XXXIX. NOTE ON THE OXYGEN CONSUMPTION OF AMPHIBIAN MUSCLE AND NERVE.

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Some observations made in 1919 with the instrument described in a recent paper [1920] showed clearly the course of oxygen consumption in excised muscle and nerve in an atmosphere of nearly pure (95 %) oxygen, when the tissues were at rest, and the different effects of electric stimulation on the two tissues. Although these experiments were incomplete, and obviously capable of much improvement, yet it seems desirable to record such results as were obtained now, since circumstances have arisen which seem likely to prevent my continuing research in this subject.

The rates of respiration observed in resting tissues were the following. Sartorius muscles, 0·10 to 0·135 cc. per g. per hour, reduced to N.T.P.; gastrocnemius muscles, 0·05 to 0·12 cc. per g. per hour; sciatic nerves, 0·05 to 0·08 cc. per g. per hour. The number of experiments done was not enough to determine whether any seasonal change occurs in the respiratory activity of these tissues.

In muscles the rate of respiration observed was usually somewhat higher than this for the first hour or so after excision, and during this preliminary period the rate fell off, reaching finally a steady value. At the end of this preliminary period, which was probably due to the recovery of the tissue from the stimulatory effects of accidental damage during the dissection, the tissue settled down to a constant rate of respiration, which was maintained for about twenty-four hours. This appeared to be the normal resting condition. Subsequently a rise usually commenced, and the rate of respiration observed accelerated continuously till it reached a value many times greater than the steady rate. This rise was obviously due to bacterial action. The general course of survival oxygen consumption thus follows Fletcher's curve [1898] for carbon dioxide output very closely.

During the period of constant respiration the exactness with which the rate was maintained was very remarkable (Fig. 1). Although variations as small as 1 % could easily have been detected from hour to hour, yet the rate was very frequently found constant within the error of reading the scale for many hours

[1] This work was carried out during the tenure of the Benn W. Levy studentship.

consecutively. It should be mentioned that irregular divergences from the steady rate were observed occasionally, but when these were most marked the tissue was generally found to have its response to an electric stimulus considerably impaired. Unfortunately the risk of damage to the tissue in the experiments was greater than is necessary, since an attempt was made to tie the very short tendons at the upper end of the muscle: it would be preferable

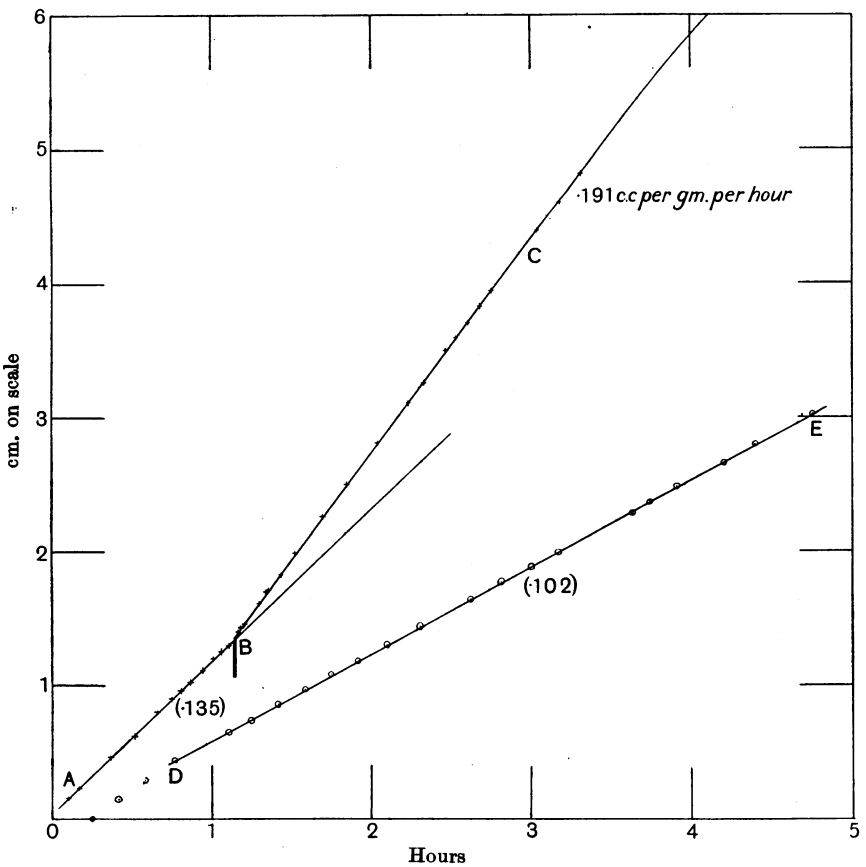

Fig. 1. The figures in brackets indicate the rate of oxygen consumption in cc. per g. per hour.

to include a small piece of bone in the preparation. These divergences were generally large in comparison with the error of reading, whereas in those experiments which show a constant rate, no divergences at all were perceptible. It would seem probable that the steady rate of respiration, remaining constant within perhaps less than 1 %, is a characteristic of a resting excised muscle, just as constant rates of metabolism appear to be characteristic of other unstimulated tissues.

Delayed oxygen consumption after stimulation.

Fig. 1, *A*, *B*, *C*, shows an experiment in which twenty single induction shocks were sent into the tissue at *B*; this was on a sartorius muscle. An immediate increase in the respiration occurred, of about 40 %, and the increased rate was maintained unaltered for about two hours after the stimulation was over, when a slow decrease commenced.

Respiration of nerve.

The experiments were done with a bundle of four or six sciatic nerves dissected out from knee to spinal cord and tied at the ends to the supporting loops of the apparatus. The nerves respired usually at a constant rate, though some slight diminution in the rate was noticed after a few hours, or after a long period of stimulation. The preliminary period of enhanced oxygen consumption, noticed with muscle, was not found with nerve; as would be expected, considering the very small effect of stimulation on the respiration of nerve, if the high initial rate is due to recovery from damage or stimulus inflicted during dissection.

The effect of stimulation was very much less than with muscle. Even tetanising currents of one minute's to half-an-hour's duration gave a very small total effect, if any, and there was practically no "delayed" consumption. Certain slight displacements of the curves of oxygen consumption were noted after long periods of stimulation, it is true, which rendered decision on the question whether the passage of the nervous impulse demands an extra supply of oxygen or not, less definite than had been hoped; but the largest displacement ever observed corresponded to an additional oxygen consumption, in excess of that which would have been used by the tissue at rest, of only 0·02 cc. per g. of tissue. This figure was obtained after fifteen minutes' continuous stimulation. Usually the effect, if any, was less than this. Whether the displacements were due to the tissue using more oxygen is very doubtful, however, since in a control experiment with moist twine in the chamber, a similar effect was actually observed. I think that the heating effect of the prolonged current passing in the chamber may be the cause of the slight displacements, though it is not easy to see exactly how this could bring about an effective increase in the volume of the chamber except by an increase of pressure so great as actually to shift the ground joints slightly.

Although the displacements were probably instrumental in origin, and therefore no positive conclusion can be drawn from them, yet the important negative conclusion appears that tetanus of fifteen minutes' duration does not cause the nerve to absorb more than 0·02 cc. per g. in excess of the resting consumption. The figures given by Tashiro [1913] for the frog's sciatic, recalculated, are for resting tissue 0·167 cc. per g. per hour, and for "stimulated," 0·43 cc. This is an additional consumption of 0·066 cc. per g. in fifteen minutes' stimulation, or three times as large as the maximum effect in my experiments, which were conducted at 22°.

Supply of energy for cell activity.

The energy for cell activities appears to be supplied ultimately by the oxidations within the cell. If however the oxygen consumption can be taken as an index of the progress of oxidation, it is clear from Fig. 1 that the supply of energy takes place, under the experimental conditions, long after the activity has ceased, an inappreciable fraction of the total energy being supplied actually during the period of contraction. That the delayed oxygen consumption is not a slow restoration of oxygen to some intracellular store (such as the "inogen") which is ready to supply large quantities of energy suddenly by oxidation at the moment of stimulation, follows from the arguments brought by Fletcher and Hopkins [1915] against the existence of any such store of oxygen within the cell; it is probable then that the oxidation occurs simultaneously (or nearly so) with the absorption of oxygen and the CO_2 evolution, and that the enhanced oxygen absorption corresponds in time with the actual oxidation or energy supplying reaction.

At the moment of contraction, the muscle fibre must work by drawing on stores of potential energy within the tissue, and it appears that the function of the oxidations is to restore the potential energy of the muscle to its normal resting level. The muscle fibre is further so constructed that the demand for replenishment of these stores of potential energy, available for future activity, is automatically supplied: for the activity of the cell leaves behind a condition leading immediately to an accelerated oxidation.

It does not seem possible to do more than conjecture the function of the steady rate of oxygen consumption in the resting tissue, nor to decide whether it is connected with the maintenance of the normal level of potential energy in the tissue or with some other activity. It may be that the resting respiration is an index of an anabolic process, compensating, and proceeding at an equal rate with, some such catabolic process as the survival formation of lactic acid, observed to occur in resting tissues at a constant rate; but direct evidence that these processes are connected appears completely lacking.

Note on the method of Shiro Tashiro [1913] *for the estimation of very small amounts of carbon dioxide.*

Miss D. M. Moyle and I, in preliminary studies made with a view to utilising the above method for estimation of the CO_2 output of nerve, met with quite unexpected difficulties, which are perhaps worth recording, as they appear to make it doubtful whether the drop of baryta solution is quite as reliable as has been supposed for *quantitative* estimations of small amounts of CO_2 in air.

The quantitative method depends on the alleged fact that a nearly hemispherical drop of baryta solution, standing at the top of a narrow column of the same liquid, in a chamber of about 15 cc. capacity, shows a visible precipitate of barium carbonate when the total CO_2 content of the chamber exceeds

1×10^{-7} g., but does not do so if the amount of CO_2 is less than this. The time required for full development of the visible precipitate is stated as ten minutes. Analyses are made by determining the volume of gas required to give the precipitate.

The first step we took was to test the accuracy of this figure, as it is fundamental to the analysis that the critical amount of CO_2 required should be accurately known as well as the limits within which it is reproducible in successive experiments; and it was clearly of importance to determine, before relying on the method, whether any modification in the design of the instrument or the conditions of use would affect the critical amount of CO_2. All measurements and manipulations of the gases were done over mercury; the apparatus consisted of (*a*) a reservoir of CO_2-free air, the preparation and storage of which according to Tashiro's instructions presented no difficulty, (*b*) measuring instruments and siphon pipettes, for transferring the gases and mixing the standard mixtures required for the calibration, (*c*) a chamber in which the drop of baryta could be formed and observed, and into which the gas mixture could be introduced.

The calibration was very disappointing. In a series of experiments the following results were obtained for (col. 2) an amount which just did give, and (col. 3) an amount which just did not give, a visible precipitate, on close examination with a good lens.

Experiment No.	Ppt. obtained	No. ppt. visible
1	$1{\cdot}13 \times 10^{-7}$	$0{\cdot}91 \times 10^{-7}$
2	0·59 ,,	0·55 ,,
3	0·97 ,,	0·83 ,,
4	1·94 ,,	1·83 ,,
5	0·75 ,,	0·71 ,,
6	0·78 ,,	0·76 ,,
7	—	1·76 ,,
8	1·46 ,,	1·27 ,,

It would appear that the drop of baryta in our hands was not more than a delicate qualitative test for CO_2 and could not be relied on for quantitative measurements.

Several variations of the technique were attempted with no better results, and the conclusion was reached not only that the method would prove extremely laborious if a long series of analyses had to be undertaken, but that we could not obtain results of even 50 % accuracy from it.

It appears not improbable that some nuclei analogous to those required for the crystallisation of supersaturated solutions may be an important factor in determining whether or no a precipitate becomes visible, and if this be the case, it is not surprising that somewhat varying amounts of CO_2 were found necessary to give the precipitate.

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