THE OXYGEN CONSUMPTION OF FROG NERVE DURING STIMULATION.

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For many years the measurement of the possible increase in the oxygen consumption of nerves during activity has been a problem of serious difficulty. During last summer at Woods Hole I was able to overcome this difficulty by making use of the large nerves of the dogfish (following in this the lead of Parker (1925, *a*)) and by at the same time modifying Thunberg's micro respirometer so as to increase its sensitivity. In this preliminary work with the dogfish (Fenn, 1927) it was possible to demonstrate a sharp increase in the oxygen consumption at the beginning of stimulation, persisting for some time after the close of stimulation, but returning to the original rate within about 30 minutes or less. The excess oxygen taken in during activity was found on the average to be equal to 0.21 c.mm. per gm. of nerve per minute of stimulation, the resting rate being 1.35 c.mm. per gm. per minute. The absolute value of this excess oxygen is about 3 or 4 times too small to account for the heat production of nerve during stimulation as found by Downing, Gerard, and Hill (1926) in the frog nerve.

For purposes of comparison with these heat values it was important to obtain similar measurement of oxygen consumption on the frog nerve. These nerves being smaller, there was less likelihood of the oxygen tension being reduced to zero in the interior of the nerve trunk and hence a possibility of obtaining a greater excess oxygen consumption during stimulation. To improve matters still further I have studied the frog nerves in an atmosphere of oxygen, which was not easily available to me at Woods Hole. In spite of these modifications the figures now available for frog nerve are little larger than those obtained with the dogfish nerve, and the discrepancy

between the values for oxygen and that for heat remains. At the present time the most obvious difference to which this discrepancy might be ascribed is the much greater duration of stimulation (20 to 30 minutes) necessary for determinations of oxygen as compared to the 10 seconds stimulation which suffices for heat measurements.

Method.

The method which I have used for frog nerve is the same as that previously described for dogfish nerve, with few modifications. The apparatus consists essentially of two 12 cc. bottles connected by a very fine capillary which carries a kerosene index drop. The nerve is laid on the stopper of one of the bottles in contact with sealed-in platinum electrodes. The other bottle serves for temperature compensation. Even so, a thermostat constant to less than 0.01°C. is necessary for accurate work, because chance currents in the bath affect the bottles independently. The temperature used was 22°C. throughout. Sodium hydroxide (M/4) is placed in each bottle to absorb carbon dioxide, so that the index drop, in response to the consumption of oxygen, will move toward the bottle containing the nerve. Positions of the drop are read at frequent intervals by means of a hand lens and two scales. One scale is just under the capillary the other 3 inches above it. In making a reading corresponding points on these two scales are kept in line, thus avoiding parallax. The capillary itself, with the lower scale, is under water. Further details of procedure may be found in the previous report.

The one difficulty encountered in using frog nerves in place of dogfish nerves is their small size. On the average the dogfish nerves weighed 5 times as much as those to be found in a good sized frog (30–40 mg. each). With the same apparatus, therefore, it should be possible to make corresponding measurements on four frog sciatic nerves and this turns out to be the case (Fig. 1, *a*). To avoid the labor of dissecting so many nerves it seemed worth while to try to make the apparatus still more sensitive. The instrument used for most of the measurements to be reported in this paper does not differ in general plan from the one previously described but it is smaller throughout. The bottles hold only 3.7 cc. instead of 12 cc. and the capillary (a piece of thermometer tubing) holds only 0.73 c.mm.

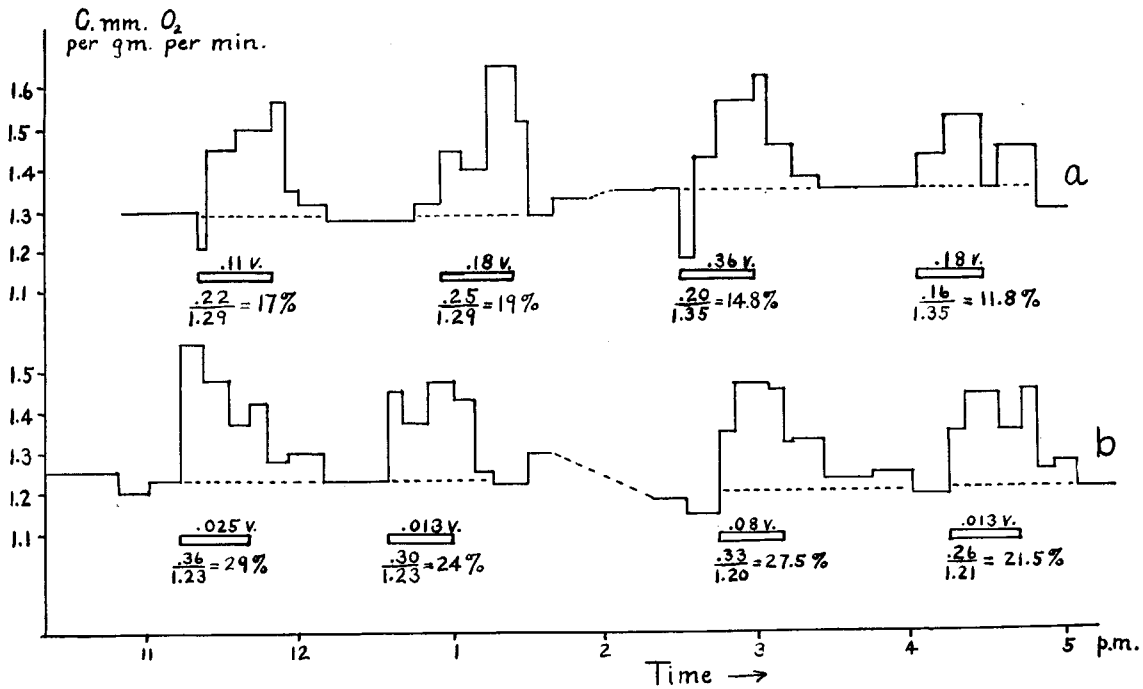

FIG. 1. Graphs showing the rate of oxygen consumption of frog nerves at rest and during stimulation periods of varying intensity. On the graph are inserted figures showing the equivalent voltage of the stimulating current derived from the secondary of an induction coil, and the percentage increase in the oxygen consumption for each period of stimulation. The total excess oxygen for each period of stimulation is represented by the area of the rise as plotted and seems to be independent of the intensity of stimulation within considerable limits.

per cm. instead of 1.94 as before. With this apparatus the index drop moves about 0.25 mm. (an amount easily measurable) per minute with a single sciatic nerve of 40 mg. in the bottle This threefold increase in sensitivity is not obtained without an increase in technical difficulties relating to the tiny index drop. A drop 2 mm. long is preferred. A longer one moves with too much friction in the small tube and a smaller one is harder to manipulate. Fortunately once a good drop is obtained it can be used indefinitely, barring accidents.

After dissection is completed the nerves are quickly weighed on a torsion balance and inserted in the apparatus so that only the central ends lie across the electrodes, the remainder of the nerve resting on the glass. The amount of solution in the two bottles is so adjusted that, after making allowance for the volume of the nerves, the air spaces are equal. Oxygen is then bubbled into the apparatus through the side arm and allowed to escape around the stopper. At the close of the experiment the nerve is weighed again. During the experiment a certain amount of blood and lymph drains out of the nerve so that the second weighing is 11 to 22 per cent (av., 16 per cent) lower than the original one. The final weight was always used in calculating the oxygen consumption. There was no perceptible drying out of the nerve in the apparatus.

Results.

The general character of the experiment does not differ from those previously reported on the dogfish nerve, except in regard to the heating effect of the stimulating current which was quite evident in the dogfish nerve but not usually perceptible in the frog nerve. The difference is partly due to the fact that a weaker stimulus was used for the frog (a Harvard induction coil set at 12 or 13 cm. (or tilted at an angle) instead of at 10 or 11 cm.), but mostly due to the larger size and hence smaller electrical resistance of the dogfish nerves. Moreover, the dogfish nerves were so long that they were brought into contact with the electrodes at more than one point, thus still further increasing the amount of current that could flow.

Intensity of Stimulation.—The results of two experiments with stimulation of varying intensity have been plotted in Fig. 1, *a* and *b*.

For *a*, four frog nerves were used in the dogfish-nerve apparatus. The stimulation periods are indicated by rectangular blocks and lasted usually 30 minutes. The figure over these blocks is the estimated equivalent voltage[1] of the stimulating current from the secondary of a Harvard induction coil vibrating at 50 per second and operated by a single dry cell. The figures underneath each stimulation block show the amount of increased oxygen measured in c.mm. per gm. of nerve per minute of stimulation, divided by the resting rate of oxygen consumption in c.mm. per gm. per minute, the quotient giving the percentage increase. No significant difference is to be seen between stimulation at 9 cm. coil distance (0.36 eq. volts) and at 10 or 11 cm., (0.18 and 0.11 eq. volts respectively), the figures for increased oxygen in these cases being 0.22, 0.25, and 0.20 c.mm. per gm. per minute of stimulation respectively. At the close of the experiment, 6 to 7 hours after dissection, this had dropped to 0.16, indicating perhaps a loss of function in some of the fibers.

In the experiment recorded in Fig. 1, *b*, two frog nerves were observed in the more sensitive apparatus. The nerves were stimulated with an induction coil as before, but a 100 per second tuning fork was used as an interrupter, there being about 0.5 volts across the primary coil during contact. The intensity of stimulation was varied by moving the secondary as before. No significant difference was found between stimulation with 11 cm. coil distance (0.08 eq. volts), 13 cm. coil distance (0.024 eq. volts), or 13 cm. coil distance with the coil tilted at 45° to the horizontal (0.013 eq. volts), the figures for these three cases being 0.36, 0.30, and 0.33 c.mm. per gm. per minute of stimulation. With an equivalent voltage of 0.013 volts the stimulus is too weak to be preceptible to the tongue, but never-

[1] The equivalent voltage corresponding to various settings of the secondary of the instrument used for these experiments has been determined by means of a thermal converter and a sensitive galvanometer. The equivalent voltage E is defined as the voltage necessary to force a direct current of the same strength through the secondary circuit. The P.D. across the primary terminals was 1 volt. The values of E for coil positions 13, 12, 11, 10, and 9 cm. were respectively 0.034, 0.055, 0.09, 0.15, and 0.3 volts. A heating effect due to high frequency radio waves direct from the spark gap without any electrical contact to the secondary has been allowed for.

theless strong enough (at least the break shocks) to produce a maximal contraction of a frog sciatic-gastrocnemius preparation. Here again at the close of the experiment a somewhat smaller figure, 0.26 c.mm., was obtained with the weakest stimulation. The irregularities in these curves represent the experimental error of the method and are due to errors in reading the position of the drop and to small irregularities in its movement. The dotted line under each rise due to stimulation represents the base line which was used in calculating the magnitude of the increased oxygen usage. In the selection of this base line there is some uncertainty. The break in the graph in Fig. 1, *a*, shown by the dotted line, represents the time necessary to move the drop back to the other end of the capillary tube. This procedure frequently upsets the reading for a short time. A similar break in Fig. 1, *b*, is of similar significance but is longer because of some technical difficulties with the index drop. No systematic attempt has been made, beyond the experiments of Fig. 1, *a*, to measure accurately the effect of varying intensity of stimulation. The experiments here reported indicate that the differences, if they exist, are not large. This indeed is to be expected from the all-or-none law and is indirect evidence that the oxygen consumption increases here observed are actually due to nerve impulses and not to electrical or other artefacts at the electrodes.

It is quite certain that curves like those of Fig. 1 cannot be obtained from bits of cotton soaked in bicarbonate solution and laid across the electrodes, nor are they obtained from dead nerves. In one experiment, for example, the negative variation and the oxygen used were being recorded simultaneously. Stimulation produced no change in either. Because of a misunderstanding the silver electrodes used for recording the negative variation had not been washed free from the strong salt solution after plating; this oversight killed the nerve and unexpectedly afforded a clean-cut control experiment.

Frequency of Stimulation.

By stimulating the nerves at varying frequencies some data have been obtained which afford a fairly satisfactory proof that the extra oxygen is actually related to the energy requirements of the nerve impulse. For this purpose tuning forks vibrating at 100 and at

50 per second were used to interrupt the primary current. Thus 200 and 100 shocks per second were delivered to the nerve through the secondary coil. In Fig. 2 there are plotted two frequency curves to show the distribution of the results obtained with 32 stimulation periods at 100 interruptions per second and 21 periods with 50 interruptions per second. Comparing the averages of these results it is evident that doubling the number of impulses per second does not double the amount of extra oxygen used but increases it only 0.315/0.268 or 1.18 times. A similar result was obtained in one experiment in which the responses of the same nerve to the two rates of

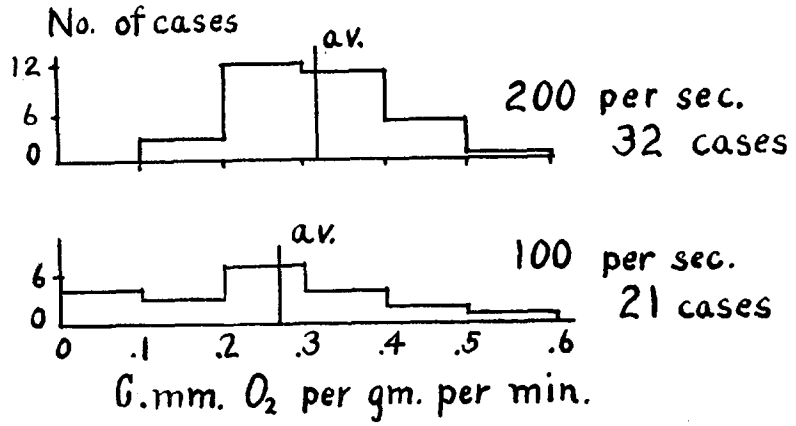

FIG. 2. Frequency diagrams to show the distribution of the values obtained for the excess oxygen consumption due to stimulation at 200 and at 100 shocks per second. The average values were respectively 0.315 and 0.268 c.mm. per gm. of nerve per minute of stimulation.

stimulation were directly compared, the current through the primary coil being equal for both frequencies. The result of this experiment is plotted in Fig. 3, *a*. In the two comparisons there recorded, doubling the frequency increased the excess oxygen used only 0.23/0.20 = 1.15 and 0.28/0.25 = 1.12 times. For purposes of comparison with these figures, the magnitude of the negative variation in nerves similarly stimulated was recorded with a Leeds-Northrup high sensitivity ballistic galvanometer of 2300 ohms resistance. The deflections obtained on stimulating the nerve with 200 shocks per

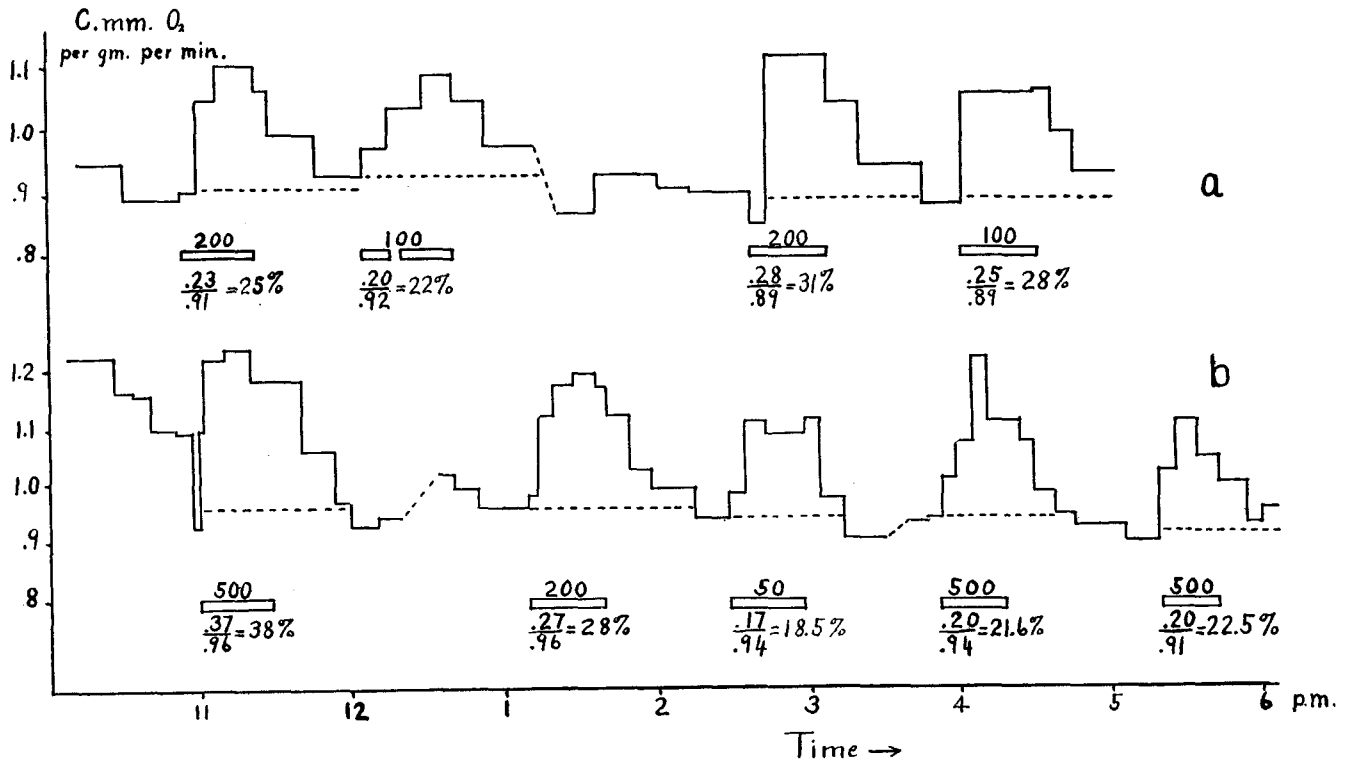

FIG. 3. Graphs showing the rate of oxygen consumption of frog nerves stimulated at different frequencies varying from 50 to 500 shocks per second. The intensity of stimulation was kept constant in each experiment, with the coil set at 13 cm. The voltage drop in the primary was 0.4 volts in *a* and 0.7 in *b*. The equivalent voltage varies in direct proportion to the frequency but for 200 per second it was 0.02 volts for *a* and 0.034 volts for *b*. Figures on the graph show the frequency of stimulation (above) and the percentage increase (below) calculated from the ratio of the excess oxygen per gm. per minute of stimulation and the resting rate per gm. per minute. In *b*, one end of the nerve was crushed which accounts, perhaps, for the high initial rate and led to a great uncertainty in the base line of the first period of stimulation.

second were on the average 1.15 times that found at 100 per second. Through the courtesy of Professor A. V. Hill I am informed that the heat production of nerve is increased 93/67 = 1.4 times for an increase in the frequency of stimulation from 100 to 280 per second or perhaps 1.25 times for an increase in frequency from 100 to 200. The fact that the response of the nerve to this change in frequency, as indicated by its oxygen consumption, is similar to its response as indicated by its negative variation and its heat production, is good evidence that the extra oxygen is actually used to supply energy for the nerve impulse.

In a few preliminary experiments simultaneous measurements have been made of the excess oxygen and of the negative variation on the

TABLE I.

Frequency	Excess oxygen	Negative variation
per sec.	*c. mm.*	*m.v.*
500	0.37	2.12
200	0.27	2.09
50	0.17	1.2
500	0.20	2.16
500	0.20	1.92

same nerve. For this purpose two silver electrodes were introduced into the nerve chamber, one of which was in contact with the injured end of the nerve and the other with its intact surface. The results of one such experiment are plotted in Fig. 3, *b*, and the figures are collected in Table I. The values for the excess oxygen cannot be determined with great precision, but there does seem to be a definite correlation between the excess oxygen and the negative variation over this range of frequencies from 50 to 500 per second. The fact that this tenfold increase in the frequency had so little effect on the oxygen consumption is to be expected from the fact that impulses set up early in the refractory period are subnormal. It may be suspected also that with certain settings of a tuning fork vibrating at 250 per second there would be some interference between the make shock and its rapidly succeeding break shock.

In Fig. 4 there are plotted two frequency curves to show the range

of values obtained for the percentage increase in oxygen consumption from stimulation at 200 shocks per second and for the resting oxygen consumption. The rather wide distribution is perhaps due to the varying conditions of the frogs (*R. pipiens*). For the earlier experiments they were kept in an indoor tank of running water; for the later experiments they were kept in water in a cold room maintained just above the freezing point, and were killed and dissected immediately after removal. No certain differences were noted in the behavior of the nerves in these two cases, however.

My most reliable data were obtained by stimulation at 200 shocks per second, and these may be used for comparison with the heat pro-

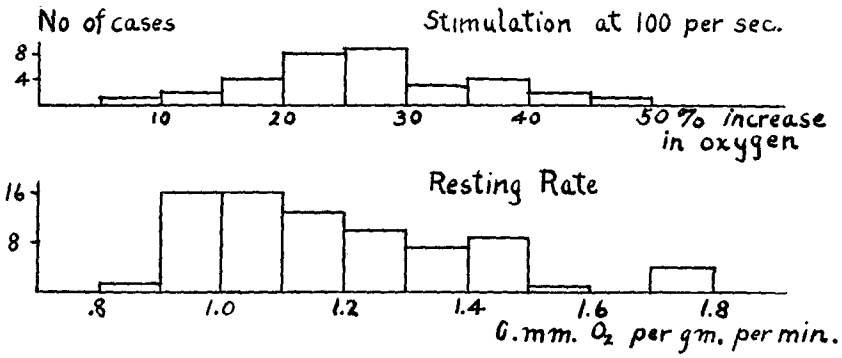

FIG. 4. Frequency diagram showing the distribution of the observed values for the percentage increase in oxygen consumption from stimulation at 200 per second (upper) and for the resting rate of oxygen consumption (lower).

duction of nerve. In 32 periods of such stimulation the resting rate was 1.23 c.mm. of oxygen per gm. of nerve per minute and the excess oxygen used in activity was 0.32 c.mm. of oxygen per gm. of nerve per minute of stimulation, or 26 per cent of the resting rate. The corresponding figures for the dogfish nerve were much the same, *i.e.* 1.35 c.mm. per gm. per minute as a resting rate and an increase on stimulation (100 shocks per second) of 0.21 c.mm. per gm. per minute of stimulation, the percentage increase varying from 10 to 32 per cent. Parker (1925, *b*) has reported a carbon dioxide elimination in the resting frog nerve of 4.46 c.mm. per gm. per minute and an increase due to stimulation of 14 per cent or 0.62 c.mm. per gm. per

minute of stimulation. In absolute magnitude these figures are higher than mine.

Downing, Gerard, and Hill (1926) found a heat production in the frog nerve which was equivalent to an oxygen consumption of 0.75 c.mm. per gm. nerve per minute of stimulation. They stimulated at a frequency of 280 shocks per second. At 100 per second the heat was 67/93 as great (personal communication from Professor Hill), which would have demanded an extra oxygen consumption of 0.54 c.mm. At 200 per second the figure would have been perhaps 0.66 c.mm. per gm. per minute of stimulation. This is about twice as large as the mean value which I have actually observed, *i.e.* 0.32 c.mm., although my highest figures have been over 0.5 c.mm. To account for this discrepancy, it is probable that during the first 10 seconds of stimulation there is a greater energy breakdown than during similar periods at the end of a half hour of stimulation. The heat was measured during the first 10 seconds only.

It is conceivable that even in the small frog nerve in an atmosphere of oxygen the central portion of the proximal end of the nerve, where its diameter is greatest, would be asphyxiated and fail to respond. This would help to explain the discrepancy between the heat production and the oxygen. By making use of Krogh's (1919) diffusion constant for oxygen, however, it can be shown that this is not the case. To do this one proceeds with a cylinder in much the same way that Warburg (1923) has done for the simpler case of a flat disc. Consider a cylinder of nerve of radius a and length l, consuming A cc. of oxygen per gm. per minute. D, the diffusion constant for oxygen in muscle tissues, $= 1.4 \times 10^{-5}$ cc. of oxygen diffusing across a surface area of 1 cm.^{2} per minute under a pressure gradient of 1 atmosphere per cm. The concentration c_0 of oxygen at the surface is kept constant. Diffusion through the ends of the nerve is neglected. In any concentric cylindrical layer, of radii r and $r\text{-}dr$, the oxygen consumption in time dt is

$$A\,[\pi r^2 - \pi\,(r - dr)^2]\,l dt = 2\pi\,A\,lr\,dr\,dt \qquad (1)$$

The oxygen diffusing *into* this layer in time dt is

$$D\,2\pi\,rl\frac{dc}{dr}dt \qquad (2)$$

The oxygen diffusing *out* of this layer is

$$2\pi\, Dl\, (r - dr)\, dt\left(\frac{dc}{dr} - \frac{d^2c}{dr^2}dr\right) \tag{3}$$

Equation (1) = equation (2) − equation (3) or, after simplification,

$$\frac{Dd^2c}{dr^2} + \frac{Ddc}{dr} = Ar \tag{4}$$

The solution of this differential equation is

$$c = c_0 - \frac{A}{4D}(a^2 - r^2), \tag{5}$$

c being the concentration of oxygen in atmospheres at a distance r from the center of the cylinder, c_0 being the concentration at the surface, *i.e.* when $a = r$. Putting $r = 0$ and $a = 0.1$ cm. which is the maximum for frog nerves I have used,

$$c = 1 - \frac{0.00123 \times 0.1^2}{4 \times 1.4 \times 10^{-5}} = 1 - 0.22 = 0.78$$

atmosphere at the center of the nerve. Thus the tension inside the larger end of the nerve is $0.78 \times 760 = 590$ mm. if the nerve is in pure oxygen. If it is in air the tension at the center of the larger end is just reduced to zero.

From (5) it is evident that when in pure O_2 the tension at the center will just reach zero if

$$\frac{A}{4D}a^2 = 1 \text{ or if } a = 0.213 \text{ cm.}$$

which is about the maximum radius of the largest dogfish nerves. The assumption is made that A is independent of the tension of oxygen. This confirms the estimate previously made (Fenn, 1927) that the rate of diffusion of oxygen was a limiting factor in dogfish nerves in air.

I am indebted to an anonymous mathematical colleague for assistance with this equation.

SUMMARY.

1. The resting rate of oxygen consumption of the excised sciatic nerve of the frog is 1.23 c.mm. of oxygen per gm. of nerve per minute.

2. During stimulation with an induction coil with 100 make and 100 break shocks per second there is an excess oxygen consumption amounting on the average to 0.32 c.mm. of oxygen per gm. of nerve per minute of stimulation, or a 26 per cent increase over the resting rate.

3. The magnitude of the excess oxygen consumption in stimulation, in agreement with the all-or-none law, is not markedly influenced by considerable variations in the intensity of stimulation.

4. Increasing the frequency of stimulation from 100 to 200 shocks per second increases the extra oxygen used only 1.12–1.18 times. The same change in frequency of stimulation increases the negative variation 1.15 times and the heat production about 1.25 times (Hill).

5. This parallelism between the excess oxygen and the negative variation argues definitely for some causal connection between the excess oxygen and the nerve impulse itself.

6. Calculation shows that the oxygen tension inside these nerves was not zero.

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