Respiration Rates in Planarians

I. The Use of the Warburg Respirometer in Determining Oxygen Consumption[1]

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The rate of respiration in planarians has been investigated by a number of workers using various techniques. One of the earliest to do research in this field was Libbie H. Hyman, who reported her findings in a series of articles published about forty years ago. Her work has been confirmed by several, while others have reported results which differ from hers to a greater or lesser degree. This conflict appears to stem both from disagreement over interpretation of results obtained and from questions concerning the accuracy of the apparatus or method used in the determination of the respiratory rate.

The Winkler method for measuring dissolved oxygen in water was used by Hyman (1919a) to determine the oxygen consumption of planarians. In a later paper (1932) she presented statistical evidence defending the accuracy of the Winkler method in reply to Shearer's criticism (1930) of her work based on his own manometric observations. Allee and Oesting (1934) presented a critical evaluation of the Winkler method, comparing it with other methods of determining oxygen consumption. They repeated some of the experiments of Hyman and others, and concluded that although some of Hyman's figures might be erroneously high, due to a nitrite error, such error "need not have affected Hyman's conclusions regarding oxygen consumption for the given experiments."

Fraps (1930) found the manometric method of Warburg adequate for measurement or oxygen consumption in planarians when certain modifications were applied. Bolen (1937), in his study of specific dynamic action in planarians, rejected both the Winkler and manometric methods in favor of Winkler micro-analysis. Wilder (1937) performed a series of comparative experiments, using both the Winkler method and respirometers in order to evaluate the relative accuracy of the two. She found statistical agreement between the two methods at the base level for calculation of oxygen consumption. Løvtrup (1953) attempted to use the Warburg method but, finding that shaking destroyed the planarians, employed instead the Cartesian diver technique for her determinations.

I am studying the oxygen uptake of planarian worms subjected to the influence of a number of related chemicals. In view of the controversies reviewed above, it seemed advisable to perform a number of consecutive tests in order to determine whether or not the proposed experimental procedures would provide accurate and consistent measurements on which valid observations and conclusions could be based. The present experiment was designed in part to obtain information in regard to the conditions under which the Warburg respirometer might be used as a research instrument for a study of planarian metabolism. Attention was directed both to technical details in manipulation of the apparatus and to the responses of the worms to the varying procedures to which they were subjected.

Also, in order that results obtained might not be invalidated by fluc-

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tuations in oxygen consumption due to extraneous factors, the experiment was so arranged that a determination might be made of the oxygen consumption of planarians as affected by feeding, starvation, and changes in temperature.

Planarian worms typically show a high increase in metabolic activity directly after feeding which later declines during starvation to a fairly constant level at which it remains for some time. Oxygen consumption rises later as the worms begin to make use of their own body tissues in the absence of other food (Hyman, 1919b, 1920). In this experiment I wished to determine how many days would elapse after feeding before the constant level of metabolic activity was established, and whether or not the constant level, in this species, would continue for a period of time sufficient for other experimental work.

Materials and Methods

The planarian worms used in this study were collected during the latter part of the summer from Buckhorn Springs, about five miles south of Sulphur, Oklahoma. Within the course of an hour about 2,000 large, sexually mature animals were obtained, together with several hundred egg capsules. Exact identification of the worms has not yet been completed. They have been tentatively identified as a variety of *Dugesia dorotocephala*. Judging from external appearance, habitat, and physiological reactions, they appear to belong to the group formerly known as *Planaria agilis* (Hyman, 1920, 1929). This group is now included in the species *Dugesia dorotocephala* by both Kenk (1935, 1944) and Hyman (1951).

The worms were taken into a laboratory room and kept at a constant temperature of 17° C. for approximately two months before being used for experimentation. They are maintained in deep, enameled pans, each with an aerator, in water obtained from Crystal Lake, north of Norman. The worms are removed to a warmer room (22-23° C.) every five days for feeding. They are given beef liver and allowed to feed 2-3 hours. After the liver is removed, the worms are washed and placed in fresh lake water. They have remained sexually mature and produce egg capsules regularly.

For this experiment, two pans of stock worms were designated as experimental animals, one group (pan # 1) to be tested at 17° C., the other (pan # 2) at 22° C. Worms in pan # 1 were fed as usual and returned to the constant temperature room where they were left without food during the course of the experiment. Water was changed every five days and aerated continuously. Worms used from this pan were returned to a separate pan, so that the same individual would not be used twice during the experiment. The first determination of the oxygen consumption of these animals was made a few hours after feeding had been completed. Additional measurements were made every other day for sixteen days.

Worms in pan # 2 were not returned to the constant temperature room, but left in the warm room overnight and fed the following day, after which the first determination of oxygen consumption at the higher temperature was made. These animals remained in the warmer room during the experiment, but in all other respects were treated exactly as were the animals in pan # 1.

On the day that the oxygen consumption of a group was to be measured, twenty-five of the largest worms were picked up with a wide-bore, large-bulb pipette and placed in a 600 ml. beaker of aerated lake water. Large, mature worms were selected in order that a smaller number of heavier individuals might be used for each determination. This provided more accuracy in weighing, due to less surface for mucus production,

and greater ease in removing excess water. Each worm was transferred individually to the weighing pan by means of a small, moistened camel's hair brush. A folded cone of Kleenex, with torn edges, was applied at the side of the group of worms, and excess water removed by capillary action. The worms were not dried, nor was any attempt made to remove the protective mucus, as this abnormal condition would have injured the worms to some degree. The worms were weighed on a Roller-Smith torsion balance accurate to 0.2 mg.

For each determination, six Warburg reaction flasks were prepared, one to be used as a thermobarometer. Two ml. of aerated lake water, pH 7.85-8.2, were placed in each of five flasks. The worms were rapidly weighed and transferred to the flasks, and 0.2 ml. of 10% KOH and a small strip of fluted filter paper added to the center well. Worms tested at 17° C. were equilibrated for ten minutes. Those tested at 22° C. were equilibrated for twenty minutes to offset the effect of their having been in the colder room during the process of being weighed and transferred to the flasks.

After the period of equilibration was completed, readings were taken every half-hour for three hours. For convenience in comparison, all oxygen uptake is expressed in μl/g/hr.

Results and Discussion

The results of the study of oxygen consumption of planarians maintained at 17° C. are given in Table I. From the data presented it can be seen that although there is a certain amount of variation, there is a definite trend from an initial higher rate of oxygen consumption shortly after feeding, through a decline of several days, to a fairly constant rate which continues until the end of the experiment. For convenience in reference I have divided this into periods as follows:

Phase *A*. The initial rise in oxygen consumption which occurs soon after ingestion of food.

Phase *B*. The marked fall in respiratory rate which begins a few hours after feeding and continues for several days.

Phase *C*. The more or less constant level of metabolic activity which succeeds phase B, and is characterized by a very slight and continuing decline.

A final period, which might be termed phase *D*, has been reported by Hyman (1919a) and others to occur. It is marked by a noticeable and continuing rise in metabolic activity. This phase was not observed in this experiment because the experiment was ended during phase *C*.

TABLE I

Average oxygen uptake in each flask, in μl/g/hr. Five mature planarians in each flask. 17° C.

	Days after feeding								
Flask	0	2	4	6	8	10	12	14	16
1	163	131	133	109	112	114	99	93	86
2	160	118	*	107	110	101	98	88	89
3	163	127	111	117	112	94	104	99	85
4	162	126	116	101	104	95	97	93	97
5	169	127	107	111	131	103	80	89	84
Avg.	163	126	117	109	114	101	96	92	88

* One worm died; data not used.

The data show that phase *A* was not as pronounced in this experiment as has been reported by other investigators. This was due, in part at least, to the following factors: 1) several hours elapsed after feeding before the first readings were taken; 2) the measurements were based on the weight of the worms after feeding, not before; the weights thus included the ingested food, which was more or less inert material. This was not considered important for the purposes of the experiment, since one object of the study was to find a sufficiently long period during which the oxygen consumption of unfed planarians would show a minimum of either rise or decline. This period, phase *C*, was found to begin about the sixth day after feeding, and to last for at least ten days, at which time the experiment was terminated. This more or less constant level of metabolic activity has been reported to extend from one to three weeks in *Dugesia dorotocephala* (Hyman, 1919a, 1920) to as long as six weeks in the variety formerly known as *Planaria agilis* (Allen, 1919).

In spite of the variation in averages shown in Table I, hourly consumption of oxygen in individual flasks was quite constant. Typical hourly readings are presented in Table II. The unvarying oxygen consumption over a period of three hours shown in flask 2 occurred a number of times during the course of the experiment. Repeated values, such as are shown in flasks 3, 4, and 5, were of quite common occurrence.

TABLE II

Hourly changes in manometer level in mm. for each of five flasks, containing five worms each. The weight of the worms in each flask is given. These data were obtained on the 12th day after feeding. 17° C.

	Flask 1 (mm/hr)	Flask 2 (mm/hr)	Flask 3 (mm/hr)	Flask 4 (mm/hr)	Flask 5 (mm/hr)
Hour 1	16	15	15	16	13
Hour 2	13	15	14	14	11
Hour 3	15	15	15	16	13
Average	14.7	15	14.7	15.3	12.3
Weight of worms	222 mg.	223.6 mg.	219.8 mg.	234.4 mg.	218.8 mg.

The data obtained for the worms maintained and tested at 22° C. showed more hourly variation in oxygen uptake, although the totals consumed during the three hours, and therefore the averages, were quite consistent. These data are presented in Table III. The figures show the same trend from day to day that was found with the worms tested at 17° C. The oxygen uptake is consistently higher, which is to be expected with a higher temperature.

TABLE III

Average oxygen uptake in each flask, in μl/g/hr. Five mature planarians in each flask. 22° C.

	Days after feeding								
Flask	0	2	4	6	8	10	12	14	16
1	164	150	149	152	145	130	138	135	135
2	194	157	*	147	133	133	120	143	126
3	174	164	143	148	147	137	129	150	133
4	186	168	144	147	149	141	150	126	125
5	185	157	151	146	146	140	147	136	140
Avg.	181	159	147	148	144	136	137	138	132

* Slow leak observed in flask during first hour; no data taken.

A comparison of the figures with those of Hyman (1920) show a much lower value than those she reported for *Dugesia dorotocephala*, and a slightly lower value than those she gave for variety *agilis*. Aside from the difference in method, this is ascribed to the fact that the worms used in this experiment were much larger and heavier than the ones used by Hyman. The lowest weight obtained here was 140 mg. for five worms after sixteen days starvation, or an average of 28 mg. per worm. Hyman reported a weight of 472 mg. for fifty worms after fourteen days starvation, or an average of less than 10 mg. per worm. The values are in agreement with Hyman's findings (1919a, 1920), however, that large and old worms consume less oxygen per unit weight than do small, young ones. The measurements obtained here agree more closely with those of Allen for variety *agilis* (1919).

Since movement of animals will increase oxygen consumption, the planarians were observed regularly in order to note their responses to the conditions of the experiment. For the most part, the worms remained attached to the glass floor of the flask. Occasionally one would be noted swinging to and fro in the water as the flask was shaken. Neither gliding nor crawling was noticed at any time, although it is presumed the variations reported may be due in part to such activity occurring during the time the apparatus was stopped for readings to be taken.

The technique employed was not perceptibly injurious to the worms. All, with the exception of one, were apparently normal in every way during and after the course of the experiment. The one exception was a planarian which crawled off the weighing pan and fell to the oily floor of the balance below. It was picked up, apparently unharmed, but one of the worms in the flask died later, presumably this one.

Summary and Conclusions

1. A study was made of the oxygen consumption of large planarians of the genus *Dugesia* during a period of sixteen days of starvation after feeding, at temperatures of 17° and 22° C.

2. The Warburg respirometer was used and found to be adequate, under the conditions of the experiment, for measurement of oxygen consumption in planarians. Worms were not injured by the techniques employed.

3. The suggestion is made that the typical changes occurring in respiratory rates in starving planarians be divided as follows: Phase *A*. Initial rise; Phase *B*. Marked decline; Phase *C*. Constant level; Phase *D*. Final rise.

4. Phase *C* was found to begin about the sixth day after feeding and to extend for at least ten days until the termination of the experiment.

5. Although oxygen consumption at 22° C. was consistently higher than at 17° C., the same phases and pattern of decline were noted.

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