# Respiration Studies With Fresh-Water Molluscs:

## II. Oxygen Consumption in Relation to Hydrogen-ion Concentration

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Considerable work has been carried on in the investigation of oxygen consumption of aquatic animals, principally with fishes, in relation to the hydrogen-ion concentration of the surrounding water. Hyman (1925) has shown that acidifying the water (lowering of the pH value) by various acids decreases oxygen consumption of *Planaria*. She stated also that there is a tendency toward acceleration of the rate of oxygen consumption with acetic and possibly carbonic acid. She also found that acidification of carbonate-free water had little or no effect upon the rate of oxygen consumption, except when acidity was produced by carbon dioxide. She gives quite a complete summary of the work up to the time of her investigation. It seems, that in the great majority of cases reported, increased acidity of the water retards oxidation.

Hall (1931) has stated that an increase in hydrogen-ions inhibits oxygen consumption of the puffer fish and that carbon dioxide causes a more pronounced effect than an addition of hydrochloric acid at the same pH.

One of us (1931) using dragon-fly nymphs found an increase in oxygen consumption when the pH of the water was lowered, either by the addition of hydrochloric acid or carbon dioxide. The results of the present work with molluscs are in agreement with those obtained with dragon-fly nymphs. Why there should be these exceptions to the general rule cannot be explained here but will require further investigation. It might possibly be thought that a decreased pH, brought about either by an addition of acid or increase in carbon dioxide, may act as a respiratory stimulus, as is true of warm-blooded vertebrates. To this should be added the more recent work of Johnson (1936) who observed an acceleration of the rate of the respiratory movements of *Squilla mantis*, *Pandalus borealis*, *Cirolana borealis*, and *Idotea neglecta*, in media either containing an excess of carbon dioxide or lacking in oxygen. Whether or not more oxygen was consumed by these crustaceans was not determined, but their increased respiratory activity indicates an attempt in that direction.

### Material and Methods

The two species of water-breathing gastropods, *Campeloma crassulum* (Raf.) and *Pleurocera undulatum canaliculatum* (Say), were used. Both of these were obtained from the Wabash River in the vicinity of Lafayette, Indiana. Being small molluscs, groups of them were used simultaneously in the determinations. The apparatus and methods of

gas analysis used were identical to those described in a previous paper appearing in this journal (Hiestand, 1937). The animals were placed in distilled water, the pH of which was varied by the addition of hydrochloric acid or sodium hydroxide, and were allowed to remain for periods of eight hours (one for a period of ten hours). The oxygen tension of the water at the start of each experiment was not exactly the same but nearly so. It was found in other investigations that even when the same stock water was used each time, the oxygen tension was not always the same. This is not unusual when one considers that slight temperature variations occur upon standing of the water; consequently the oxygen dissolved in the stock water will likewise vary. After each experiment the snails were removed from the animal jar and returned to distilled water. After the experiments were completed, the snails were weighed, killed, and removed from the shells. The shells were then weighed separately and the live weight of the snails (less shells) determined.

1. **Experiments with Campeloma.**—Twenty individuals with a weight (less shells) of 19.97 gm. were placed in the animal cage and the latter suspended in laboratory distilled water having a pH value of 5.4. Determinations of oxygen (cc. per liter) were made at intervals of two hours for a ten hour period. A constant temperature of 23° C. was maintained through this and the following experiments. The results are shown by Table I and Figure 1. Following this a second trial was run under as nearly as possible the same conditions. The same stock water as before was used, however, with slightly more (0.08 cc. per liter) oxygen than before. This trial was conducted for an eight hour period as were the following two trials. The results are given in Table II and Figure 1.

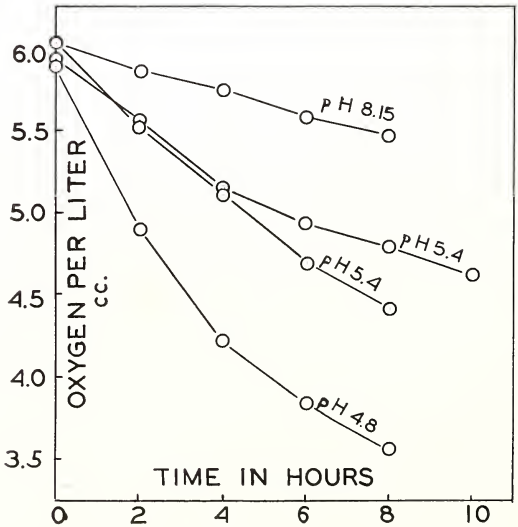


Fig. 1.

TABLE I.

*Campeloma crassulum:* 20 specimens. Wt. 19.97 gm. pH. 5.4

| Hours | Water vol. ml. | Thios. ml. | $O_2$ per L. mgm. | $O_2$ per L. cc. |
|---|---|---|---|---|
| 0 | 960 | 4.24 | 8.48 | 5.94 |
| 2 | 949 | 3.98 | 7.96 | 5.57 |
| 4 | 938 | 3.68 | 7.36 | 5.15 |
| 6 | 927 | 3.52 | 7.04 | 4.93 |
| 8 | 916 | 3.42 | 6.84 | 4.79 |
| 10 | 905 | 3.30 | 6.60 | 4.62 |

TABLE II.—Same conditions as Table I. pH 5.4

| Hours | Water vol. ml. | Thios. ml. | $O_2$ per L. mgm. | $O_2$ per L. cc. |
|---|---|---|---|---|
| 0 | 960 | 4.30 | 8.60 | 6.02 |
| 2 | 949 | 3.95 | 7.90 | 5.53 |
| 4 | 938 | 3.65 | 7.30 | 5.11 |
| 6 | 927 | 3.35 | 6.70 | 4.69 |
| 8 | 916 | 3.15 | 6.30 | 4.41 |

After these two determinations were made at a pH of 5.4, the hydrogen-ion concentration of the stock water was decreased by addition of three drops of a saturated solution of sodium hydroxide until a pH of 8.15 was reached. The results of the increased pH are shown in Table III and Figure 1.

TABLE III.—Same conditions as preceding tables except pH 8.15

| Hours | Water vol. ml. | Thios. ml. | $O_2$ per L. mgm. | $O_2$ per L. cc. |
|---|---|---|---|---|
| 0 | 960 | 4.30 | 8.60 | 6.02 |
| 2 | 949 | 4.18 | 8.36 | 5.85 |
| 4 | 938 | 4.10 | 8.20 | 5.74 |
| 6 | 927 | 3.98 | 7.96 | 5.57 |
| 8 | 916 | 3.90 | 7.80 | 5.46 |

It then seemed desirable to determine the effects of raising the hydrogen-ion concentration. One drop of concentrated hydrochloric acid was added to the stock water lowering the pH to 4.8. Otherwise the conditions were the same as previously. The results are shown in Table IV and Figure 1.

TABLE IV.—Same conditions as preceding tables except pH 4.8

| Hours | Water vol. ml. | Thios. ml. | $O_2$ per L. mgm. | $O_2$ per L. cc. |
|---|---|---|---|---|
| 0 | 960 | 4.20 | 8.40 | 5.88 |
| 2 | 949 | 3.50 | 7.00 | 4.90 |
| 4 | 938 | 3.02 | 6.04 | 4.23 |
| 6 | 927 | 2.75 | 5.50 | 3.85 |
| 8 | 916 | 2.55 | 5.10 | 3.57 |

## Discussion

The foregoing experiments definitely show a marked effect of the hydrogen-ion concentration of the water on the rate of oxygen consumption of the snail, *Campeloma*. An inverse relationship exists between pH and oxygen intake. So marked is the effect of the hydrogen-ion concentration that (at the end of an eight hour period) the oxygen consumed at a pH of 4.8 was approximately 50% more than at a pH of 5.4, and about 300% more than at a pH of 8.15. Whether the consumption of oxygen in molluscs behaves as a simple diffusion process cannot be stated until more information is available. Since increased acidity of the surrounding water causes an increase in oxygen used, one might look for a more complicated explanation than diffusion alone. In general, increasing the acidity of the medium tends to inhibit cellular activity so one would expect to find a similar effect with the respiration of molluscs unless a separate mechanism exists that responds to an increased hydrogen-ion concentration such as a respiratory center.

**2. Experiments with Pleurocera.**—These gastropods, being smaller than the former, were used in larger numbers. A group of 30 snails was used for the following tests. The weight without shells was found to be 10.22 gm. Three determinations at different hydrogen-ion concentrations were made. Although the results obtained were not as marked as with *Campeloma*, nevertheless comparable results were obtained. The experiment was started at a pH of 5.4, using the same stock water as previously. Determinations were made at two hour periods until eight hours had elapsed . The results of the first trial are shown in Table V and Figure 2. At the end of this eight hour period the oxygen concentration of the water had been reduced to 4.65 cc. per liter.

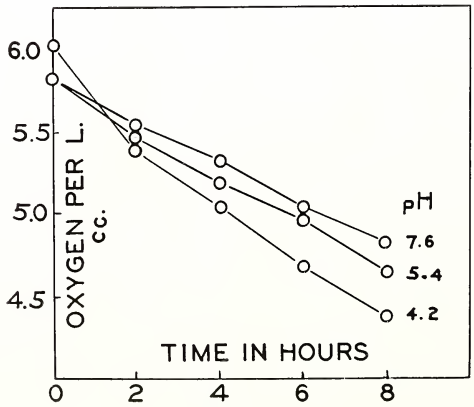


Fig. 2.

TABLE V.—*Pleurocera undulatum:* 30 individuals Wt. 10.22 gm. pH 5.4

| Hours | Water vol. ml. | Thios. ml | $O_2$ per L. mgm | $O_2$ per L. cc |
|---|---|---|---|---|
| 0 | 960 | 4.15 | 8.30 | 5.81 |
| 2 | 949 | 3.90 | 7.80 | 5.46 |
| 4 | 938 | 3.70 | 7.40 | 5.18 |
| 6 | 927 | 3.55 | 7.10 | 4.97 |
| 8 | 916 | 3.32 | 6.64 | 4.65 |

Following this trial the water was acidulated with concentrated hydrochloric acid, lowering the pH to 4.2. At the end of this trial the oxygen had been reduced to 4.37 cc. per liter, which indicates a greater amount used at a pH of 4.2 than at 5.4. The results are incorporated in Table VI and Figure 2.

TABLE VI.—Same conditions as Table V except at pH 4.2

| Hours | Water vol. ml. | Thios. ml. | $O_2$ per L. mgm. | $O_2$ per L. cc. |
|---|---|---|---|---|
| 0 | 960 | 4.30 | 8.60 | 6.02 |
| 2 | 949 | 3.85 | 7.70 | 5.39 |
| 4 | 938 | 3.60 | 7.20 | 5.04 |
| 6 | 927 | 3.34 | 6.68 | 4.68 |
| 8 | 916 | 3.12 | 6.24 | 4.37 |

Following this experiment the same individuals were placed in water with a pH of 7.6, prepared by adding sodium hydroxide to the stock water. The results are given in Table VII and Figure 2. It can be seen that at this higher pH oxygen consumption is less than at the other levels.

TABLE VII.—Same conditions as Tables V and VI except at pH 7.6

| Hours | Water vol. ml. | Thios. ml. | $O_2$ per L. mgm. | $O_2$ per L. cc. |
|---|---|---|---|---|
| 0 | 960 | 4.16 | 8.32 | 5.82 |
| 2 | 949 | 3.89 | 7.98 | 5.54 |
| 4 | 938 | 3.80 | 7.60 | 5.32 |
| 6 | 927 | 3.60 | 7.20 | 5.04 |
| 8 | 916 | 3.44 | 6.88 | 4.82 |

## Discussion

Even though the variations in oxygen consumption at different pH values of the water of this series of experiments are less significant

than those with the snail, *Campeloma*, nevertheless, they are in the same direction; that is to say, at higher concentrations of hydrogen-ions the respiration becomes augmented.

Since the original experiments were performed, other individuals of the same species were used as checks and were found to behave in the same manner in respect to the hydrogen-ion concentration of the water. Also *Vivipara japonica* was tried at different pH levels. Although this species does not respire at as uniform rates, even at the same pH, as the above snails, nevertheless there is a definite trend in the same direction. *Vivipara* is not as reliable an experimental animal as it often remains retracted in its shell for periods of several hours, at which time the oxygen consumption may be practically negligible. After extension of the foot, however, the oxygen consumption progresses at a faster rate so that in a period of six or more hours a fairly definite reading of oxygen consumption can be taken.

## Summary

The rate of oxygen consumption of the gastropod molluscs, *Campeloma crassulum, Pleurocera undulatum,* and *Vivipara japonica* is increased when the hydrogen-ion concentration of the surrounding water is increased. The opposite condition is also true if the hydrogen-ions are decreased. With *Campeloma* the effect is very marked, with *Pleurocera* less so. The respiration of *Vivipara japonica* is much less regular than that of the two preceding species of snails.

## References

Hall, F. G., 1931. The respiration of the puffer fish. Biol. Bull. **61**:457-467.

Hiestand, W. A., 1931. The influence of varying tensions of oxygen upon the respiratory metabolism of certain aquatic insects and the crayfish. Physiol. Zoöl. **4**:246-270.

————, 1937. Respiration studies with fresh water molluscs: I. Oxygen consumption in relation to oxygen tension. In press in this volume.

Hyman, L. H., 1925. On the action of certain substances on oxygen consumption: VI. The action of acids. Biol. Bull. **49**:288-322.

Johnson, M. L., 1936. The control of respiratory movements in Crustacea. Jour. Exp. Biol. **13**:467-475.

Powers, E. B., 1930. The relation between pH and aquatic animals. Amer. Nat. **64**:342-366.