

## THE INFLUENCE OF OXYGEN TENSION UPON METABOLIC RATE IN INVERTEBRATES.

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Since the work of Thunberg (1) on air breathing invertebrates (*Lumbricus*, *Limax*, *Tenebrio*) it has been recognized that the oxygen consumption of some of the lower animals is at least partially dependent upon the oxygen tension in the surrounding air or water. Henze (2) reports that the metabolic rate of some of the simpler marine invertebrates (*Actinia*, *Anemonia*, *Sipunculus*) shows a dependence upon the oxygen tension in the water, decreasing as it decreases; whereas the metabolism of high invertebrates (*Carcinus*, *Scyllarus*, *Aplysia*, *Eledone*) is independent of the oxygen tension over a considerable range. McClendon (3) finds that the oxygen consumption of *Cassiopea* diminishes as the tension of the oxygen in the water is reduced. Lund (4) reports that the oxygen tension becomes a limiting factor in the oxygen consumption of *Planaria* when the tension is reduced below one-third saturation of the water, but finds (5) that *Paramecium* is independent of the oxygen tension over a wide range. Krogh (6) accepts Henze's observations and interpretation and arrives with him at the general conclusion that the metabolic rate of cold-blooded animals is independent of the oxygen tension in the surrounding medium except in some of the simpler organisms, where, because of inadequate respiratory or circulatory mechanisms, the oxygen tension in some of the tissues becomes zero. In such forms the rate of diffusion of oxygen into the tissues must vary with the oxygen tension in the environment, and the metabolic rate must be similarly affected. Krogh believes that Thunberg's results are to be explained in this manner, the animals which he investigated having somewhat imperfect respiratory mechanisms.

We have secured evidence, which indicates clearly that Henze's distinction between the simpler and the more complex invertebrates is by no means absolute. We are able to show that the metabolic rate in some of the higher marine invertebrates, with well developed respiratory mechanisms, is closely dependent upon the oxygen tension in the water over a wide range. We have conducted the experiments in such a way that the data assume a dynamic form, the experimental values giving a consistent series, adapted to mathematical treatment and related by a simple law.

For the determination of dissolved oxygen we have used Birge and Juday's modification (7) of the Winkler method. We have followed the rate at which various animals remove oxygen from a quantity of sea water, the reduction of oxygen tension being effected by the metabolism of the animals themselves. We have conducted the experiments in glass aquaria of various sizes, in glass bottles, and in other similar vessels. Determinations of the oxygen content of the water are made at various times during the course of the experiment, test samples being withdrawn through a siphon into small test-bottles of about 35 cc. capacity. The loss in volume due to the removal of water for testing is thus reduced to the minimum consistent with accurate analysis. In many experiments we have replaced the water withdrawn by a like amount of water made up to an oxygen tension identical with that indicated by the preceding analysis. Constant volume is thus maintained. In no case has the amount withdrawn constituted more than 5 per cent of the total quantity of water used. In all cases the water surface has been covered with a thick layer of paraffin oil, to retard the diffusion of oxygen into the water from the air. Suitable control tests have shown that under our experimental conditions the diffusion of new oxygen into the water from the air is negligible in amount.

Using this simple technique we find that when the oxygen tension diminishes slowly, the marine fish, *Fundulus heteroclitus*, is able to remove equal amounts of oxygen in equal times down to about 10 per cent oxygen saturation, showing thereby that its metabolism is independent of the oxygen tension over a wide range, as Winterstein (8) previously found for fresh water fishes. With more rapid rates of reduction a slight dependence of metabolic rate upon oxygen

tension may become apparent. In the metabolic regulation respiratory and other reflexes are apparently involved.

In striking contrast with the relative independence of these vertebrate forms we find that the oxygen consumption of the lobster,

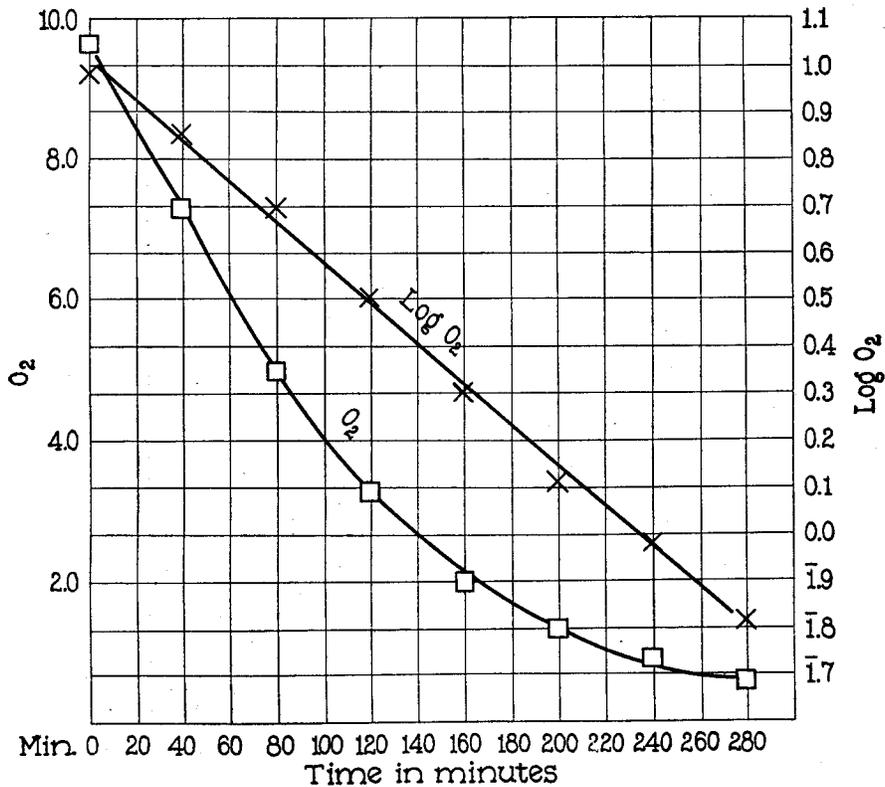


FIG. 1. Rate of oxygen consumption by *Homarus americanus*. Squares represent oxygen tensions remaining in the water plotted against time; crosses represent the logarithms of these tensions plotted against time. Oxygen values are expressed in cc. of N/300 thiosulfate. Capacity of test-bottle = 35 cc., 1 animal. Temperature = 20°C.

*Homarus americanus*, and of the annelid worm, *Nereis virens*, is quite dependent on the oxygen tension in the water. The lobster possesses respiratory and circulatory mechanisms whose efficiency compares favorably with those of aquatic vertebrates. Those of

*Nereis* are less well developed. In the lobster experiments single animals have been used; in the *Nereis* studies groups of from 25 to 100 worms. The lobsters stir the water of the aquaria very efficiently by means of the constant movements of the respiratory apparatus, and the occasional activity of the legs and swimmerets. The worms are

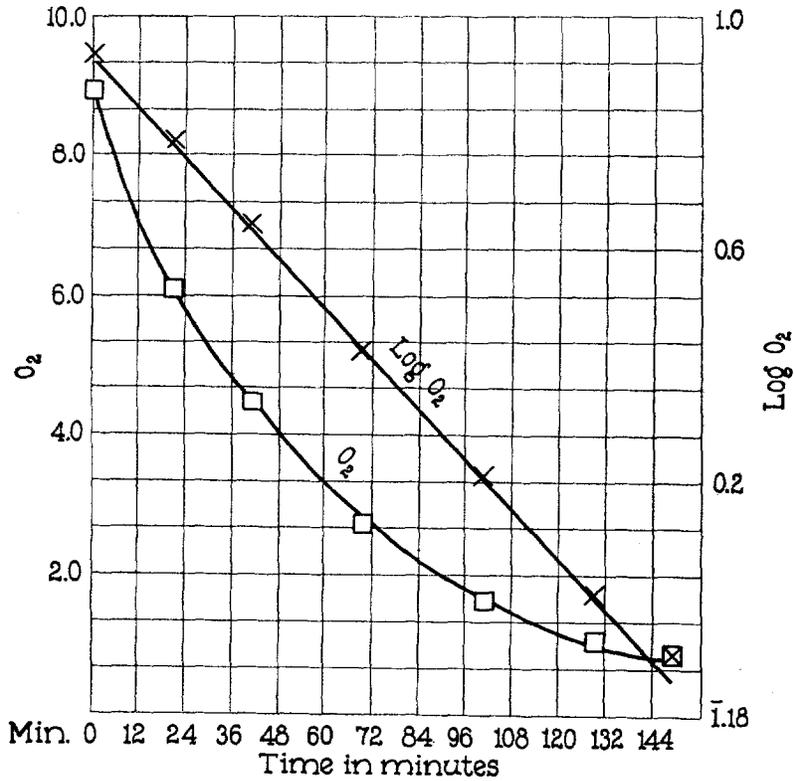


FIG. 2. Rate of oxygen consumption by *Nereis virens*. Squares represent oxygen tensions remaining in the water plotted against time; crosses represent logarithms of these tensions plotted against time. Oxygen values are expressed in cc. of N/300 thiosulfate. Capacity of test-bottle = 35 cc., 82 animals. Temperature = 20°C.

relatively sluggish, and in all experiments the water has been continually mixed by a motor-driven stirrer projecting into the water through the oil seal.

For both animals the experimental values assume the same form. Fig. 1 shows a typical curve of oxygen consumption given by *Homarus*; Fig. 2 a similar curve given by *Nereis*. In both it is seen that as the oxygen tension is reduced the animals consume smaller and smaller absolute amounts of oxygen, so that when oxygen remaining in the water is plotted against time, a curve of exponential form is obtained. When the logarithms of oxygen tension are plotted against time a straight line relationship is obtained, as shown in the same figures. This relationship indicates that at every instant the oxygen consumption is directly proportional to the oxygen tension in the sea water at that instant. The relationship has come out with surprising consistency in dozens of experiments and holds closely over a range of oxygen tensions from full saturation of the water down to 10 per cent saturation. In some cases, as in Fig. 2, it has held down to 7 per cent saturation, and we believe that with refinements in the technique it will be possible to show that it holds for even lower values. Moreover, the curves are reproducible and the same animals, under identical experimental conditions, again and again show the same rate of oxygen consumption, so that the determinations made for several successive experiments fall upon a single curve.

We have satisfied ourselves that this diminution in oxygen consumption with lowered oxygen tension is not due to the accumulation and inhibitory action of  $\text{CO}_2$  in the water, since identical curves are obtained when the same animals are placed in sea water with different  $\text{CO}_2$  tensions. The production of  $\text{CO}_2$  does cause a slight change in the pH of the water (from pH 8.1 to 7.6 when the oxygen tension is reduced to 10 per cent saturation of the water) but we find that changes in  $\text{CO}_2$  tensions much exceeding this amount have no effect upon the rate of oxygen consumption.

The mechanism of the observed effects is not clear. The evidence suggests that some simple physical process, such as the diffusion of oxygen into tissues where the oxygen tension is zero, is the controlling factor. Our data accord exactly with the theoretical expectation for such a diffusion process, since the amount of oxygen delivered by diffusion into such tissues would always be proportional to the external tension. The complexity of the organisms, however, renders impossible any final judgement as to the mechanism of the observed

effects. We hope to push the analysis further, and are continuing the study.

In a few preliminary experiments we find evidence that the oxygen consumption of the king crab, *Limulus polyphemus*, and of the blue crab, *Callinectes sapidus*, is also directly dependent upon the oxygen tension of the water. On the other hand the shrimp, *Palaemonetes vulgaris*, appears to be able to regulate its metabolism down to about 50 per cent oxygen saturation of the water, extracting equal amounts of oxygen in equal times. At lower oxygen tensions its curve of oxygen consumption swings over to the exponential form, similar to that of *Homarus*. The squid, *Loligo pealei*, shows even better regulation, down to about 30 per cent.

It would appear, then, that no general law can be stated for the higher invertebrates; but it is clear that some of them are quite dependent upon the oxygen tension of the surrounding medium, and it is suggested that this dependence is a factor of importance which should be recognized in all studies in which measurements of oxygen consumption are being made. Many workers have assumed that oxygen consumption is independent of oxygen tension, and by the determination of two values, at the beginning and at the end of the experiment, have made calculations of the oxygen intake per unit of weight and time. For all animals in which the oxygen consumption is controlled by the external oxygen tension, such measurements will obviously give incorrect values.

#### SUMMARY.

It is shown that in several of the higher invertebrate animals, oxygen consumption is directly proportional to the oxygen tension in the sea water, over a wide range.

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