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THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Edna Mary Wolf final oral examination for the degree of Master of Science. We recommend that the degree of Master of Arts be conferred upon the candidate.

Minneapolis, Minnesota

May 26 1919

E. J. Lund
Chairman

F. H. MacDougall

J. F. McClendon

Henry F. Nachtrieb

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Edna Mary Wolf for the degree of Master of Arts. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

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QUANTITATIVE STUDIES

ON THE RELATION OF OXYGEN CONCENTRATION

TO OXYGEN CONSUMPTION IN THE LEECH

ERPOBDELLA PUNCTATA

By

Edna M. Wolf

A THESIS

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL

OF THE

UNIVERSITY of MINNESOTA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER of ARTS

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I Introduction

The results presented in this paper include a few preliminary determinations in the oxygen consumption of a number of aquatic invertebrates and a more intensive study of the relation of oxygen concentration to oxygen consumption in the leech, Erpobdella punctata.

The work was undertaken as a preliminary study of the conditions and suitability of material and methods for a further intensive study of the respiratory metabolism of lower invertebrates.

I am indebted to Dr. E.J.Lund who made this work possible by suggesting the problem and the methods of investigation, also for his assistance in collecting material and his kindly criticism and advice in interpreting and presenting results.

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II MATERIAL

The animals used in the experiments were chosen at random from whatever material was available in larger quantities after a collecting trip.

The leeches, of the species Erpobdella punctata, occur abundantly in the Mississippi river and may be obtained by overturning the rocks to which they cling. They are reddish brown in color and "may be plain or more usually more or less marked with irregular black spots with light centers, arranged in two or four longitudinal lines leaving the middle of the back and the margins clear", Moore (12).

One important condition that must be met in accurate studies on respiratory metabolism is that the organism used should be constant in its rate of metabolism over short as well as long periods of time. The leech will live for months in the laboratory without food and hence it was thought that this fact would eliminate one source of variation in rate of metabolism, namely that due to feeding.

Thruout the work, unless difference in size is mentioned, animals of approximately uniform size and activity were selected with care. The temperature of the water in which the animals lived was kept constant.

III METHODS FOR OBTAINING KNOWN CONCENTRATIONS OF OXYGEN

All the bottles in which the animals were kept during the experiment had the same volume, 134c.c. They were closely fitted with ground glass stoppers.

The analyses for oxygen content were made according to Winkler's method as given by Treadwell and Hall (17) except that 5 c.c. instead of 3 c.c. of concentrated HCL was used. The thiosulphate solution was standardized against known weights of freshly resublimed iodine at frequent intervals.

In order to obtain water of constant oxygen content at atmospheric pressure of oxygen, a large carboy was filled with tap water and allowed to stand until it reached room temperature. A stream of air was then passed through it for several hours. 134 c.c. of aerated water at about 20°C contain from 0.750 to 0.770 c.c. of oxygen N.T.P.

Before filling, each bottle was rinsed with water from the carboy. The end of the siphon from the carboy was placed on the bottom of the bottle, a stream of water was allowed to flow through until the bottle was thoroly flushed. The siphon was then withdrawen slowly leaving the bottle full to overflowing with uniformly aerated water.

One after another of the bottles was filled in this way and placed in the order of filling, none were stoppered until all were ready for the next step, so that the change in oxygen content due to diffusion, if any, did occur, was eliminated.

TABLE I.

Showing uniformity of low and high concentrations obtainable by the method.

	Bottle	Oxygen Concentration cc. O_2 per 134 cc. H_2O		
		Experiment 1	Experiment 2	Experiment 3
High concentration of O_2 obtained by passing pure O_2 thru H_2O in a carboy.	1	2.00	1.296	2.434
	2	2.00	1.300	
	3	1.99	1.298	2.452
Low concentration of O_2 obtained by passing tap water thru heating coil.	1	0.200	0.035	0.087
	2	0.203	0.026	0.078
	3	0.196	0.028	

As controls the bottle first filled, the one last filled and an intermediate one were chosen. The oxygen content of each was determined and the average of these three determinations was taken as the oxygen content of each of the bottles in the set. A very high degree of accuracy can be obtained in this way. Table 1.

It is a more difficult problem to obtain and to fill the bottles with water of concentrations below or above that at air saturation. After some preliminary trials the following procedure was finally adopted.

To obtain water of minimum oxygen concentration a slow current of tap water was conducted through a heated copper coil into a large carboy. When the carboy was filled the water was allowed to cool to room temperature. Boiled water was added from time to time so that a minimum surface of the water should be exposed to air.

After the water had cooled and just before filling the 134 cc. bottles ~~the~~ water was gently stirred to prevent the occurrence of layers of different oxygen concentration. The water was siphoned from the bottom of the carboy. In this way oxygen concentration as low as 0.06 c.c. oxygen N.T.P. per 134 cc water at 21.5°C was obtained, which concentration is about one twelfth that of air saturated water at the same temperature.

Water of high oxygen content was obtained by allowing a stream of oxygen from an oxygen tank to pass through a carboy of tap water. Concentrations as high as 2.43 c.c. oxygen per 134 cc. water at 21.5°c were obtained in this way. This concentration is about three times that of air saturated water at the same temperature and about forty times that of low oxygen concentration obtained.

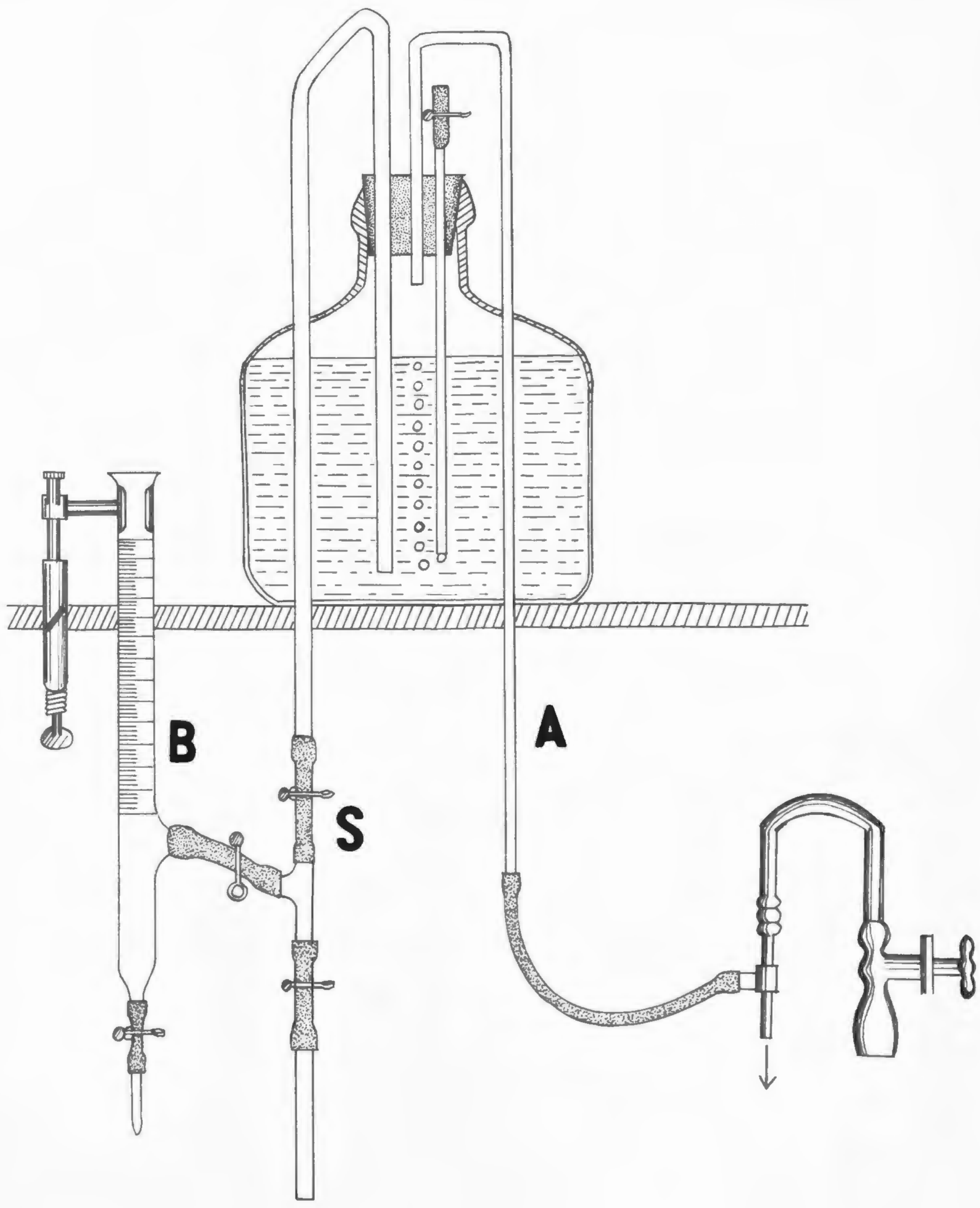
To obtain intermediate concentrations the bottles were first filled with water of low oxygen content. By means of a burette B (see Plate 1) connected with a carboy containing water of high oxygen content a definite number of cubic centimeters of water of low oxygen concentration in the bottle was displaced by the same volume of water of high oxygen content. Experiment 1 and 2 of Table II. The greatest uniformity in filling was obtained when the mouth of the burette was placed almost on the bottom of the bottle and when the water of low oxygen content was still warm, being displaced by water of higher density and high oxygen content.

Since the concentration resulting from such a mixture can be but somewhat roughly estimated, it is necessary, whenever a certain definite concentration is desired to make preliminary tests so that the number of cubic centimeters of water to be displaced may be definitely determined.

Plate 1 shows the arrangement of the apparatus for aerating and oxygenating the water in the carboy. The siphon S for filling the bottles and the burette B for displacing a definite volume of water in the bottle are also shown.

Table I shows that a high degree of accuracy can be obtained in determining the oxygen concentration in bottles of the same volume when the bottles are flushed and filled with water of uniform oxygen content from a large carboy.

PLATE I



~~oxygenated water to be added may be definitely determined~~.

Table 2 shows the uniformity and accuracy obtained when a certain volume of water in a 134 c.c. bottle was displaced by the same volume of water of different oxygen concentration.

Table 2

In order to prevent loss or gain in oxygen by the water in the bottles the glass stoppers were greased with vaseline or else the bottles were immersed under water to prevent any evaporation around the glass stoppers. The leeches and the larger animals used were transfered by means of long handled flexible forceps. All the animals excepting Corethra larvae, Daphnia gammarus and Ostracods were removed before titration.

In those cases where the animals were not removed, correction was made for iodine absorption. Three controls containing the same number of animals, of same size and weight were titrated immediately. The difference between this determination and that of the controls containing water only, gives the amount of iodine absorbed.

The following experiment was performed to determine whether the insertion of the leeches and their removal before determining the O_2 content causes a measurable error. Two medium sized leeches, average volume of half a cubic centimeter were put into a 134 c.c. bottle and again removed as quickly as possible. The reagents were them added. Three controls gave an oxygen content of 0.374 c.c., 0.379 and 0.376 (considerably below concentration at air saturation). The bottles to which the leeches had been added and removed showed 0.374, 0.374 and 0.372 c.c. oxygen, the bottles to which the leeches had been added contained 0.232, 0.232 and 0.230 c.c. oxygen. The diffusion of oxygen is not sufficient to change the oxygen concentration measurably.

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IV UNIFORMITY IN OXYGEN CONSUMPTION BY THE SAME INDIVIDUAL AT DIFFERENT TIMES AND DIFFERENT INDIVIDUALS AT THE SAME TIME.

It is essential to determine the degree of uniformity in oxygen consumption in an animal and in animals of the same size and degree of starvation under similar conditions, in order to know the characteristic behavior of the animal under given conditions. If oxygen consumption is found to be uniform under the same conditions, then if upon changing a factor in the environment a corresponding change occurs in oxygen consumption, a relation between the two changes may be infered.

Other conditions being the same, and oxygen supply being plentiful, a leech consumes approximately the same quantity of oxygen per unit time. That is leech No. 1, table 3 consumed per hour 0.116,0.103, 0.107 and 0.107 c.c's oxygen during 2,3,4 and 5 hour periods respectively.

Table 3

A similar degree of uniformity was found during six hour periods on successive days as shown in table 4.

Table 4

That leeches of approximately uniform weight consume approximately uniform quantities of oxygen during a given time when the oxygen supply is the same for all, is shown in table 14 In this experiment the leeches were subjected to sudden changes in oxygen concentration but uniformity among the leeches in amount of oxygen consumed persists, even though the rate may be dependent upon oxygen concentration.

TABLE III.

Individual variation per hour during periods successively increased by 1 hour.
Leeches fasting for three days. Two leeches in each bottle.
Temperature 20° C.

Length of period	2 hours	3 hours	4 hours	5 hours	6 hours
Cc. O_2 in each bottle of 134 cc. at beginning of exper.	0.759	0.759	0.766	0.763	
Bottles	Cc. O_2 consumed per hour				
1	0.116	0.103	0.107	0.107	
2	0.107	0.092	0.099	0.094	
3	0.103	0.085	0.094	0.087	
Cc. O_2 in each bottle of 134 cc. at beginning of experiment.		0.750	0.766	0.771	0.784
4		0.080	0.075	0.062	0.078
5		0.076	0.066	0.064	0.078
6		0.089	0.073	00071	0.076
7		0.075	0.082	0.082	0.089
8		0.089	0.089	0.078	0.092
9		0.089	0.107	0.096	0.103

TABLE II

Showing the uniformity and accuracy with which water of any concentration of O_2, above or below air saturation, may be obtained. Low and high concentrations of Experiments 1, 2 and 3 of Table I are used respectively in Experiments 1, 2 and 3.

134 cc. Bottles filled with water of low oxygen concentration				134 cc. bottles filled with water of high oxygen concentration.		
No. of cc. of H_2O of high O_2 content added to a bottle filled with H_2O of low O_2 content.	Bottles	Cc. of O_2 in 134 Cc. H_2O. Exper. 1	Cc. of O_2 in 134 Cc. H_2O. Exper 2	No. of cc. of H_2O of low O_2 content added to a bottle filled with H_2O of high O_2 content.	Bottles	Cc. of O_2 in 134 cc. H_2O Experiment 3
100	1	1.178	0.706	100	1	1.029
	2	1.170	0.706		2	1.177
	3	1.180	0.706			
50	1	0.785	0.419	50	1	1.718
	2	0.785	0.427		2	1.727
	3	0.785	0.436			
25	1	0.497	0.218	25	1	2.076
	2	0.523	0.226		2	2.094
	3	0.514	0.226			
15	1	0.384	0.157	15	1	2.198
	2	0.401	0.148		2	2.181
	3	0.384	0.148			
10	1	0.322		5	1	2.364
	2	0.322			2	2.320
	3	0.314				
5	1	0.261	0.061			
	2	0.253	0.069			
	3	0.256	0.067			

TABLE IV.

The degree of variation of oxygen consumption by different individuals during six hour periods on each of six successive days. Leeches collected June 1. Temperature 18°.

Cc. O_2 in each 134 cc. bottle at beginning of experiment.	0.750	0.763	0.750	0.750	0.737	0.763
Time	June 4, 1:00-7:00	June 5, 1:00-7:00	June 6, 11:00-5:00	June 7, 1:00-7:00	June8 8:00-2:00	June9 9:00-9:00
Leech Number	Oxygen Consumption per hour, cc. O_2, N.T.P.					
1	0.166	0.161	0.157	0.157	0.179	0.135
2	0.139	0.143	0.157	0.139	0.143	0.179
3	0.209	0.179	0.192	0.139	0.205	0.170
4	0.227	0.222	0.218	0.209	0.222	0.222

V RELATION OF SIZE TO OXYGEN CONSUMPTION

Due to the influence of Rubner's experiments on dogs and other warm blooded animals the belief has been widely held that somehow the rate of metabolism is regulated by the relative amount of body surface. Rubner found that, calculated per kg., the metabolism increased regularly with decreasing size. When, however, the surface of the animals was taken into account a practically constant metabolism per square metre of surface was found for all.

Considerable work to prove or dis prove this contention, has been done on warm blooded animals, where the relation of surface to heat radiation is much more significant than in cold blooded animals. Oftentimes the constancy of the relation is marked. On the other hand, however, Hill (4) found that the ratio of heat production to unit surface in fasting rats is not constant. Here the heat production is not determined solely by the heat loss.

In warm blooded animals where the mechanism for regulating the temperature of the body is influencing the radiation from the surface, the true relation of surface to metabolism cannot be arrived at. Curarized animals that have lost the power of maintaining their body temperature behave as do cold blooded animals toward external temperature. Surface is only one factor in a complex, determining the amount of heat radiation. Lusk (9)

Less work has been done on cold blooded animals. Morgulis[13] actually measured the surface of flounders of different sizes and determined their oxygen consumption. He summarizes his results thus;-" The metabolic processes of the flounder bear direct relation neither to the weight nor surface of the flounder, the oxygen consumption per gram hour diminishing but increasing per square centimeter-hour as the flounders become larger."

Removal of the fins, causing a reduction in surface of over 30 per cent without materially changing the mass of the flounders, does not affect the oxygen consumption. "Under these conditions the oxygen consumption is proportional to the mass."

The oxygen consumption of several different species of aquatic invertebrates was determined. The results given in Table 5 show that oxygen consumption varies in different animals. There is no definite relation between oxygen consumption and live or dry weight,- nor to the water or ash content.

Size as such is not the factor which determines the rate of oxygen consumption. This is in agreement with the observations of Benedict and others who have shown that animals of the same size and weight may differ considerably with regard to their basal metabolism. Krogh (7).

Table 5

Within the same species, however, where the difference in size is probably due to difference in age, in every case reported in Table 6, those animals lowest in weight have the highest consumption per unit weight. Those intermediate in weight consume less oxygen, those of greatest weight have the lowest consumption of oxygen per unit weight.

That these differences could not be due to differences

TABLE V.

Oxygen consumption of different species.
All are averages of three sets of determinations.
Temperature 18° C.

Animal	No. of individuals per set.	Live wt. per set.	% H_2O	Weight of ash	Cc. O_2 in 24 hours N:T:P.	Cc. O_2 per gram live weight in 24 hours.	Cc. O_2 per gram dry weight in 24 hrs.
Damsel Fly	6	0.1552	0.823	0.00145	0.4376	2.819	15.9
Corethra larvae	45	0.2938	0.850	0.00283	1.7367	5.91	40.6
Daphnia	20	0.0556	0.888	0.0004	0.3852	6.928	62.1
Gammarus	15	0.0785	0.796	0.0030	0.5242	6.663	32.8
Ostrocods	900	0.0300	0.706	0.0025	0.5068	16.89	52.5

TABLE VI.

Showing the relation of size to oxygen consumption within the species. The figures for Branchippus and small cray fish are averages of three sets of determinations. Temperature 18° C.

Animal	No. in a set	Live weight in grams	% H_2O	Weight of ash	Oxygen consumed: Cc. O_2 in 24 hours N.T.P.	Cc. O_2 per gram live wt. in 24 hours.	Cc. O_2 per gram dry weight in 24 hours.
Branchippus.	45	0.3973	0.955	0.0021	1.9062	4.78	108.6
Branchippus.	45	0.1035	0.949	0.001	0.7262	7.01	138.02
Crayfish	1	17.1100	0.76	1.7971	14.8826	0.869	3.76
Crayfish	1	0.9303	0.808	0.0465	1.1166	1.187	6.55
Stonefly larvae	8	0.4012	0.724	0.0067	1.7663	4.402	16.0
Stonefly larvae	8	0.1273	0.774	0.0012	1.3030	10.234	45.4
Mayfly larvae	8	0.257	0.745	0.003	2.2163	8.623	33.8
Mayfly larvae	8	0.123	0.781	0.0017	1.3528	11.007	48.4
Mayfly larvae	8	0.086	0.792	0.0006	1.0074	11.67	58.2

in activity or food intake is made reasonably certain from the following facts;- The animals were collected at the same time from the same environment. They were kept at the same temperature before the experiment and during the time of the experiment. The intensity of light was the same for all.

This inverse relation was found to exist also in leeches. Those weighing between 0.01 and 0.05 grams are considered as one group. Those weighing from 0.05 to 0.10 grams are taken as the second group, those weighing from 0.10 to 0.15 as a third and those weighing near 0.3 grams as a fourth group. The average weight and oxygen consumption of each group is given.

Table 7

These data are from a lot of leeches collected from the Mississippi River at the same time and were apparently of different but closely related species. Therefore they are not really comparable, even though they show in general an inverse relation between oxygen consumption and weight.

The relations of oxygen consumption to size within the same species of leech, Erpobdella punctata, is shown in Table 8. Here a perfectly definite inverse relation between size and rate of oxygen consumption per gram weight, appears.

Table 8

The results are plotted and are shown in a curve in Plate II.

Plate II

There is probably very slight radiation of heat from the surface of the leech, because its temperature is but slightly above that of the medium in which it lives. Rogers (15 The surface also varies greatly depending on whether the body

TABLE VII.

The relation between O_2 consumption and weight. Leeches of closely related species, fasting for three months. Temperature 18° C. 0.796 cc. O_2 per 134 cc. H_2O.

Leech	Live weight in grams.	Cc. O_2 consumed in 24 hrs. per gram weight.
1	0.0093	3.812
2	0.0328	2.309
3	0.0483	1.568
Average	0.0301	2.829
4	0.0591	2.944
5	0.0883	1.173
6	0.0925	3.091
Average	0.0799	2.402
7	0.1069	1.083
8	0.1089	1.583
9	0.1457	0.729
Average	0.1204	1.132
10	0.2974	0.927
11	0.3273	0.758
12	0.3397	0.562
Average	0.3215	0.749

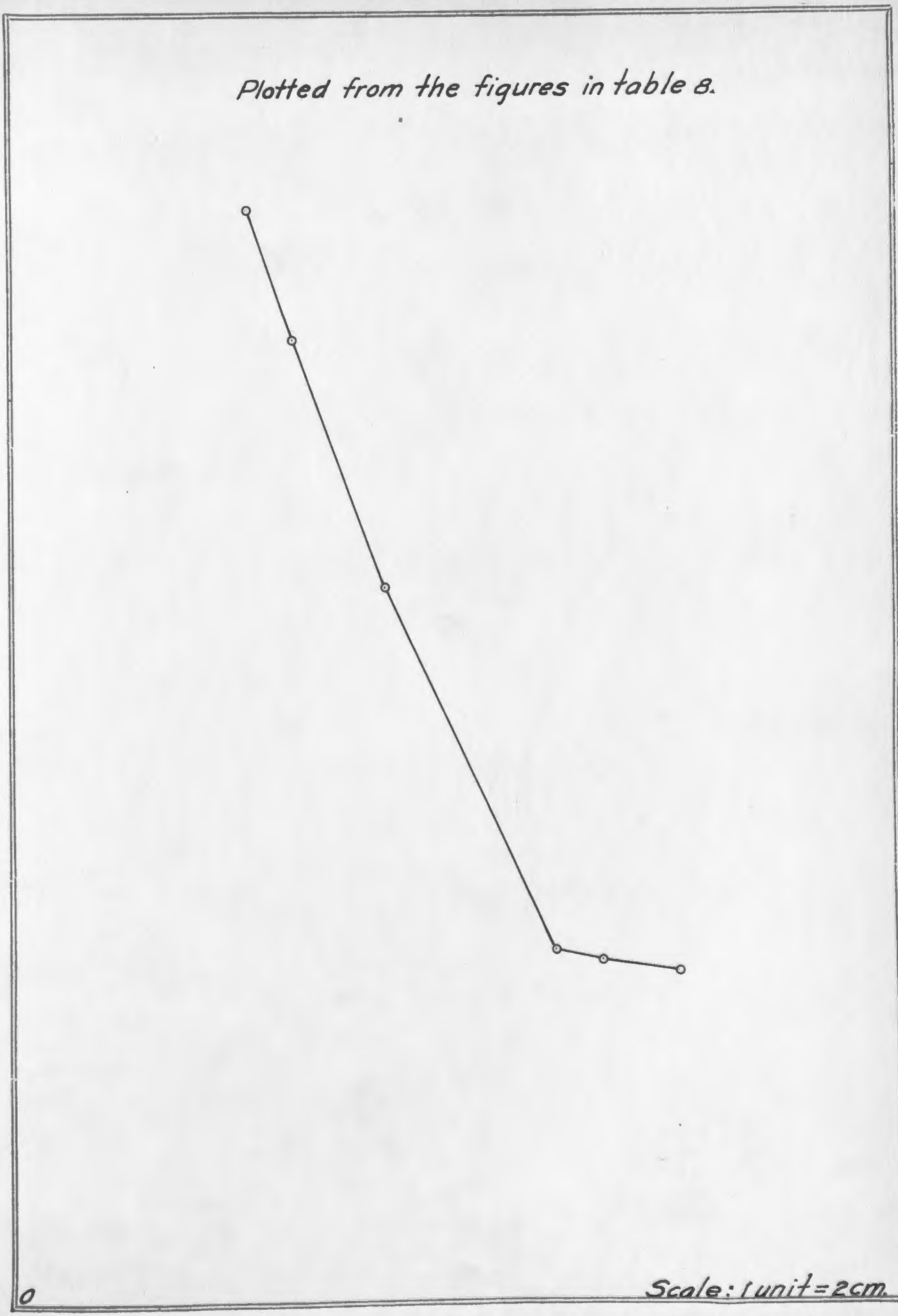
TABLE VIII

Relation of weight of leeches, *Erpobdella punctata* to oxygen consumption. Leeches collected the day experiment was begun. Temperature 18° C. 0.814 cc. O_2 per 134 cc. H_2O

Leech	Live weight in grams	Cc. O_2 consumed in 24 hours per gram weight.
1	0.3004	5.253
2	0.3209	4.708
3	0.3424	4.315
4	0.9099	2.967
5	1.2923	2.401
6	1.5027	1.920

PLATE II

Weight of leech in grams   Scale: 1gr. = 10cm.



Oxygen consumption   cc's. oxygen per gram and 24 hours.

of the leech is contracted or elongated.

Mr. Allen (1) has found that for Planaria agilis small size means increased oxygen consumption per gram weight. The rate of oxygen consumption decreases per gram weight with increase in size. He found no reason for considering the extent of surface area as being an important factor in determining the rate of oxygen consumption in Planaria.

VI THE RELATION OF OXYGEN CONSUMPTION TO OXYGEN CONCENTRATION

The oxygen consumption of a number of animals has been studied in more or less detail. From these investigations it appears that in some species the oxygen consumption is dependent upon oxygen concentration while in others the consumption is independent of the concentration within wide limits.

Pütter (14) states that when the amount of oxygen is decreased below a certain minimum (depending on the size of the animals). Hirudo medicinales consumes less than that necessary for normal respiration even though that amount may be available. He interprets this to mean that the anaerobic mechanism begins to be effective as soon as the oxygen supply is below a certain minimum.

Thunberg (1905) found that in Limax, Tenebrio and Lumbricus oxygen consumption is dependent upon oxygen concentration. In Limax the increased consumption in 96% oxygen is 1.22 times the amount consumed in air, Tenebrio 1.26 times as much and Lumbricus shows an average increase of 1.36 times that in air. In every case he finds that when oxygen pressure is decreased below that of air the oxygen consumption falls below that when in air.

Hense (1910) using Winkler's method while working with the Coelenterates Actinia, Anemonia and Sipunculus, found that the oxygen consumed by each species is dependent upon oxygen pressure. For Actinia he reports a consumption in high oxygen concentration of twice or even two and one half times the normal amount. This high rate is maintained thruout its existence (24 hours) under high oxygen pressure. As soon as the animals are returned to air saturated water, the oxygen consumed returns to normal. In the Crustacean Carcinus, the molluscs, Aplysia and Eledone, and the fishes, Coris and Sargus, the amount of oxygen taken up is independent of oxygen concentration within wide limits.

Similar results are reported by McClendon (1918) for the jelly-fish, Cassiopea.

Hense[(3)] also states that oxygen consumption by sea urchins eggs is independent of oxygen concentration when the eggs are kept moving about, each one surrounded by water, but dependent on oxygen concentration when not so surrounded. This condition of massed eggs he considers to be analagous to masses of cells in multicellular animals where oxygen is obtained by diffusion. The exterior cells have a greater supply of oxygen while the most interior cells are living under partly anaerobic conditions. Under high oxygen pressure oxygen penetrates the mass more thoroughly and there is a larger amount absorbed throughout, the maximum being reached when every cell receives all the oxygen it can consume. This maximum occurs at a lower concentration in Sepunculus (which may live for days without oxygen) than in Actinia. It is not at all certain, however, that Hense's comparison between the behavior of masses of eggs and cells in metazoa is permissable for echinoderm eggs are very sensitive to stimulation and to changes in chemical conditions which occur when eggs are massed together. C. Juday (5) while studying conditions in the deep waters of Lake Mendota, was convinced that various protozoa, worms and rotifers live under anaerobic conditions for about three months of the year suffering no ill effects by the changed environment nor showing decrease in activity.

Dr. Lund (9) showed that Paramecium caudatum was killed by too high oxygen concentration, that the rate of oxygen consumption was independent of oxygen concentration and also that " in a pure line of Paramecium from the same culture different cells differ in respect to the minimal oxygen concentration in which they continue to live".

Krogh (7) makes the following statement:-"It would seem to be a more or less general rule that the oxygen consumption for cold blooded animals is independent of the oxygen pressure of the surrounding medium when there is a positive tension of the gas in the tissues of the organism and the oxygen pressure becomes the limiting factor only when the oxygen supply fails and the oxygen tension in some of the tissue becomes zero."

The purpose of the following experiments is to find how the rate of oxygen consumption in the leech, Erpobdella punctata, is related to oxygen concentration of the medium in which it lives.

A preliminary experiment, the results of which are given in Table 9 shows that the oxygen concentration may be increased more than three times that of aerated water, at 20 degrees Centigrade, and decreased to less than half of aerated water without affecting oxygen consumption during two and a half hour periods.

TABLE IX.

Relative oxygen consumption in aerated water compared with high and lower oxygen concentrations.
Leeches kept for 15 days in a starving condition before beginning the experiment. Temperature 20° C.

Bottle	Leeches per bottle	Total Weight	Oxygen consumption in 2 1/2 hours.	
			Aerated Water 0.766 cc. per 134 cc. H_2O.	High O_2 concentration 2.51 cc. per 134 cc. H_2O.
1	2	0.2899	0.142	0.098
2	2	0.2543	0.116	0.142
3	2	0.2283	0.134	0.98
			Avg. 0.130	Avg. 0.112
			Aerated Water 0.799 cc. per 134 cc. H_2O.	High O_2 concentration 2.70 cc. O_2 per 134 cc. H_2O.
4	3		0.172	0.119
5	3		0.186	0.244
6	3		0.173	0.173
			Avg. 0.178	Avg. 0.179
			0.918 cc. O_2 per 134 cc. H_2O.	Less than half the conc. air saturation. 0.299 cc. per 134 cc. H_2O.
7	2	0.2663	0.080	0.077
8	2	0.3397	0.098	0.130
9	2	0.5061	0.160	0.148
			Avg. 0.113	Avg. 0.118

The effect of greater differences in concentrations of oxygen on the same individuals was then determined.

Two methods were employed to obtain different concentrations of oxygen.

In the first type of experiment, the results of which are summarized in Table 10 and in the curve on Plate III, the exact oxygen concentration was obtained as given under method page 6

From these results it appears that for leeches weighing about 0.14 grams, the oxygen consumption is not affected by concentrations above 0.24c.c!s oxygen per 134 c.c's water, (which is about one third the concentration of aerated water). At concentrations below 0.24 c.c. per 134 c.c. water the oxygen consumption per gram hour decreases with decrease in oxygen concentration.

Table 10

The results given in Table 10 are reproduced in the "curve" in Plate III. The first two of the sixteen results given in Table 10 that is those where the highest oxygen concentrations were taken, are averaged to give test 1. The next two give test 2, and so on, test 8 being an average of the two trials where the oxygen concentration was lowest.

The relative slope of the two curves shows that where the amount of oxygen consumed is dependent upon the oxygen available, the decrease in the oxygen consumption is less than the corresponding decrease in oxygen concentration.

Plate III

For the second type of experiment, the results of

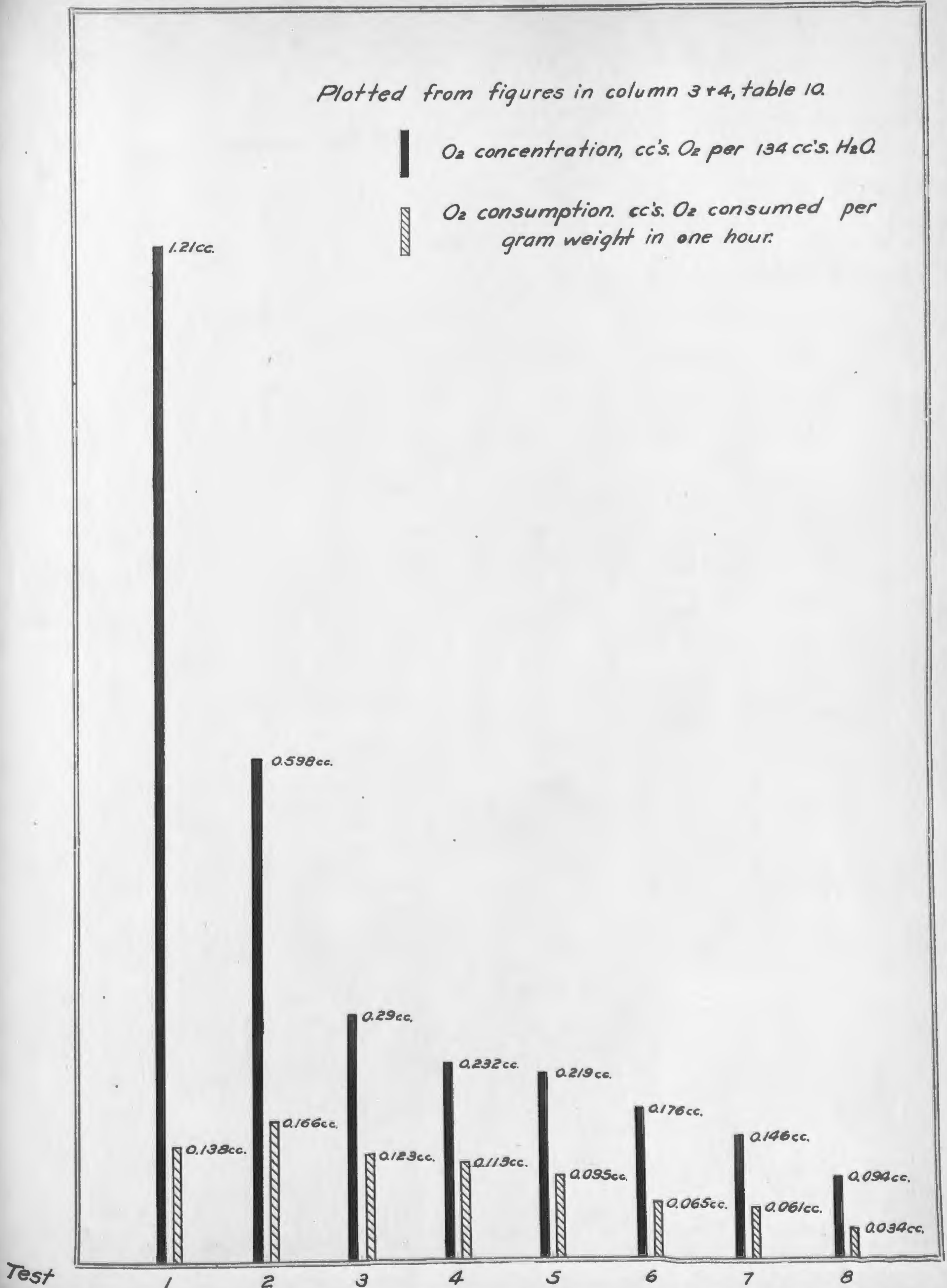
TABLE X.

Leeches kept in the laboratory for 15 days before beginning the experiment. Average weight of one pair for the pairs numbered 1, 2 and 3 is 0.2862 grams, average weight of one pair for pairs numbered 4, 5 and 6 is 0.3707 grams, for pairs numbered 7, 8 and 9 is 0.3354 grams. Temperature 20°C. Each result is the average of the three pairs at the same O_2 concentration. Leeches kept in aerated water between experiments. Duration of experiment 2 1/2 hours.

	1	2	3	4	5	6
Trials	Date	Pairs used	O_2 concentration cc. O_2 in 134 cc. H_2O. N:T:P	Cc. O_2. consumed per gram weight in 24 hrs.	$K=\frac{O_2 \text{ consmd}}{\sqrt{O_2 \text{ concentration}}}$ K	$a= k\sqrt{p}$. O_2 consumption $= 4.51\sqrt{O_2 \text{ conc}}$
1	July 1	1, 2,3	1.513	3.76	3.05	5.542
2	July 3	4, 5,6	0.918	2.91	3.06	4.321
3	June 27	1,2,3	0.766	4.36	5.01	3.947
4	June 27	7, 8,9	0.4312	3.67	5.60	2.962
5	July 2	4, 5,6	0.299	3.04	5.55	2.410
6	June 29	4, 5,6	0.2816	2.91	5.48	2.393
7	June 27	4, 5,6	0.2405	3.04	6.00	2.212
8	June 28	4, 5,6	0.2245	2.63	5.56	2.137
9	June 29	1, 2,3	0.2156	2.26	4.87	2.095
10	July 1	4, 5,6	0.2138	2.30	4.98	2.085
11	July 2	7, 8,9	0.196	1.68	3.80	1.987
12	June 28	7, 8,9	0.157	1.47	3.72	1.787
13	June 28	1, 2,3	0.157	1.73	4.38	1.787
14	June 29	7, 8,9	0.135	1.22	3.59	1.657
15	July 1	7, 8,9	0.116	1.17	3.04	1.536
16	July 2	1, 2,3	0.073	0.48	(1.80 (Not included in avg)	

Average K 4.51

PLATE III



Plotted from figures in column 3 + 4, table 10.
O_2 concentration, cc's. O_2 per 134 cc's. H_2O.
O_2 consumption. cc's. O_2 consumed per gram weight in one hour.
1.21cc.
0.138cc.
0.598cc.
0.166cc.
0.29cc.
0.123cc.
0.232cc.
0.113cc.
0.219cc.
0.095cc.
0.176cc.
0.065cc.
0.146cc.
0.061cc.
0.094cc.
0.034cc.
Test
1
2
3
4
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8

which are shown in Table 11, the animals were placed in air saturated water, about 0.760 c.c. oxygen per 134 c.c. water. They were left for two hours, removed, and immediately placed into air saturated water and left for three hours, then removed and placed into aerated water for four hours---and so on for successive periods of five hours, six hours and up to twenty hours.

The amount of oxygen left at the end of the second hour was taken as the oxygen content for the third hour, that left at the end of the third hour was considered to be the concentration for the fourth hour, and so on.

The difference between the oxygen consumption during the second hour period and the third hour period is taken as the oxygen consumption during the third hour, that between the fourth and fifth hour period as the oxygen consumed during the fourth hour, and so on.

Although this method does not give the exact oxygen content of the water for any definite time, still the approximate concentration may be obtained and the point at which the oxygen consumption is decreased below the normal is fairly definitely established.

Table 11.

In aerated water the leeches consumed a rather uniform amount of oxygen for six hours,--that is the consumption during the sixth hour is as great as it is during the first hour. This amount, average 0.080 c.c. is taken as the "normal" oxygen consumption per hour, At a concentration of 0.383 c.c. per 134 c.c. water.

TABLE XI.

The relation of oxygen consumption to oxygen concentration. Leeches three days in the laboratory before beginning the experiment. Each figure in columns 2, 3, 4, 5 is the average of six pair of leeches. Average weight of each leech is from 0.15 to 0.20 grams. Temperature 20° C.

Column	1	2	3	4	5
Trials	Hours	Original O_2 content in cc. O_2 per 134 cc. H_2O	Cc. O_2 left after the experiment	Cc. O_2 consumed during the experiment	Cc. O_2 consumed per hour.
1	2	0.766	0.642	0.142	0.071
2	2	0.752	0.588	0.164	0.082
3	3	0.750	0.490	0.260	0.085
4	4	0.766	0.438	0.328	0.082
5	5	0.771	0.388	0.383	0.077
6	6	0.784	0.262	0.522	0.087
7	7	0.714	0.264	0.451	0.064
8	8	0.766	0.224	0.542	0.067
9	9	0.778	0.134	0.645	0.071
10	10	0.775	0.159	0.618	0.060
11	11	0.778	0.158	0.620	0.055
12	12	0.771	0.076	0.695	0.057
13	13	0.777	0.098	0.679	0.052
14	15	0.727	0.034	0.691	0.044
15	17	0.697	0.030	0.666	0.039
16	20	0.739	0.025	0.714	0.035

Table 11 column 5, five hour period, there is still sufficient oxygen for the leech to consume the normal amount for another hour, or, until the oxygen supply is depleted to about 0.262c.c. per 134 c.c. water. By taking an average of the amounts of oxygen which are left after six hours, Table 12, column 4, a concentration of 0.279 c.c. per 134 c.c. water is obtained, being slightly higher than 0.262 c.c. Near this concentration, therefore, the decrease in oxygen consumption due to lowered concentration becomes measurably evident.

Table 12

Plate IV

The results given in Table 11 column 2 and 5 are also used to produce the curve in Plate IV. To obtain the amount of oxygen available per hour for the duration of the experiment, the original oxygen concentration was divided by the number of hoursduration of the test. That is for trial I a supply of 0.383 c.c. oxygen per hour is available for the pair. For the duration of 10 hours 0.0775 c.c. oxygen is available per hour and for the duration of 20 hours only 0.0369 c.c.

The average of the first two tests is reported as test I in the curve, the next two are averaged as test 2 and the last two (duration of experiment being 17 and 20 hours respectively) are averaged as test 8.

Plate IV

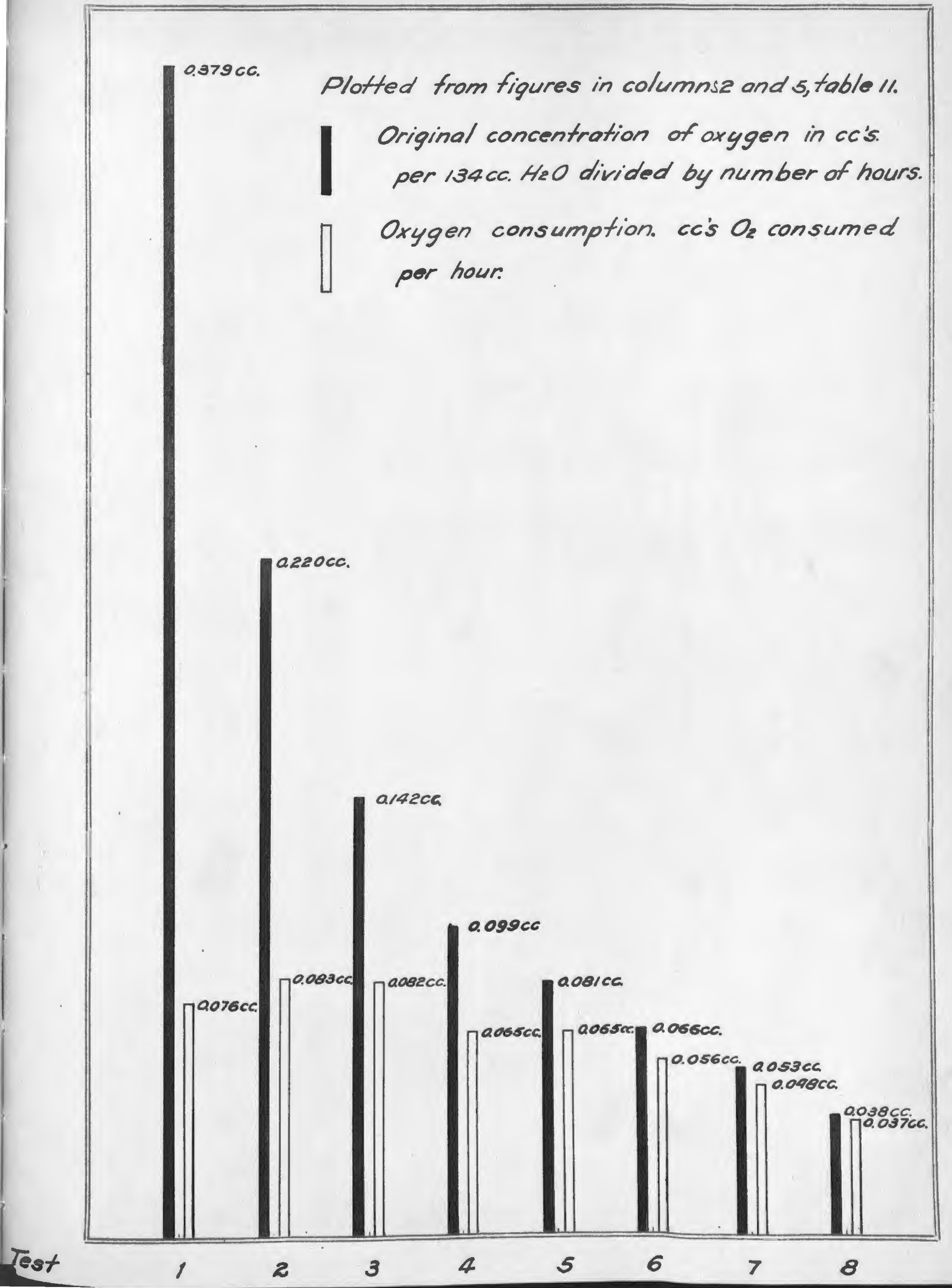
The relative slope of the two curves shows that where the amount of oxygen consumed is dependent upon the amount of oxygen available, the decrease in the oxygen consumption is less than the corresponding decrease in oxygen

TABLE XII.

Using the results from columns 3 and 5 of Table 11, of the first six tests, where the O_2 consumption is approximately uniform, to calculate the oxygen which would be consumed during six hour periods at the same ratė, column 2. The oxygen concentration at this rate which would remain after these six hour periods is given in column 3.

Column	1	2	3
Duration of the test in hours	Cc. O_2 consumed per hour.	C. O_2 consumed during 6 hours.	Cc. O_2 left after six hours.
2	0.071	0.426	0.340
2	0.082	0.492	0.260
3	0.085	0.520	0.230
4	0.082	0.492	0.274
5	0.077	0.459	0.309
6	0.087	0.522	0.262
Average	0.0806	0.485	0.279

PLATE IV



Plotted from figures in columns 2 and 5, table 11.
Original concentration of oxygen in cc's. per 134 cc. H2O divided by number of hours.
Oxygen consumption. cc's O2 consumed per hour.
0.379 cc.
0.076 cc.
0.220 cc.
0.083 cc.
0.142 cc.
0.082 cc.
0.099 cc
0.065 cc.
0.081 cc.
0.065 cc.
0.066 cc.
0.056 cc.
0.053 cc.
0.048 cc.
0.038 cc.
0.037 cc.
Test
1
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concentration.

This decrease in oxygen consumption as found in both types of experiment indicates a change in respiratory metabolism. The aerobic respiration is evidently supplemented by an anaerobic respiration which becomes more prominent as the oxygen supply becomes less. The gradual decrease in oxygen consumption per hour, up to 20 hours, as the oxygen concentration is decreased beyond about 0.27 c.c. per 134 c.c. water indicates the increase in importance of the anaerobic metabolism.

The results from these two methods of changing oxygen concentration are similar. Since this is true there is no evidence that acclimatization plays a prominent part in the respiratory processes of the leech during periods of six or seven hours of gradual change in oxygen concentration.

Preliminary experiments indicate that this probably is not true for Planaria.

Konopacki (1907) maintains that the formula $a = k\sqrt{d}$ expressed the relation between oxygen consumption to oxygen pressure in Lumbricus where at concentrations below air saturation a stands for oxygen consumption, k a constant and d oxygen concentration. This formula has also been applied by Konopacki to Thunberg's and Hense's results to show that within certain limits the oxygen consumption increases as the square root of the oxygen pressure increases. In different animals, the limits, between which the oxygen consumption conforms with the above formula, are different--and above a certain pressure the consumption is independent of the concentration.

From the results of the experiment recorded in Table 10 column 5 and 6 the relation of the oxygen consumed to the square root of the oxygen concentration is not a constant.

##############

VII EFFECT OF LIFE IN WATER OF LOW OXYGEN CONCENTRATION ON SUBSEQUENT OXYGEN CONSUMPTION IN HIGHER OXYGEN CONCENTRATION

As an attempt to explain life in oxygen free media the view that the cell may store intramolecular oxygen in some loosely combined form is still accepted by some.

This storage is accomplished by " affinities " of the protoplasm according to Pflüger's theory or by "Biogen molecules" according to Vernon. Miall suggested that the haemoglobin functions as a store for oxygen.

Winterstein (19) quotes with reference to worms, many experiments to demonstrate the greater power of resistence to lack of oxygen of those possessing respiratory pigments.

Leitsch (8) after experiments on chironomons larvae concluded that these animals do not store oxygen.

Putter (14) found that Hirudo Medicinales may live for ten days in an atmosphere of nitrogen and recover completely after two days of anaerobic life. The oxygen consumption of the leeches when placed in aerated water is increased from 40 to 100% over the previous rate of oxygen consumption before placing the animal under anaerobic conditions. The oxygen consumption returned to normal after two days in aerated water. In another experiment at higher temperature the abnormal consumption was still evident after four days.

He concludes from his experiments that oxygen storage is possible but that the amount has been overestimated.

The following two experiments were performed for the purpose of determining whether the rate of oxygen consumption in aerated water is changed when a leech which has lived in water of low oxygen concentration is suddenly transfered from it to aerated water.

After a period as short as four hours in water of low oxygen concentration there was an increase in oxygen consumption when the leech was put into aerated water.

1) The results of an experiment given in Table 13 show that starting at an initial oxygen concentration of 0.034 c.c. per 134 c.c. water the average oxygen consumption per hour during twelve hours was 0.013 c.c. of oxygen on only about 1/70 of the rate of oxygen consumption per hour during the previous two hour period at air saturated water.

2) The average rate of oxygen consumption by the leech after return to aerated water is over two times that during the previous period in aerated water.

3) This high rate during the third period descends again to a level at the end of the fourth period (2½ to 3 hours each) to a rate which is approximately that of the first period.

Another experiment was performed to determine more accurately the time at which normal oxygen consumption re-occurs. Two determinations were made to get the normal rate of consumption (period 1 and 2, Table 14). The anaerobic period was prolonged to 12 hours, during which time the oxygen consumption for the larger leeches was 0.004 c.c. per hour (period 3)

TABLE XIII.

Leeches collected July 10. Kept from July 11-12 at a temperature of 20°C as also during the experiment. Experiment begun July 12, finished July 13.

	1st Period	2nd Period	3rd Period	4th Period
Duration of test in hours	2	12	1	2
Time	4. P.M-6.P.M	6.P.M-6.A.M	6.A.M.-7.AM	7.AM-9.A.M.
Cc. O_2 per 134 cc Orig. Concen.	0.711	0.034	0.731	0.766
Medium Sized Lchs One in each bottle.	Oxygen Consumption			
Bottle 1	0.151	0.0172	0.155	0.181
" 2	0.160	0.0086	0.215	0.224
" 3	0.134	0.0172	0.198	0.232
" 4	0.306	0.0172	0.249	0.396
" 5	0.186	0.0172	(limp)	
Average	0.187	0.0155	0.204	0.258
Average per hour	0.093	0.0013	0.204	0.129
Smaller Leeches Two in each bottle	Oxygen Consumption.			
Bottle 1	0.246	0.0172	0.232	0.241
" 2	0.246	0.0174	(limp)	
" 3	0.255	0.0170	0.206	0.241
" 4	0.237	0.0260	0.163	0.249
" 5	0.272	0.0260	0.155	0.232
Average	0.251	0.0207	0.189	0.241
Average per hour	0.125	0.0017	0.189	0.120

TABLE XIV.

To determine more accurately the time at which the normal consumption reoccurs for Erpobdella punctata after living for twelve hours under anaerobic conditions.

Six larger leeches collected July 10-'17. Ten medium sized leeches collected July 17. All animals were kept at 20° C for 24 hours immediately preceding the experiment as also during the experiment.

		Larger Leeches. One in a bottle								
Period		1	2	3	4	5	6	7	8	9
Duration of the test in hours		2	2	12	1	1 1/2	1 1/2	2	2	2
Time		3-P.M 5-P.M	5-P.M 7-P.M	7-P:M 7-A.M	7-A.M 8-A.M	8-A.M 9.30AM	9.30 11AM	11-AM 1-PM	1-PM 3-PM	3PM 5PM
Cc. O_2 N.T.P. per 134 cc. H_2O org. concentration.		0.748	0.748	0.069	0.740	0.714	0.752	0.752	0.745	0.716
Leech	Wt. grms	Oxygen Consumption								
1	0.81	0.206	0.155	0.052	0.155	0.198	0.149	0.158	0.151	0.174
2	1.36	0.275	0.267	0.043	0.172	0.249	0.236	0.279	0.205	0.268
3	0.848	0.181	0.146	0.052	0.198	0.189	0.149	0.158	0.168	0.165
4	1.665	0.258	0.284	0.052	0.172	0.292	0.253	0.270	0.255	0.234
5	1.756	0.378	0.387	0.052	0.275	0.430	0.425	0.356	0.280	0.346
Avg.	1.295	0.259	0.248	0.050	0.194	0.271	0.242	0.244	0.212	0.237
Avg. per hour		0.129	0.124	0.004	0.194	0.181	0.161	0.122	0.106	0.118
Bottle	Wt. of 2	Smaller Leeches. Two in a bottle.								
1	0.484	0.275	0.181	0.052	0.258	0.163	0.115	0.167	0.151	0.139
2	0.487	0.361	0.292	0.043	0.206	0.198	0.167	0.227	0.203	0.208
3	0.682	0.241	0.181	0.043	0.146	0.172	0.149	0.236	0.168	0.191
4	0.521	0.267	0.224	0.043	0.155	0.172	0.149	0.184	0.177	0.165
5	0.498	0.271	0.184	0.047	0.181	0.241	0.210	0.236	0.177	0.156
Avg.	0.528	0.281	0.208	0.046	0.189	0.189	0.158	0.210	0.175	0.172
Average per hr.		0.140	0.104	0.004	0.189	0.126	0.105	0.105	0.087	0.086

as compared with the normal of 0.126 per hour. After the anaerobic period the amount of oxygen consumed was above normal but gradually decreased so that at the end of four hours the return to normal consumption had reoccurred.

The smaller leeches resumed normal oxygen consumption after two and one-half hour periods (period 5 and 6 Table 14).

From the results given in Table 14 the average amount of oxygen which would be consumed during twelve hours at the normal rate obtained from periods 1 and 2 is 1.512 c.c. for the larger leeches. The amount actually consumed during the twelve hour anaerobic period is 0.050 c.c. If the leech, during this period, obtained its energy by consuming oxygen which had been stored in the tissues, to use the amount normally consumed, 1.462 cc must have been available. Table 15. If the amount of oxygen represented by the increase over and above normal in the period immediately following the anaerobic period is used to replenish this depleted supply of "stored" oxygen then only 10.4% of the amount needed is actually furnished by the increase.

Similarly for the six pairs of smaller leeches the increased oxygen consumption supplies 11.6% of the amount needed to replenish the store. Table 15.

From the results given in Table 14, the average oxygen consumed by the six larger leeches during the four hours preceding the twelve hour anaerobic period was 0.507 cc oxygen. During the four hours immediately following the anaerobic period 0.707 cc oxygen were consumed, which is 0.200 cc in excess over the four hour period preceeding the anaerobic period.

The average volume of leeches weighing 1.295 grams is

TABLE XV.

Showing the relation of the averages obtained from the results given in table 14. The amount of oxygen which would normally be consumed in aerated water during 12 hours is obtained from the averages of periods 1 and 2.
Column 4 of Table 15 gives the averages obtained from period 3, Table 14. Column 6, Table 15 gives the total increase in oxygen consumption above normal which occurred in periods 4, 5 and 6, Table 14.

Column	1	2	3	4	5	6	7
Leeches	Avg. cc O_2 consumed per hour in aerated H_2O	Avg. cc. O_2 consumed per gram hour in aer H_2O	Cc. O_2 normally consumed during 12 hrs. at avg. rate in aerated H_2O	Cc. O_2 actually consumed during 12 hrs. at conc. of .034 cc. H_2O on the exprm.	Difference between 3 and 4. The amount to be supplied.	Cc. which the increase in O_2 consumption supplies	Per cent of the amount needed for normal consumption which the increase supplies
5 Larger Avg. Wt. 1.3 gm.	0.126 cc.	0.1⁻	1.512	0.050	1.462	0.158	10.4
6 Pairs Smaller Avg. Wt. per pair 0.52 gms,	0.122 cc.	0.2⁺	1.464	0.046	1.418	0.171	11.6

1.3 cc. Suppose that an amount as great as one-sixth of the total volume of the leech is blood, namely 0.25cc. and that the haemoglobin of the leech has the same combining power as that of human blood, i.e. 1cc of human blood may hold 0.185 cc. oxygen - then 0.046 cc O_2 might be stored in a leech of this size. The amount which must be stored to supply the oxygen for normal consumption is 0.200 c.c.

Either the haemoglobin of the leech has an enormously greater capacity for storing oxygen than that of man, which is very improbable, or the excess oxygen consumed after anaerobic existence is obtained for purposes other than for storage.

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VIII ANAEROBIOSIS

It has been previously shown that oxygen consumption is nearly uniform for uniformity in weight under the same conditions, therefore the following deductions seem permissible.

Two leeches-Table 10 weighing 0.2899 grams, volume 3 cc. consumed 0.0568 cc. oxygen per hour from aerated water. These leeches lived for twenty hours in aerated water (0.739 cc. of O per 134 cc. of H_2O) and removed all but a trace of the oxygen. Two leeches of similar weight, 0.2835 grams, table 17 column 5, lived for more than one hundred hours in oxygen free water.

Suppose that an amount as great as one sixth the total volume of the leech is blood, namely 0.05 cc. and that the blood of the leech has the same combining power as that of human blood, then 0.00925 cc. oxygen may be stored by this pair of leeches, while 5.68 cc. oxygen would have to be stored in order to supply the amount normally consumed during one hundred hours.

In the case of the larger leeches. One leech Table 8, weighs 0.9099 grams. volume 1cc consumed 0.1125 cc. oxygen per hour. A leech of similar size and weight of 1.059 grams had exhausted the oxygen from aerated water. Number 5, Table 17 in twenty-four hours. Leeches of similar size and weight, namely .8665, 8785 and 1.00 gram (no's. 3 and 4, Table 17) lived for one hundred and twelve hours in water from which the oxygen had been exhausted.

Suppose again that one sixth of the total volume of the leech is blood, namely 0.166 cc., this with a combining capacity for oxygen equal to that of human blood could store 0.0307 cc. of oxygen, While the amount which would have to be stored to keep the leech living normally for this period is 2.60 cc. oxygen.

There is here no evidence of storage of oxygen in the blood in amounts sufficient to allow the same type of metabolism to continue when oxygen is removed,

Lätch (8) after working with Planorbis and chironomons larvae concluded that haemoglobin as a practical store for oxygen is insignificant. The oxygen which Planorbis can store lasts three minutes while in chironomons it lasts twelve minutes. He says, "Its function is to make oxygen available for binding it chemically in quantities sufficient for the needs of the animals at oxygen tensions so low that the necessary amount is not supplied by physical solution. Its use is to supply a constant circulation of a current of alternately oxidized and reduced blood."

The fact that the leech continues to live actively in the absence of oxygen indicates that the metabolism is changed during anaerobic conditions, either the animal gets its oxygen from its own substance, intra molecular respiration, or it consumes none. The increase in oxygen consumption following an anaerobic condition over and above the normal consumption, may be due to the oxidation of material which was incompletely oxidized during life without oxygen,-or perhaps the rise is simply due to the stimulating effect of higher oxygen pressure following an anaerobic existence.

Survival time in oxygen free water.

The results of the next experiments show that the survival time of the leech in oxygen free water is increased with decrease in temperature, Table 16 and increased with increase in size, Table 16.

For want of more accurate methods for determining the condition of the animal, I use the word 'normal' to indicate tenacious use of the suckers, not loosening when the bottle is slowly inverted, and energetic swimming when the animal is stimulated by a slight shaking of the bottle. 'Sluggish' indicates less-tenacious use of the suckers and less energetic swimming when stimulated. 'Limp' refers to the condition in which the animal seldom uses its suckers if at all, mostly lying on the bottom of the bottle and falling again when the bottle is inverted. Death in the leech is often indicated by bursting of the blood vessels. In table 14 l refers to limp, s means sluggish and d dead.

These results also give an index to the conditions which cause a decrease and final loss of irritability and the time when it occurs under given conditions.

Winterstein (1907) explains the loss in irritability in tissues as being due to "an accumulation of asphyxial products which require oxygen to remove them". He found a temporary increase in oxygen consumption in the frog muscle following a period in an oxygen-free atmosphere and concluded that the length of time before normal oxygen absorption is resumed depends upon the rate at which these products may be oxidized.

Similarly, Pütter suggests that this accumulation of material which results from incomplete oxidation accounts for

Table XVI.

Showing that the survival time in oxygen free water is longer for leeches of medium size than for smaller leeches. Also that the survival time of both medium sized and smaller leeches is decreased by increase in temperature in oxygen free water. Leeches one week in the laboratory before beginning the experiment. Original oxygen concentration 0.730 cc. per 134 cc. H_2O.
l = limp, s = sluggish, d = dead.

Temperature 11°-13° C.

Smaller Leeches						Time in hours	Medium Sized Leeches					
1	2	3	4	5	6		1	2	3	4	5	6
Removed. O_2 exhausted						63						Removed. O_2 exhausted.
	d	d		d	d	67		d			d	
			d			77						
						97			S			
						112	l		d	l		
						122	d			l		
						136				d		
						184				S	Removed. cont to live norma	

Temperature 24°-26° c.

1	2	3	4	5	6		1	2	3	4	5	6
					d	46		d		d		d
d	d	d	d	d		63	d		d		d	

TABLE XVII

Showing that larger leeches retain their irritability longer and continue to live longer in oxygen free water than smaller leeches. n = normal, l = limp, s = sluggish, d = dead. Temperature 20° C. Leeches collected two weeks before beginning the experiment.

1	2	3	4	5	Bottles	1	2	3	4	5
0.5282	.02915	0.3224	0.3468	0.2835	Tot. Wt.	0.7929	0.1000	0.8765	0.8665	1.0589
Two leeches in each bottle					Time in hours	One leech per bottle				
Removed all O_2 exhausted					24					Removed all O_2 exhausted
	(a) n (b) l		(a) s (b) s		40					
	(a) n (b) l		(a) l (b) l		51					
	(a) n (b) d	(a) n (b) s	(a) d (b) d		63					
		(a) n (b) l			68					
		(a) n (b) d			72					
				(a) n (b) l	116					
				(a) n[1] (b) d	123					
				(a) s	136		l		l	
				(a) s	142		d		d	
				(a) d	157			s		
					167			l		
					185	d		d		
					232				l	
					240				l	
					255				d	

[1] (a) Leech left in the bottle
(b) Leech removed. Glass rod inserted.

the fact that the leech may not live indefinitely without oxygen.

He concludes that oxidation is the most rapid and effective way of supplying free energy for the animal. Hydrolytic processes are less so. As the oxygen supply becomes insufficient, oxidation becomes less and less effective and hydrolytic processes assume predominence. The two processes go on side by side.

The fact that the rate of oxygen consumtion in Erpobdella punctata decreases gradually with a decrease in oxygen concentration below a certain minimum concentration, similarly indicates that perhaps hydrolytic processes in the leech take the place of oxidation. Erpobdella punctata, however, may not live indefinitely without oxygen, therefore hydrolytic processes ~~of metabolism~~, it seems, in this leech may not permanently replace respiratory metabolism.

SUMMARY

1. A method is described by which any desired oxygen concentration between 0.019 cc. and 2.43 cc N.T.P. per 134 cc H_2O may be obtained.
2. Under the same conditions of starvation and of oxygen supply, the oxygen consumption of a leech is uniform within the limits of error of the method for periods varying from 2 to 20 hours.
3. Leeches of the same species and approximately the same weight consume approximately the same quantities of oxygen per gram weight, other conditions being equal.
4. In individuals of the same species the oxygen consumption decreases per unit weight as the size of the animal increases.
5. The oxygen consumption in leeches weighing from 0.15 to 0.20 grams is independent of oxygen concentration, above concentrations of about 0.25cc per 134 cc water, both when the oxygen concentration is suddenly changed or when the leech is gradually subjected to the change. The maximum consumption of oxygen occurs at this concentration which is about one-third air saturated.
6. Oxygen consumption above the normal occurs after four hours in water of low oxygen content. The degree of increase and the length of time required before return to normal consumption is resumed, increases with increase in size of the leech and the length of time the leech lived in water of low oxygen concentration.
7. Intramolecular storage of oxygen in haemoglobin of the blood is insufficient as a supply to account for the

oxygen consumptaon during the life of the leech in oxygen free water.

8. Length of life in oxygen free water is longer for larger leeches and longer at low temperatures for leeches of similar size.

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