ON THE UTILISATION OF OXYGEN BY *MYA ARENARIA*

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(With Seven Text-figures.)

A study of the rather scarce and widely scattered literature concerning the respiratory exchange of Lamellibranchia shows that even under constant external conditions the oxygen intake of a given specimen is extremely variable. This phenomenon was observed by Mitchell (1912) and Galtshoff and Whipple (1930) in *Ostrea*, by Weinland (1918) in *Anodonta*, by Collip (1921) in *Mya*, by Bruce (1926) and Bouxin (1931) in *Mytilus*.

Weinland (1918), for example, states that the oxygen intake of *Anodonta* in one experiment was thirteen times greater than in another, though he used the same specimen under precisely the same external conditions. No doubt, this variability is partially due to variations in the quantity of water moved through the gills. The importance of this factor was already realised by Parnas (1910) but was pointed out more emphatically by Mitchell (1912). However important these variations in the rate of flow of water through the gills may be, it is evident that they cannot be the only cause of the fluctuations in oxygen intake. Mitchell (1912), Weinland (1918), Galtshoff and Whipple (1930) and Bouxin (1931) all state that considerable variations in oxygen intake remain, even when all obvious factors—rate of flow, temperature, etc.—are taken into consideration.

Mitchell supposed "that the nutritive condition of the individual might account for some, at least, of these discrepancies"; Galtshoff and Whipple thought that varying muscular activity might be one of the causes. Weinland concluded "dass die Teichmuschel (*Anodonta*) in verschiedenen Zuständen sich befinden kann, die in ihrem Sauerstoffverbrauch sehr stark verschieden sind."

To this problem of different "conditions," causing a difference in oxygen intake, the investigations described in the present paper may yield a clue.

It is evident that the oxygen intake of those aquatic animals which move a continuous water current over their gills must equal the product of two factors, viz. (1) the ventilation volume, *i.e.* the quantity of water moved over the gills (expressed in terms of litres per hour), and (2) the quantity of oxygen withdrawn from each litre of water[1].

This last factor was called "Ausnutzung" by Winterstein (1908). I will call it *utilisation*, speaking, for example, of an *absolute utilisation* of 1·6 c.c. per litre and a *utilisation coefficient* of 20 per cent., if the inhalant current contains 8·0 and the exhalant current 6·4 c.c. O_2 per litre.

[1] Cutaneous respiration is here left out of consideration.

So far as I am aware, the utilisation was studied only in the case of two fishes (by Winterstein (1908) in *Leuciscus* and by Hall (1931) in the puffer fish, *Spheroides*) and of one cuttlefish (*Octopus*, Winterstein, 1925). In *Leuciscus* Winterstein passed a stream of water over the gills by means of a glass cannula inserted in the mouth. There is one serious objection to be raised against this method: the amount of water driven over the gills is determined by the investigator, and since the utilisation coefficient was found to be inversely proportional to the quantity of water used, the normal utilisation—as was already pointed out by Winterstein himself (1908, p. 79)—cannot be determined in this way.

In this respect Hall's experimental technique is better, though not ideal; this author inserted a cannula in the rounded opercular aperture of the puffer fish, and in this case the animal itself pumped water over its gills. The only drawback is that the velocity of the respiratory current was, in all probability, not *exactly* the same as under natural conditions; assuming it to be somewhat diminished by the resistance of the cannula, we must conclude that the figure found for utilisation (approx. 46 per cent.) was somewhat too high. It is impossible to decide whether the difference was important or not.

In his experiments on *Octopus* Winterstein fastened a rubber tube to the outside of the funnel. Here too, of course, the experimental technique may have caused the utilisation to deviate from its normal value, but in this case I am inclined to think that the deviation was rather insignificant. The utilisation was found to be as high as 70 per cent. and more.

By means of the micromethod described in the preceding paper I studied the utilisation of oxygen in the Lamellibranch *Mya arenaria*; moreover, some observations concerning the ventilation volume were made. The experiments were carried out in the Zoological Station at Helder (Holland), during the last three months of 1933 and in January 1934.

The animal used was *Mya arenaria* L., the soft clam, a species which is of common occurrence along the coast near Helder. The animals were placed in a small aquarium and were, as in their habitat, deeply buried in the sand, the tip of the siphon being flush with the surface (Fig. 2).

This situation was reached in the following way: the clams were placed vertically in a layer of sand on the bottom of an aquarium, the end of the siphon (which was then, owing to the handling, wholly retracted) being about 1 cm. below the surface; water was then admitted. After a shorter or longer interval, during which the siphon renewed communication with the water, a new layer of sand (about 1 cm.) was added, and so on till a siphon length of 15–17 cm. was reached (after about 5 days). In this way the animal was kept in a natural position, which has the further advantage that the sampling manipulations could easily be performed. The aquarium, in which three animals were buried, was placed in a large water bath supplied with a gas thermoregulator; above the sand of the aquarium about $2\frac{1}{2}$ litres of water was present. This water was very intensively aerated by small bubbles (about 100 litres of air per hour), in order to maintain, as far as possible, constancy of O_2 and CO_2 pressure. The aeration was only interrupted during the sampling, which usually

lasted only 3–5 min. The *p*H of the water was frequently compared with that of freshly drawn sea water by means of Merck's Universal Indicator. In no case could I observe any difference in colour. Once a day the water was renewed and freshly caught plankton was added. Before the renewal of the water the animals were left dry for an hour or so, in order to imitate as closely as possible the conditions prevailing in their habitat, which was (as is usually the case, cf. Kellog, 1905) above the low-water line.

For the study of the utilisation of oxygen the micromethod, described in the preceding paper, was used. In order to eliminate errors caused by nitrites, eventually present, NaN_3 was added to the NaOH + JK solution (Alsterberg, 1925)[1]. Two syringes were firmly fastened in a large adjustable screw stand, so that they could be accurately and easily adjusted in all directions (Fig. 1). The capillary of one pipette was adjusted just above the aperture of the branchial siphon, the capillary of the other pipette was introduced about 3–5 mm. into the anal siphon (Fig. 2). Water samples of about 1 c.c. were then simultaneously drawn with both syringes, using the screws for moving the plungers (cf. preceding paper, p. 82). Care was taken to avoid any contact between capillary and siphon, the latter being highly sensitive to the slightest mechanical stimulus.

The difference in oxygen content between branchial and anal water gives the absolute utilisation; this difference expressed in percentage of the oxygen content of the inhalant current gives the utilisation coefficient.

The relative ventilation volume was determined in the following way: by means of a syringe, fastened in the stand, a small quantity of a suspension of solid, powdered Chinese ink in sea water was conveyed right into the centre of the branchial siphon aperture[2]; with a stopwatch I measured the time which elapsed until the first visible trace emerged from the anal siphon, using a plate of white glass as a background. Of course the absolute ventilation volume (expressed in litres per hour) cannot be measured in this way, but I was able to observe changes in ventilation volume which for my purpose was sufficient.

Though there may perhaps be various objections to this method, I preferred it to the methods of Wallengren (1905), Babak (1921) and Hopkins (1933). The method of Galtshoff (1926, 1928) was, in this case, impracticable. The principle of these methods is to measure the ventilation volume by the force, exercised by the exhalant current on a revolving wheel (Babak) or on a system of levers, the movements of which are recorded (Wallengren and Hopkins). Against these methods the following objection may be raised, as was already pointed out by Babak for his own method: if the aperture of the anal siphon is narrowed a little, it seems quite possible that, although the ventilation volume is actually somewhat decreased, a larger one may be registered, since the linear velocity and therefore also the force

[1] When trying to check my micromethod with the macromethod of Winkler, also in the case of sea water, I was unable to attain a full agreement; the cause of this discrepancy is not yet clear, but since the individual trials of the micromethod corresponded very closely, it does not invalidate the results of my experiments. It must, however, be borne in mind that all oxygen figures given in this paper may be from 1 to 4 per cent. too low.

[2] The Chinese ink apparently did not irritate the animal.

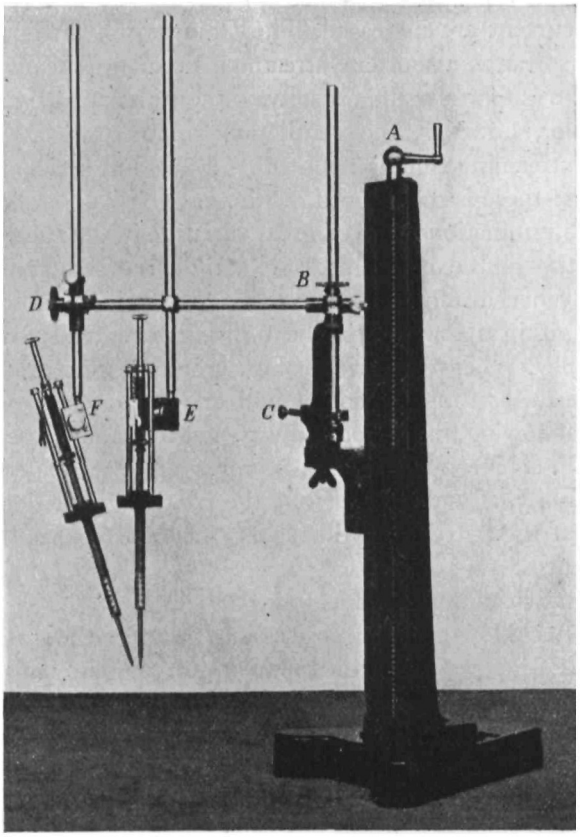


Fig. 1. Two syringe pipettes mounted on Palmer's large adjustable screw stand. The screws *A*, *B* and *C* move both pipettes simultaneously (*A* up and down, *B* to the left or to the right, *C* in a direction perpendicular to the plane of the paper). Screw *D* moves the left syringe pipette separately (up and down); the finer adjustment of each pipette is made possible by turning it on its right bar as an axis (clamps *E* and *F*).

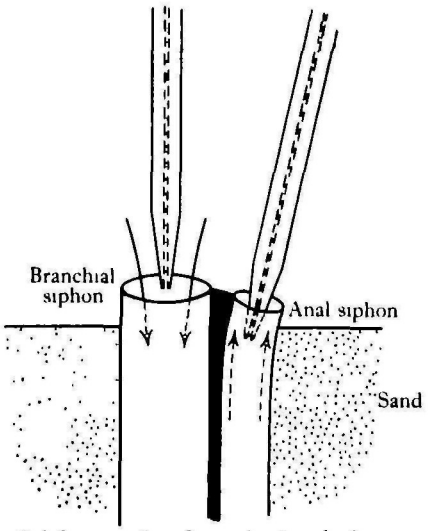


Fig. 2. Position of siphons and syringe pipettes during sampling (diagram).

of the exhalant current are increased. The Chinese ink method yields, I think, useful results in so far as a shorter "streaming time" will really indicate a larger ventilation volume (supposing the capacity of the siphons and mantle cavity to be constant); whether, however, the ventilation volume is exactly inversely proportional to the streaming time is doubtful. In all, four series of determinations were made; one of these is reproduced in Fig. 5.

The earlier determinations showed great variation in the value of the utilisation coefficient, even though the oxygen pressure and the temperature of the water were kept constant[1]. When a number of successive determinations were made on the same specimen within a few hours, the utilisation generally seemed to show a decreasing tendency. When the periods of anaerobiosis were prolonged (low tide), and when I succeeded in making some eight or ten consecutive trials during the first 3 or 4 hours following this period, I always got the same decreasing trend of the utilisation values[2]. Three out of a total number of seven experiments made are reproduced as Figs. 3, 4, and 5. In Fig. 5 the relative ventilation volume (*i.e.* the reciprocal value of the "streaming time" as measured by means of Chinese ink) is also represented.

The results may be summarised as follows:

(1) Shortly after a low-tide period of about 20 hours the utilisation coefficient is rather high (about 20 per cent.); in the next 3 or 4 hours it decreases gradually until a more or less constant level of 5–10 per cent. is reached[3].

(2) The longer the period of low tide has lasted, the higher is the utilisation coefficient immediately afterwards (Fig. 6).

(3) Immediately after anaerobiosis the relative ventilation volume is somewhat increased (streaming time, *e.g.* 9 sec.); during the next 2 or 3 hours it gradually decreases (streaming time, *e.g.* 15 sec.) (Fig. 5).

DISCUSSION.

It is a remarkable fact that the utilisation coefficient of *Mya arenaria* is normally (*i.e.* when the after-effects of a period of anaerobiosis are over) extremely low, viz. about 5–10 per cent. (as mentioned on p. 87, former investigators have found an utilisation coefficient of 46 per cent. in *Spheroides* and of 70 per cent. in *Octopus*). As far as oxygen supply is concerned, a much slower water current would be sufficient; yet it need occasion no surprise that the animal ventilates so strongly, since the same water current must also provide it with food.

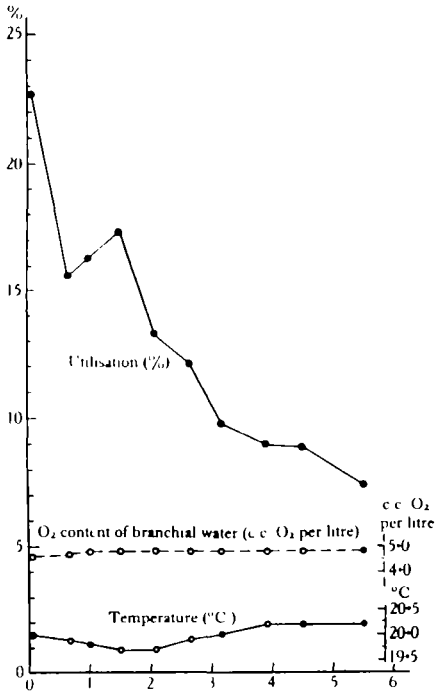
It is evident that the increase in utilisation after anaerobiosis is not due to a decreased ventilation, and that not only the utilisation but also the oxygen con-

[1] All determinations concerning utilisation were performed on the same specimen.

[2] That this phenomenon is not due to an initially high and then gradually diminishing CO_2 content of the anal water (the CO_2 reacting with the Mn^{++} of the $MnCl_2$ solution, thus forming $MnCO_3$ which does not react with oxygen) was shown by adding a threefold quantity of reagents; in this case the same results were obtained.

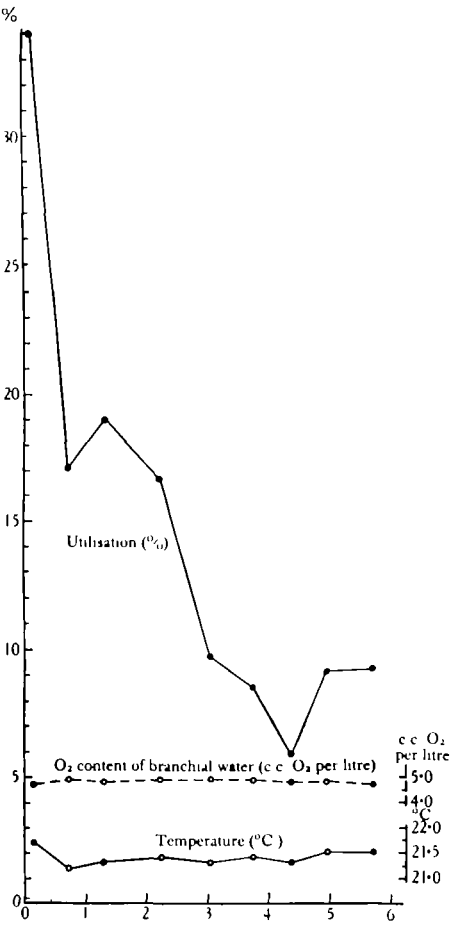
[3] The three experiments represented all show a slight irregularity at the beginning. I do not know whether this temporary increase of the utilisation is a coincidence or not. These irregularities are obviously too great to be accounted for by the inaccuracy of the method for determining the oxygen content as such.

sumption per kg. and hour must be increased. When the animal is deprived of oxygen during low tide, an oxygen debt appears to be formed, which is paid during the first hours of the subsequent aerobic period. The same phenomenon has repeatedly been observed in several species of Lamellibranchia, viz. by Mitchell (p. 213) in *Ostrea*, by Collip in *Mya* and by Jatzenko[1] (1928) in *Sphaerium*. None



Time in hours since end of low-tide period

Fig. 3. Course of the utilisation coefficient after a low-tide period of 13 hours.



Time in hours since end of low-tide period

Fig. 4. Course of the utilisation coefficient after a low-tide period of 21 hours.

of these authors, however, has connected it with the irregularities in oxygen intake of undisturbed, ventilating animals, apparently because none of them realised that an interruption of the ventilation current (either spontaneously or in response to an external stimulus) in the period preceding the experiment will influence the oxygen consumption immediately afterwards.

[1] Some other conclusions reached by Jatzenko are open to objection, as, for example, where it is stated that the circulatory system of Lamellibranchia has external outlets (cf. Quagliariello, 1925), and that the haemocyanin is contained in and the O_2 is transported by the amoebocytes.

With regard to a spontaneous interruption of the ventilation current in Lamellibranchia few observations are recorded in literature. Milne-Edwards (1857) states, speaking of Lamellibranchia in general: "Les courants respiratoires...ne sont pas

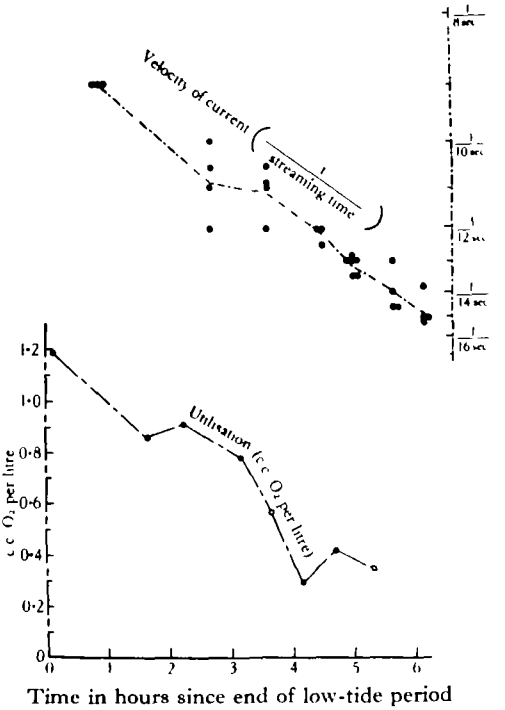


Fig. 5. Course of the utilisation and of the velocity of the respiratory current after a low-tide period of 20 hours.

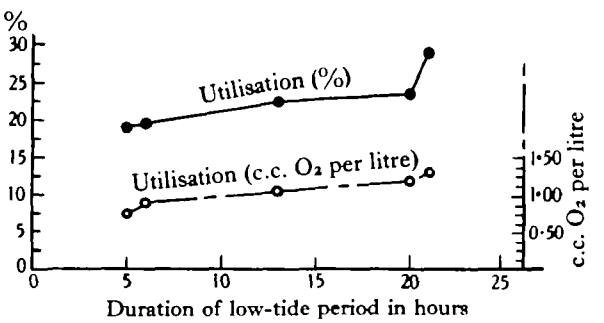


Fig. 6. Influence of duration of low-tide period on the utilisation immediately following this period.

continus; souvent l'animal les interrompt, non seulement quand il est inquiété, mais aussi quand il est dans un état de calme parfait. Du reste il n'y a rien de régulier, ni dans la fréquence de ces temps, ni dans leur durée...." Gartkiewicz (1922) states: "J'ai constaté que l'Anodonte subit alternativement deux états qui se manifestent par l'écartement ou par la fermeture forte et prolongée des valves de

la coquille. Ainsi l'Anodonte est un animal qui absorbe périodiquement l'oxygène. Une période de quelques heures de l'absorption de l'oxygène est suivie d'une autre où l'absorption de l'oxygène s'arrête." In *Mya* I frequently observed that the animals stopped the ventilation for longer periods (*e.g.* some hours) by closing the siphons, sometimes drawing them back under the sand. I found it impossible to say what external stimulus could cause this phenomenon. It evidently never occurred immediately after a period of low tide, but only after the animal had been ventilating for a considerable time, so that its oxygen debt might be supposed to have been paid either wholly or very largely. From the foregoing facts it is clear that the oxygen intake of *Mya*—and, I think, of all Lamellibranchia so far investigated—will depend to a great extent on the oxygen intake in the period immediately

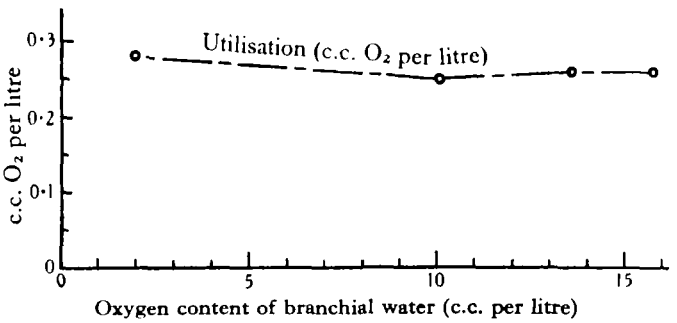


Fig. 7. Showing that the absolute utilisation is within wide limits independent of the oxygen content of the water.

preceding the experiment, *i.e.* on the "oxygen condition" of the animal. Even during continuous ventilation, the oxygen intake will be found to be sometimes high and sometimes low, depending on whether the animal has lived anaerobically shortly before the experiment or not.

The normal absolute utilisation seems to be practically independent of the oxygen tension within wide limits (Fig. 7); the data at hand, however, are rather scanty.

SUMMARY.

1. In *Mya arenaria* the normal utilisation coefficient of oxygen amounts to only 3–10 per cent.

2. Immediately after a period of low tide (anaerobiosis) the utilisation is considerably increased (*e.g.* to about 25 per cent.); during the next few hours it gradually decreases till the normal level is reached. Since, after a low-tide period, the ventilation volume is also temporarily increased, the same must be true for the oxygen consumption per kg. and hour.

3. The great variability in oxygen intake of Lamellibranchia in general, as found by previous authors, is probably due to an interruption of the ventilation current—either spontaneously or in response to an external stimulus—in the period just preceding the experiment.

4. In studies concerning the basal respiratory metabolism of Lamellibranchia it is necessary to work with individual animals.

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