |
| 9 | 4.08 |

Table IV (high oxygen)

| Hrs. | Mgr. $O_2$ per liter |
|---|---|
| 0 | 25.04 |
| 3 | 21.64 |
| 6 | 19.28 |
| 8 | 15.68 |
| 24 | 3.64 |
| 26 | 2.20 |
| 27 | 1.68 |
| 28 | 1.40 |
| 29 | 1.24 |
| 30 | 1.08 |
| 31 | 1.04 |
| 32 | .90 |

Table V (high oxygen plus high carbon dioxide)

| Hrs. | Mgr. $O_2$ per liter |
|---|---|
| 0 | 25.80 |
| 3 | 22.60 |
| 6 | 18.36 |
| 8 | 16.84 |
| 11 | 14.24 |
| 24 | 3.32 |
| 26 | 1.96 |
| 27 | 1.60 |
| 28 | 1.36 |
| 29 | 1.28 |
| 30 | 1.00 |
| 31 | .86 |
| 32 | .76 |

Table VI

| Hrs. | Previous to feeding Mgr. $O_2$/L. | 1st day following Mgr. $O_2$/L. | 2nd day following Mgr. $O_2$/L. | 3rd day following Mgr. $O_2$/L. | 4th day following Mgr. $O_2$/L. |
|---|---|---|---|---|---|
| 0 | 10.20 | 10.30 | 10.40 | 10.30 | 10.36 |
| 3 | 7.12 | 5.02 | 4.90 | 6.70 | 6.24 |
| 6 | 4.34 | 2.64 | 2.44 | 3.70 | 3.76 |
| 9 | 2.80 | 1.90 | 2.14 | 2.64 | 2.40 |

**Effects of High Oxygen Tensions.** Since it was determined by the preceding tests that oxygen consumption decreased at low oxygen tensions it was thought advisable to determine if higher oxygen tensions than normal air-water equilibrium would accellerate respiration. Tap-water which had been washed free of dissolved carbon dioxide was shaken with oxygen from a supply tank until it contained 25.04 mgr. oxygen per liter. The same six individuals were placed in this supersaturated water and samples were taken for a period of 8 hours. On the following morning the experiment was continued, the leeches being left in the chamber over night. Samples were taken until a total period of 32 hours had elapsed. The results are indicated in Table IV. It can readily be seen that the oxygen consumption took place at a greater rate at very high oxygen tensions than at normal saturation. When a tension of 2.20 mgr. per liter was reached the respiration rate was considerably reduced. At the conclusion of this experiment the question was raised as to the effect of combined high oxygen and carbon dioxide.

**Effects of High Oxygen and High Carbon Dioxide.** Tap water was mixed with oxygen and carbon dioxide from tanks until the oxygen content was raised to 25.80 mgr. per liter and the carbon dioxide content increased to 489.5 mgr. per liter. The same procedure as in the preceding experiment was followed. The results are recorded in Table V. It will be noted that the results are practically identical with those of high oxygen content alone. The differences are so slight as to be considered more or less insignificant.

Since respiration had practically ceased at the end of the two preceding tests it was questioned if the animals actually formed an oxygen debt.

**Determination if Leeches Can Form an Oxygen Debt.** The procedure followed was that of confining the animals for a period of 15 hours in the respiration chamber, determining the oxygen tension of the water at the end of this period, and immediately transferring the leeches to normal stock water, the oxygen tension of which was known. Then in the following eight hour period, with determinations made regularly at 2 hour intervals any increased oxygen consumption of the leeches (which would indicate a debt) could be ascertained.

Two such determinations were made, the data of which are not included in this paper. The results, however, show a slight acceleration in respiration for the first 2 hours in the fresh stock water after a previous period of low tension, but at the end of the 8 hour period no more oxygen was consumed than normally. We do not believe this evidence as being indicative of an oxygen debt formation. In all probability the return to water of normal oxygen tension from that of low tension acted as a mild stimulation to the respiration of the leeches, but the resultant accelleration soon diminished and at the end of a given period (8 hours) approximately the same volume of oxygen was consumed as in the normal tests, which fact would indicate the absence of an oxygen debt formation.

**Effect of Circulation of Water on Respiration.** In a previous paper one of us (Hiestand, 1931) demonstrated the effect of volume of water on the rate of oxygen consumption of the crayfish, *Cambarus virilis*. He found that whether this species could be said to be dependent upon or independent of oxygen tension of the surrounding water, involved not only the gaseous tension of the water but also the volume of the water, i.e., total quantity of oxygen available. Thus the possibility that water in motion might influence the oxygen consumption presented itself. For investigating this possibility, the circulating device previously described was built. Four determinations with normal stock water were made and compared with the normals in quiet water of the same stock.

The results showed no appreciable change in the rate of consumption, the only visible difference being in the behavior of the leeches. They did not remain as motionless as in still water but often set up an undulating motion which indicated their sensibility to the water in motion. The same type of undulating motion was apparent also when leeches were transferred from low tension water to higher. It is apparently a reaction brought about by the stimulation of water of high oxygen content as well as by circulation of water for it occurred in water supersaturated with oxygen. It was also noted that, although the water was kept in motion, the leeches could not respire at lower tensions than in quiet water.

**Effect of Ingestion of Blood on Respiration Rate.** Five leeches whose normal respiration rate had been determined were fed by being placed upon the inner surface of the ears of a live rabbit. After they had become gorged with blood they were weighed. Their gain in weight is shown by the following table:

| | |
|---|---|
| Total weight previous to feeding................. | 7.726 gms. |
| Total weight after feeding...................... | 23.574 gms. |
| Weight of blood ingested....................... | 15.848 gms. |

On the following four days oxygen determinations were made as comparisons with those before feeding. In each case an increase in oxygen consumption above that of inanition occurred, being most marked during the second day following feeding. At the end of a six hour period in the animal chamber the oxygen consumption showed about a 30 per cent increase after feeding above that of a similar period during inanition. The increase was less marked in the remaining three hours of the test. The results are shown in Table VI.

## DISCUSSION

The authors have been aware of two facts throughout this work. One is the fact that slight variations occur from time to time under supposedly identical conditions. This is to be expected when one deals with living forms, however, and is experienced by all who come in contact with problems of respiratory metabolism. The other fact is the acclimatization that occurs among the animals. As has been stated earlier in this paper, leeches show an adjustment to conditions, such that if successive tests are conducted on successive days, the oxygen consumption will diminish slightly as the animals become adapted to the lowering tensions. This, in itself, is an important fact, for it is one means of regulation that the animals possess.

In his paper, Lindeman (1932) shows graphically a "straight-line" relationship between oxygen tension and oxygen consumption until low tensions are reached. Our results do not quite agree. As the tension diminishes the consumption gradually recedes but this recession becomes much more marked at tensions less than 2 mgr. $O_2$ per liter. Thus we cannot say that an actual dependence exists except at tensions above normal equilibrium of air and water. We feel safe in saying, however, that the animals are virtually independent over most of the range below normal air and water equilibrium. Above normal saturation a complete independence exists. This is not surprising, when one considers that the leech lives in an external environment where the water is relatively rich in oxygen most of the time.

Also we do not find that removal of a small amount of water by sampling affects the respiration of the leeches. Our method does not involve replacing the water withdrawn and therefore little disturbance occurs within the jar.

## Conclusions

1. Oxygen consumption of *Hirudo medicinalis* is independent of oxygen concentration above normal air and water equilibrium.
2. As the tension of oxygen is reduced from normal air and water equilibrium by the respiration of the leeches, the oxygen consumption rate gradually decreases until a tension of about 2.0 mgr. per liter is reached. Below this value respiration decreases gradually and ceases at about .80 mgr. per liter which is approximately 1/10th that of normal air-water equilibrium.
3. Oxygen saturation above normal air-water equilibrium accellerates respiration but throughout this range the oxygen consumption is quite uniform.
4. Water rich in $CO_2$ as well as $O_2$ does not increase the rate above that of water rich in $O_2$ alone.
5. No evidence that the leeches can form an oxygen debt was found.
6. Circulating water does not materially affect the $O_2$ consumption.
7. Ingestion of blood increases the rate of $O_2$ consumption markedly.

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