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RESTING POTENTIALS OF DIAPHRAGM MUSCLE AFTER PROLONGED ANOXIA

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Experiments on isolated mammalian tissue at body temperature require vigorous oxygenation in most cases, for these tissues are usually fairly thick and their oxygen consumption is high. In many situations there is some doubt regarding the adequacy of the supply of oxygen. It is shown in the present study that exploration of muscle by the use of micro-electrodes may be used to provide a criterion of adequate oxygenation, for anoxia characteristically produces lowered resting potentials in the depth of the muscle. The findings may also be extended to give some information regarding oxygen requirements and oxygen diffusion through mammalian muscle at body temperature. In addition, certain physical dimensions of the tissue, including thickness, mean cell diameter and interfibre space, may be estimated from the results of the experiments.

METHODS

Saline solution. The composition was (mM): Na^+ 145, K^+ 5·0, Ca^{2+} 1·2, Mg^{2+} 1·2, Cl^- 137·5, HCO_3^- 12·5, SO_4^{2-} 1·2, HPO_4^{2-} plus $H_2PO_4^-$ 1·2. The glucose content was 100 mg/100 ml. The solution was gassed with a mixture containing 2% (v/v) carbon dioxide and variable oxygen content, and the pH was 7·5 at 37° C.

Handling of tissues. Left diaphragm muscles from rats of 150–250 g were used for measurement of resting potentials. It was necessary to strip the pleura from the muscle before impaling with micro-electrodes. This was facilitated during dissection by removal of a lower lobe of the left lung, which was often invaginated between the diaphragm and part of the heart. The pleura, which was here loose, could then be seized with forceps and stripped from the muscle, the process being started near the central tendon and proceeding towards the rib margin.

The hemidiaphragm was attached in a vertical position to a plastic frame which was partly immersed in an organ bath filled with saline (Fig. 1). The tendon was tied to the lower cross-piece of the frame, while the ribs were attached to threads which passed up through holes in the frame and hence to a weight of 25–50 g, which was required to keep the muscle sufficiently steady for impalement by micro-electrodes. The muscle was arranged so that the pleural surface faced the electrode. The plastic organ bath was made with rectangular faces and was jacketed on two sides, the saline being maintained at 37° C by water which was circulated from a heated reservoir by a

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centrifugal pump. One wall of the organ bath was polished, and this enabled the surface fibres of the muscle to be viewed by means of a dissecting microscope. Dissections and other manipulations were performed in a shallow bath containing saline which was continuously oxygenated.

Oxygenation. Two gas mixtures were obtained, one containing 2 % (v/v) carbon dioxide and 98 % oxygen, the other containing 2 % carbon dioxide and 98 % nitrogen. The contents of the two cylinders were allowed to mix in suitable proportions and the gas was then led to the saline through a distributor, formed from a length of plastic tube whose end had been plugged with glass wool dipped in chloroform and clamped. This produced a fine cloud of gas bubbles which enveloped the tissue. Auxiliary gas jets were used to ensure that all parts of the saline were well aerated. The inflowing gas was analysed from time to time by means of a magnetic oxygen analyser, and the oxygen content in the mixture was adjusted if necessary. Samples of the gas mixture were also withdrawn by a needle and syringe from a short length of thick-walled rubber tubing which led to the organ bath, and the contents were analysed by conventional manometric methods.

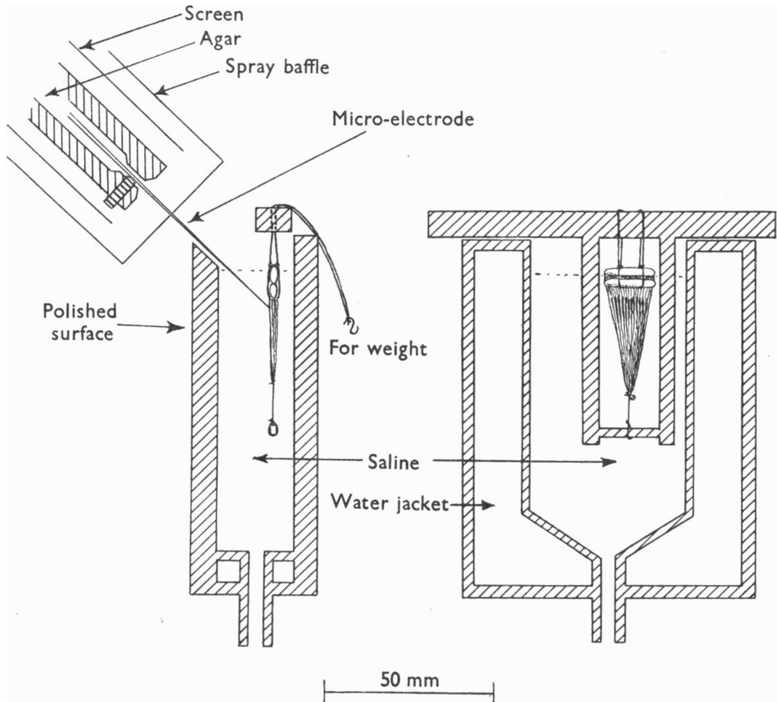

Fig. 1. Arrangement used for recording resting potentials. Two sections at right angles are shown. The muscle was held vertically, and the micro-electrode approached at an angle of 42° to the tissue. The electrode was driven slowly through the muscle by a motor. The gas distributor, which produced a fine cloud of gas bubbles around the tissue, has been omitted. One wall of the plastic bath was polished, so that the muscle could be viewed by a microscope.

Micro-electrode technique. The methods in general resembled those of Nastuk & Hodgkin (1950). The electrodes were 7 cm long, one end being clamped in a plastic holder (Fig. 1), so that contact was made with the agar jelly (1·5 % with 2·5 M-KCl), which also contained the end of a short chlorided silver wire which was soldered to the control grid of the first valve. The electrode holder and cathode-follower circuit were attached to the rack taken from a microscope fitted with the usual mechanism for fine adjustment, and the whole was mounted on a universal adjustable milling table. By this means the electrode could be advanced or withdrawn or moved from side to side.

The electrode approached the muscle at an angle of 42° with the vertical, and could be slowly driven down and through the substance of the tissue at a rate of 0·37 mm/min by a synchronous motor which was used to rotate the pinion of the fine adjustment mechanism.

The cathode-follower input circuit was formed from a miniature pentode, Raytheon CK 5886, and both the heater and the high tension were supplied by small mercury batteries. This valve had an anode current of $6\mu A$, and in conditions of low humidity the grid current was below 10^{-14} A, as measured by inserting a grid resistance of $10^{12}\ \Omega$. In humid conditions the grid current was approximately 10^{-12} A. The cathode resistance was 650 kΩ, and in response to a signal this resistance produced a long time constant which was not a disadvantage for the present measurements. The output of the cathode-follower stage was led to an amplifying pen recorder having a high input impedance and a rise time of approximately 0·4 sec for a deflexion between 5–95% of the range. An oscilloscope with a d.c. amplifier was also used as a monitor.

Electrodes were prepared from capillary tubing (Kimble Glass Co.) of outside diameter 0·7 mm and wall thickness 0·2 mm, a machine similar to that described by Alexander & Nastuk (1953) being used. Careful selection of electrodes was made and only those having resistances between 10 and 30 MΩ were retained. Results obtained by penetration of muscle fibres were only accepted if no change in the resistance of the electrode occurred and if there was no change in the base line of the oscilloscope or pen recorder (see later). Some batches of electrodes gave frequent sudden increases of recorded potentials accompanied by an increase in the electrode resistance, as described by del Castillo & Katz (1955) and by Adrian (1956); results from these electrodes were discarded. The recording system was calibrated by means of a Weston cell.

Precautions were necessary to ensure that spray did not reach the recording system and produce spurious potential changes. For this reason the electrodes were long, and during recording the bath and contents were covered with a cellophane wrapper having a slit for the electrode. At times it was necessary to blow away the spray by means of an air jet directed on to the exposed shaft of the electrode.

Measurement of linear dimensions. Horizontal movement of the pen recorder (see Fig. 2) was related to the horizontal component of the movement of the electrode through the tissue by measurement of: (*a*) the relation between movement in the direction of the electrode shaft with rotation of the pinion of the microscope holder (which carried the electrode assembly); this was 185μ/rev; (*b*) the speed of the synchronous motor, which was in most cases 2 rev/min; (*c*) the angle θ between the shaft of the electrode and the vertical muscle (42°, so that $\sin\theta$ was 0·70); and (*d*) the speed of the paper used for the pen recorder, which was 2 in. (51 mm)/min. Movement in a horizontal plane through the tissue was magnified by a factor of 205.

The thickness was obtained from records similar to that of Fig. 2 by measuring between the first and the last full deflexion. Extrafibre space was obtained by measuring the intervals between deflexions and expressing the sum as a fraction of the thickness. Only records which were technically satisfactory (see above) were used.

In some cases the cell thickness was also measured at approximately the mid point of the deflexion. No correction was made for the lag of the pen recorder, which corresponded to a movement of the electrode of about 2μ for a full-scale deflexion. It was considered that any error recorded in piercing the cell would be balanced on leaving it. Similar considerations may be applied to possible denting of the cell membrane. In general, however, the electrode passes not through the diameter of the fibre but through a chord, and the thickness as recorded will be smaller than the true value and must be corrected. The distribution of these apparent values is also expected to show marked skewness, and this problem is analysed in an Appendix (see below) which also gives methods of applying corrections to the results.

Measurement of oxygen consumption. Oxygen consumption was measured in independent experiments by a method which was similar to that described by Whalen (1956, 1957). Diaphragm muscles were secured in small plastic vessels whose total volume, including manometers, was 11 ml. The vessel contained 8 ml. saline and the contents were saturated with a mixture of 2% carbon dioxide and 98% oxygen. The consumption was estimated manometrically, and during runs the

concentration of carbon dioxide in the space above the saline was maintained at 2% by the use of diethanolamine (Krebs, 1951). A magnetic stirrer rotated inside the saline and caused sufficient turbulence and bubble formation to aerate the muscle. The whole vessel was immersed in a water-bath at 37° C. The tension on the muscle was adjusted to 25 g wt. by a thread which was attached to the tendon and passed upwards through a mercury seal to a strain gauge mounted on a rack.

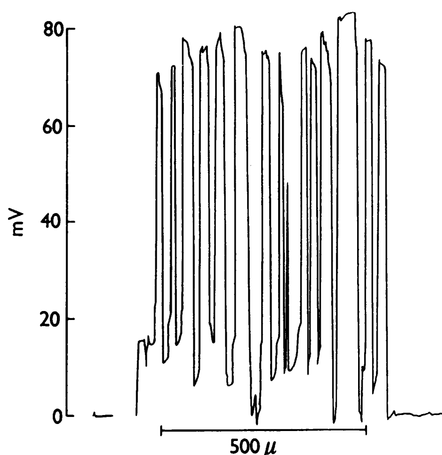

Fig. 2. Potential changes recorded by a pen writer during penetration of micro-electrode through normal diaphragm muscle from right (pleural surface) to left (peritoneal surface). Upward deflexion indicates a potential at the electrode tip which is negative to the earthed saline. The deflexions are presumably due to entry into and exit from the fibres which are encountered.

RESULTS

Penetration of normal muscle

Fig. 2 shows the record obtained when the micro-electrode was steadily propelled through diaphragm muscle by means of a motor. Penetration was from right to left, from the pleural to the peritoneal surface, and took over 2 min for completion. Entry into the tissue was accompanied by a series of deflexions of the pen recorder in a direction such that upward movement indicated a negative potential relative to the earthed saline in the bath. The potentials were of the order of 70–80 mV and were presumably produced by penetration of the electrode into, through and out of muscle fibres which were encountered. The figure shows some fifteen deflexions. This seems to be somewhat less than the number of cell layers seen previously in transverse sections of diaphragm (Creese, 1954), but is in agreement with the smaller thickness of the muscles used in the present study (see below).

The pen writer does not return completely to the base line between deflexions, for reasons which are not clear. The electrodes frequently broke if they were pushed through the peritoneum on the far side of the muscle. When deflexions corresponding to fibres ceased, the motor was stopped and was then reversed to bring the electrode back into saline. The base line was again recorded, and

this explains the break in the record at the left-hand side of Fig. 2. Changes in the base line or in the electrode resistance were attributed to plugging of the tip (Adrian, 1956) and the records were usually discarded. A second penetration at exactly the same position showed a series of low potentials, presumably due to damage produced by the passage of the electrode. If the electrode was moved a few cells away in a lateral direction, normal records could again be obtained, and with a suitable electrode the process could be repeated many times. In some cases the first deflexion, on the extreme right-hand side of the records, was markedly lower than normal. This corresponds to the cell layer immediately beneath the pleura. It is likely that the underlying cells were sometimes damaged by removal of the pleural membrane. It was necessary to put the muscles under tension to keep them sufficiently steady for impaling, and the effects here reported have all been obtained on stretched muscle. The effects of stretching have been summarized by Ling & Gerard (1949*a*), who detected no effects in the resting potential in the case of normal amphibian muscle. Irregularities in the base line were mainly caused by the vigorous direct aeration which was employed.

Muscle thickness, extrafibre space, and fibre diameter

The thickness of left diaphragm muscles from rats weighing 200–280 g ranged from 419 to 630μ, with a mean value of 500μ (twenty-seven penetrations from five muscles). The distance was measured from the first to the last deflexion, and the contribution made by the membranes was not taken into account.

These measurements, obtained on stretched muscle, resembled the values of 400–500μ recorded by Pearson, Hsieh, Dutoit & Hastings (1949). Higher values of 600μ were recorded by Holmes, Jenden & Taylor (1951), and 590μ by Creese (1954) in rats of 120 g. It is clear that there is considerable local variation.

The extrafibre space was estimated by measuring the intervals between the deflexions and expressing the sum as a fraction of the thickness. The fraction by volume occupied by the extrafibre space ranged from 0·23 to 0·39, the mean being 0·28 ± 0·014 (S.E. of seventeen measurements from nine diaphragm muscles). Results from frog sartorius muscle, obtained by methods similar to those used by Blum, Creese, Jenden & Scholes (1957), give a value of 0·19 ± 0·003 (S.E. of 7; range 0·17–0·21).

The apparent cell diameter was obtained by measuring the distance between the upward and downward movement of each deflexion in records similar to that of Fig. 2, and some 284 cells from twelve diaphragm muscles from rats of 200–300 g were measured. The distribution was markedly skewed in a positive direction. The skewness, as indicated by the ratio (3rd moment about mean): (standard deviation cubed), was +1·12. This type of skewed distribution is to be expected from the manner in which the measurements were made (see below). The mean value as measured on the records was 26·7 ± 15·7μ (S.D. of individual measurements). These figures require corrections which allow for the systematic biasing of the results caused by the arrangement whereby chords are measured instead of diameters. The corrections to be applied are considered later in an Appendix. The corrected mean diameter was 32μ in stretched

muscles from rats of 200–300 g. This may be compared with a value of about 20 μ obtained from histological sections of soaked diaphragms from rats of 120 g (Creese, 1954).

Effects of poor oxygenation

A stream of gas bubbles flowing past the muscle may hinder manipulations with micro-electrodes. During certain manoeuvres the oxygen supply was reduced to a trickle or was cut off for various periods of time. After such procedures it was noticed that the potentials recorded from the centre of the tissue were often below normal. Fig. 3 is an example, and shows a region containing low potentials in the middle of the muscle.

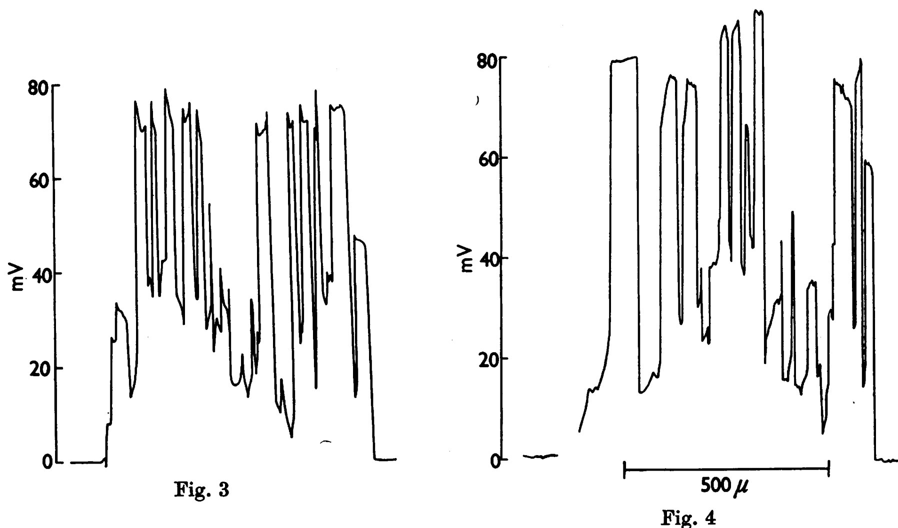

Fig. 3. Record obtained as before, from muscle which had been intermittently deprived of oxygen. The cells in the centre are depolarized.

Fig. 4. Record from muscle aerated by a trickle of oxygen on the left (peritoneal) surface only. The region of depolarization is asymmetrical.

Fig. 4 shows a record obtained after a trickle of oxygen (containing 2% of carbon dioxide) had been allowed to play on the peritoneal side of the muscle (left side in Fig. 4) for 2 hr. There is a gap in the record which shows low potentials, and this time the region is asymmetrical in position, being shifted towards the side which has had least oxygen. There was apparently enough oxygen in the saline over the pleural surface (right side in Fig. 4) to provide normal potentials in the case of the first few layers of cells.

When low concentrations of oxygen were used it was found that cells in the depths of the tissue might take up to 2 hr for depolarization. Superficial cells were rapidly depolarized by a mixture of 2% carbon dioxide and 98% nitrogen,

and recovery was sometimes seen, as found by Boyd & Martin (1956). These effects of anoxia appeared to be reached much more quickly than in the amphibian muscle studied by Ling & Gerard (1949*b*).

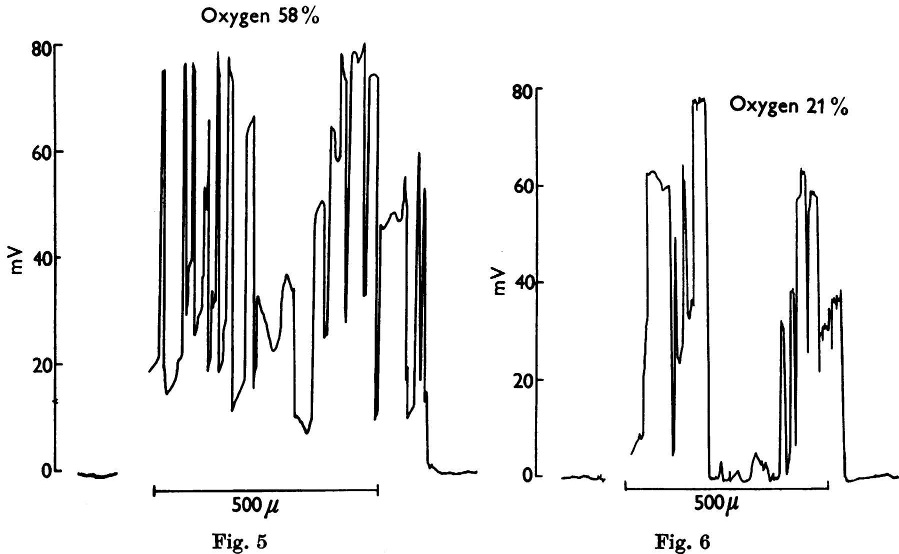

Fig. 5. Potentials from muscle aerated for 3 hr with a gas mixture containing 58% oxygen. There is a region of depolarization in the centre. The low potentials of cells on the right (pleural surface of diaphragm) may be due to damage produced during stripping of the pleura.

Fig. 6. As before, using 21% oxygen for 3 hr. The depth within which resting potentials are found is narrowed.

Quantitative effects of oxygen variation

Fig. 5 shows the effect of aerating the tissue for 3 hr with a mixture containing 58% oxygen and 2% of carbon dioxide, the balance being nitrogen. Potentials are reduced in the centre of the muscle. Fig. 6 shows the effect of 21% oxygen; the gap indicating absent or reduced potentials is very obvious. Repeated penetration of the middle third of the muscle revealed no apparent change after 2 hr, and in practice at least 3 hr were allowed for a steady state to be produced in each case. The solution was changed at intervals so that the external ionic environment was approximately constant, and this procedure would also serve to maintain a constant composition for the intercellular fluid.

Fig. 7 demonstrates the effect of reducing the oxygen content to 1% for 3 hr, the carbon dioxide concentration being maintained at 2% as before. Only the outer few cells now show measurable potentials. Frequent blocking of the micro-electrode occurred when it was pushed through anoxic muscle, and the break in the record of Fig. 7 is attributed to this. In a majority of cases the outer cell shows a high potential, which suggests that a partial pressure of

7 mm Hg, corresponding to 1% oxygen in the gas mixture, is normally sufficient to maintain cellular function. In a few records a resting potential was not detected in the outer cell, and Fig. 8 shows an example of this.

The results suggest that lowering the oxygen content of the gas used to aerate the saline has the effect of diminishing the depth of tissue in which cell potentials could be recorded.

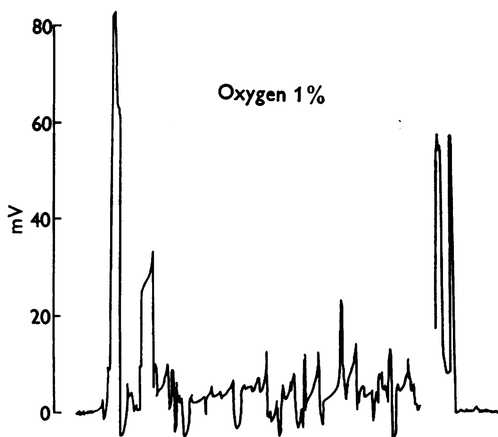

Fig. 7

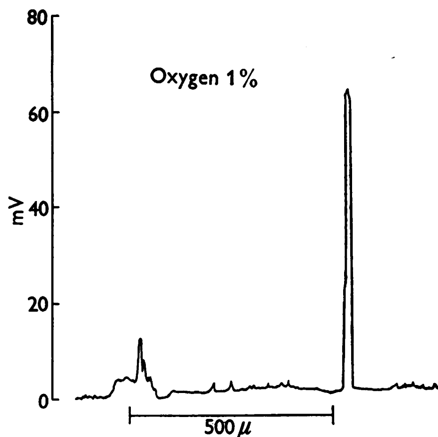

Fig. 8

Fig. 7. Record obtained with 1% oxygen, with thick muscle. Only the outer cells show resting potentials. The break in the record was caused by removal of an artifact (see text).

Fig. 8. Record from another muscle aerated for 3 hr with 1% oxygen.

The relation between these two variables was examined in six diaphragms. Each muscle was set up in 98% oxygen (and 2% carbon dioxide), and two normal records were obtained by penetration with a micro-electrode. The oxygen content was then suitably reduced, the carbon dioxide concentration being maintained constant as described above. After 3 hr several recordings were made in the middle third of the muscle. The oxygen concentration was usually lowered again for another period of 3 hr and further recordings were taken.

The records were later measured and the depth of tissue which maintained resting potentials was estimated. Measurements were made from the first deflexion and included cells having potentials greater than an arbitrary value of 15 mV. Two values were obtained from each record in all but one muscle. In any particular record a decision had to be made regarding the inclusion or otherwise of a single cell, and the estimations therefore resembled those obtained from an 'all or none' or quantal response (Gaddum, 1933). In a few records similar to the left side of Fig. 8, the critical thickness was taken to be zero. Measurements were converted to microns by the calibration factor.

The results are shown in Fig. 9. For reasons which are considered in the following paragraph, the depth in which potentials are found is plotted against

the square root of the oxygen concentration. The points suggest that the critical depth, as measured above, is related to the concentration of oxygen which is in contact with the muscle.

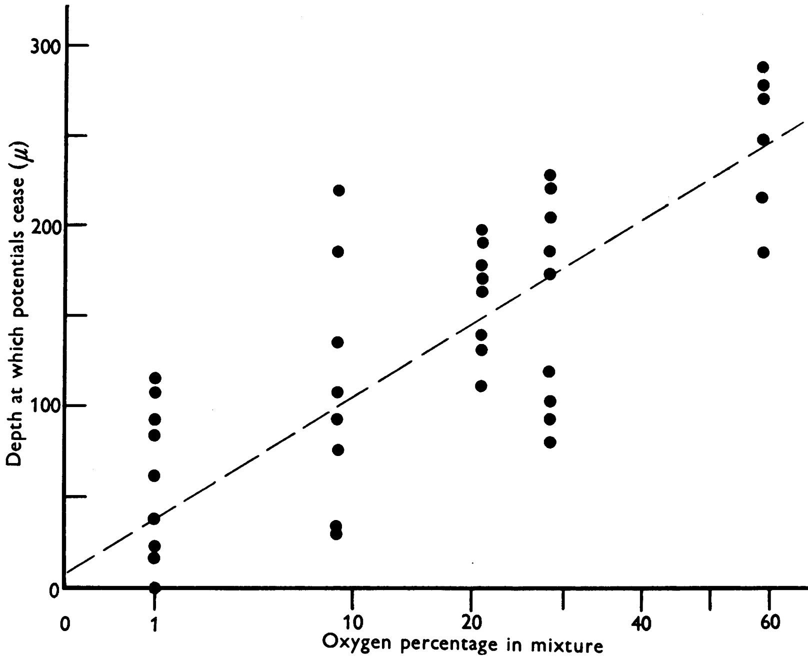

Fig. 9. Scatter diagram showing the depth at which potentials cease (or become very small) plotted against the square root of the oxygen fraction in the gas mixture. For convenience the abscissa shows values of the oxygen percentage. The broken line gives the regression. Some points represent more than one pair of values.

Interpretation of quantitative results

Oxygen reaches the depths of the muscle by a process of diffusion, and the partial pressure is progressively reduced towards the centre by oxygen consumption. If this consumption were independent of oxygen tension, then the curve relating oxygen tension to the distance from the surface would be parabolic, and the depth b at which the oxygen tension becomes zero would be

$$b=\sqrt{(2Dy_0/a)}, \tag{1}$$

where y_0 is the external oxygen tension, a is the oxygen consumption in c.c. (s.t.p.)/c.c. wet tissue/min, and D is the Krogh diffusion coefficient of oxygen through the tissue. The nomenclature is similar to that of Hill (1928). The use of the Krogh coefficient has recently been discussed by Rehberg (1955).

If the depth b could be measured for various oxygen concentrations and plotted against the square root of the oxygen concentration, eqn. (1) shows

that a straight line through the origin should result, provided the above conditions are fulfilled. The measurements of the critical depth d, obtained from records similar to Figs. 5–8, give the depth at which the potential has fallen to less than 15 mV. The oxygen tension at this depth is likely, from Figs. 7 and 8, to be less than 7 mm Hg. Hence instead of b, which cannot be measured, we plot d, which will be somewhat smaller. If d is plotted against the square root of the oxygen tension the plot should still be linear with the same slope, though the origin will not now be zero. This reasoning assumes that the oxygen consumption is independent of oxygen tension down to low values, and this point is discussed later. Another assumption is that the gas mixture is in equilibrium with the saline around the muscle, so that y_0 equals $q\,(\mathrm{P}-\mathrm{p})/\mathrm{P}$ where q is the fraction of oxygen in the gas mixture, P is the atmospheric pressure and p is the water vapour pressure. Then the slope of the critical distance d when plotted against $\sqrt{q}$ will be

$$\text{slope} = \sqrt{\{2D(\mathrm{P}-\mathrm{p})/a\,\mathrm{P}\}}. \tag{2}$$

From the results plotted in Fig. 9 it can be seen that the scatter is considerable for any particular oxygen value, and the range in most cases exceeds 100μ. There is a trend in the expected direction such that greater oxygenation increases the depth of tissue in which potentials are found. The results are not inconsistent with a linear relation, and the regression equation is

$$d = 6{\cdot}7 + 311\sqrt{q}, \tag{3}$$

where d is measured in microns.

The standard error of the regression coefficient is ± 26, obtained from 54 pairs of measurements. A slope of this magnitude is unlikely to be fortuitous ($t \simeq 12$, $P < 0{\cdot}001$). The correlation coefficient is $+0{\cdot}85$. We expect the intercept on the ordinate to be negative; in fact it has a small positive value of $+6{\cdot}7$. The standard error of this predicted value is $\pm 11{\cdot}6$, so the value obtained has little significance. The permissible thickness consistent with adequate oxygenation is given by $2d$, and from eqn. (3) the maximum value, obtained with 100% oxygen with q equal to unity, is 635μ. This is similar to critical values obtained in various tissues by other methods as summarized by Field (1948).

Oxygen consumption and diffusion coefficient

Left diaphragms were used from rats of 150–200 g. In the presence of 2% carbon dioxide, the mean value of the oxygen consumption at 37° C was $7{\cdot}07\,\mu$l. $\pm 0{\cdot}51$ (S.E. of 12)/mg final dry weight/hr, the gas volumes being reduced to 0° C and a pressure of one atmosphere. The determinations were made during the first 30 min after the muscles had been placed in the plastic chambers for manometric estimation. The mean water content was 78·2%, and the specific gravity was taken as 1·055 (Creese, 1954). The oxygen consumption

may therefore be expressed as 0·027 c.c./c.c. wet tissue/min. The oxygen consumption recorded under these conditions is higher than that usually reported for rat diaphragm muscle, though the results of Pearson *et al.* (1949), who used phosphate saline, are comparable. They recorded a Q_{O_2} of 6·9. The values obtained on rats of 150–200 g are assumed to apply to the muscles from rats of 150–250 g which were used for electrical recording (cf. von Bertalanffy & Pirozynski, 1951).

The apparent diffusion coefficient may now be calculated. In eqn. (2) the slope is 0·0311 cm, a is 0·027 c.c./c.c./min, P is taken as one atmosphere and p, the vapour pressure of saline at 37° C, is 47 mm Hg. Then the Krogh diffusion coefficient of oxygen through diaphragm muscle at 37° C is $1{\cdot}4 \times 10^{-5}$ cm^2/min. The standard error of the mean, calculated according to Dahlberg (1948), is $0{\cdot}20 \times 10^{-5}$.

DISCUSSION

This investigation was started with the object of finding a method which could provide a convenient criterion for the occurrence of anoxia. The possibility that the oxygen supply is inadequate is often of pressing concern in the case of isolated mammalian tissues maintained at body temperature. Such preparations have a high oxygen consumption and oxygen must diffuse a considerable distance to reach the inner depths of the tissue. Frog muscle at room temperature consumes about 40 mm^3 oxygen/g/hr (Meyerhof & Schulz, 1927), which corresponds to about 0·0007 c.c./c.c./min. The oxygen consumption of diaphragm under the present conditions is about 0·027 c.c./c.c./min, which is nearly forty times greater.

Criteria of oxygenation previously adopted include the twitch tension, and the potassium and sodium content, which seems to be a sensitive indication of anoxia (e.g. Creese, 1954). An inadequate oxygen supply will result in a regional difference in resting potentials, for the outer portions of the tissue must receive more oxygen than the deeper parts. The present findings support the suggestion that regional differences in which the inner cells show reduced potentials may be used as a criterion of anoxia in isolated preparations.

Using the criteria both of the ionic content and of the micro-electrode method, the present authors have been able to obtain continuous adequate oxygenation only when the gas jet was arranged so that bubbles of oxygen were able to impinge directly on to the tissue. When the muscle was placed in a horizontal position and saline which had been aerated previously was passed over the tissue, it was found that the muscles showed a fall in potassium and a gain of sodium, and these findings were taken to indicate anoxia (Creese, 1954). The latter method of oxygenation would probably be adequate, however, for electrical studies on surface fibres.

The method used in the present work has the additional advantage that the records allow the estimation of the common physical dimensions of the stretched

muscle, including thickness, fibre size and extrafibre space. The value of the inulin space of diaphragm muscle is 0·28, which is the same as the mean value found by micro-electrodes, and the space estimated by means of labelled sodium is also similar (Creese, 1954). In the case of frog sartorius muscle, however, there is a discrepancy. The fraction by volume of sartorius muscle into which inulin will penetrate is about 0·13 (Desmedt, 1953). The micro-electrode method gives 0·19. This last figure is in agreement with the results of Causey & Harris (1951), who obtained a value of 0·20 both with labelled phosphate ions and with labelled hexose phosphate. Harris & Burn (1949) similarly obtained a mean value of 0·18 in experiments with radioactive sodium. The discrepancy might be due to different and arbitrary degrees of wiping of muscles before estimations, or may be attributed to connective tissue. It is known that gaps between deflexions as recorded by the micro-electrode method may be produced by the presence of connective tissue, and this would be recorded as extrafibre space (Creese, Dillon, Marshall, Sabawala, Schneider, Taylor & Zinn, 1957).

Some attempt has been made to put on a semi-quantitative basis the results which relate the records of cell potentials to the oxygen concentration. Only steady states have been considered, and at least 3 hr were allowed before recording. The depth in the muscle at which depolarization below 15 mV occurs has been used as a bio-assay procedure to indicate the presence of a particular oxygen tension, which is itself unknown but which is likely to be below 7 mm Hg. This depth varies with the external oxygen concentration, and the relation of these variables has been obtained. It is assumed that oxygen consumption is independent of oxygen tension down to low values of the latter. In the case of frog muscle the work of Hill (1948) has shown that this relation is valid at 15–20° C down to 2 mm Hg or less. No reliable investigations are available in the case of mammalian muscle, but careful studies on other mammalian tissues at body temperature give support to this view. Thus Warren (1942), who worked with cell suspensions from bone marrow, and Elliott & Henry (1946), who studied suspensions of brain tissue, have shown that oxygen consumption is independent of oxygen tension down to at least 4–6 mm Hg, providing the results are free from distortion produced by diffusion and solubility factors affecting the amount of oxygen in the saline solution. The present experimental findings though variable are consistent with these assumptions, and there seems therefore no reason to invoke more cumbersome mathematical treatment.

The interpretation of the quantitative results is complicated by the possibility that lactic acid is being produced by the anaerobic muscle in sufficient quantity to affect markedly the consumption of oxygen. Lactic acid is unlikely to be produced by cells with normal potentials nor by cells with very low potentials which are probably functionless; but cells which are somewhat

depolarized probably produce lactate or other 'debt metabolites'. The zone of partial depolarization does not extend over many cell layers. Hill (1948) has shown that under similar conditions the parabolic relation between oxygen tension and distance is maintained down to very low tensions in frog muscle, though the oxygen consumption was raised in the anoxic zone. Hannon & Cook (1956) have detected a transient increase in oxygen consumption after short exposure to nitrogen in the case of mouse diaphragm. If the figure used for oxygen consumption in eqn. (2) is too small then the calculated value of the diffusion coefficient will be somewhat smaller than the true value, and this may account for the rather low figure actually obtained. The altered potassium content of cells in the zone of depolarization might also be expected to alter the metabolic rate to some extent. It is known that a high external potassium concentration increases the oxygen consumption of frog muscle (Keynes & Maisel, 1954), and in the present experiments the external saline was therefore changed frequently.

The value of the Krogh diffusion coefficient as calculated from the slope of the regression line is $1{\cdot}4 \times 10^{-5}$ cm^2/min at 37° C. There is no direct measurement made on mammalian muscle with which the results may be compared. Writers on oxygen diffusion for the last forty years have used the value of the mixed diffusion coefficient originally published by Krogh in 1919 (*a*), which was based on measurement made on abdominal muscle of the frog, at 20° C. The figure of $1{\cdot}64 \times 10^{-5}$ cm^2/min at 37° C was calculated by Krogh (1919*b*) by extrapolation of results obtained at lower temperatures, and the value found in the present study is some 15% lower than that used by Krogh. This difference is not significant since the S.E. is $\pm 0{\cdot}20 \times 10^{-5}$. Hill (1928) and others have used the figure $1{\cdot}7 \times 10^{-5}$ cm^2/min at 37° C. Considering the limitations of the methods employed, the result in the present experiments is not unsatisfactory. It is difficult to devise a method of measuring the diffusion coefficient directly in mammalian muscle while maintaining the tissue properly supplied with oxygen, and such information as is known about oxygen transport in mammalian tissues has been obtained largely by indirect means, as in the present study.

SUMMARY

1. A method of exploring muscle is described in which a motor is used to push a micro-electrode slowly through the tissue. Resting potentials of fibres which are encountered are registered by a pen recorder.

2. The method may be used to measure certain physical dimensions of the tissue. For stretched diaphragm muscle of the rat the extrafibre space was 0·28 of the total volume. The value for the sartorius muscle of the frog was 0·19. The mean fibre diameter of diaphragm muscles from rats of 200–300 g was 32μ, after corrections for systematic bias during measurements.

3. In the presence of a constant carbon dioxide concentration, reduction of the oxygen content in the gas mixture used to aerate the tissue results in depolarization in the centre of the muscle, while the superficial fibres are normal. These findings are characteristic, and may be used as a criterion of anoxia.

4. The oxygen consumption, obtained by manometric methods in the presence of 2% carbon dioxide, was 0·027 c.c. (s.t.p.)/c.c. wet tissue/min at 37° C during the first 30 min after diaphragm muscles from rats of 150–200 g were placed in the chambers.

5. The method was also used to obtain information regarding oxygen requirements and oxygen transport in mammalian muscle. The Krogh diffusion coefficient for oxygen at 37° C in diaphragm was estimated as $1{\cdot}4 \times 10^{-5}$ cm^2/min.

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APPENDIX, BY R. CREESE AND D. J. JENDEN

Expressions are required which give the mean thickness and standard deviation of muscle fibres from values obtained by random impaling by micro-electrodes in which chords are measured instead of diameters.

The cells are treated as cylinders and their radii are assumed to be distributed in a lognormal manner. There is some evidence for this latter assumption (Creese, Neil & Stephenson, 1956; Creese, D'Silva & Hashish, 1955). It is also considered that the probability of encountering a cell of given radius is proportional both to its distribution frequency and also to its radius.

Consider first a single fibre of radius r. It is convenient to compare r with the semichord l, where l is half the measured thickness. The frequency with which a given class of lengths is encountered is

proportional to the class interval along the X-axis corresponding to it. Then $f(l)$, the probability density function, may be constructed as follows.

The conditions are

$$\int_0^r f(l)\,\mathrm{d}l = 1 \tag{4}$$

and

$$\mathrm{d}N = f(l)\,\mathrm{d}l, \tag{5}$$

where $\mathrm{d}N$ gives the number of semichords encountered in the interval $\mathrm{d}x$, and $\mathrm{d}N$ is proportional to $\mathrm{d}x$. Now x is given by $(r^2-l^2)^{\frac{1}{2}}$ and $\mathrm{d}x/\mathrm{d}l$ is $-l/(r^2-l^2)^{\frac{1}{2}}$, and to satisfy eqn. (4) we have

$$f(l) = l/\{r(r^2-l^2)^{\frac{1}{2}}\}. \tag{6}$$

The first moment α gives the mean semichord:

$$\int_0^r lf(l)\,\mathrm{d}l = \pi r/4. \tag{7}$$

The second moment β gives the mean squared semichord:

$$\int_0^r l^2 f(l)\,\mathrm{d}l = 2r^2/3. \tag{8}$$

The third moment γ gives the mean cubed semichord:

$$\int_0^r l^3 f(l)\,\mathrm{d}l = 3\pi r^3/16. \tag{9}$$

The skewness statistic g, given by (3rd moment about mean):(standard deviation cubed), is $-1{\cdot}15$, so that the distribution of semichords (and of chords) in a single cell has a markedly negative skew.

Now consider the distribution of the radii. If this is lognormal then the logarithms of the radii have a Gaussian distribution such that

$$f(\ln r) = (2\pi\lambda^2)^{-\frac{1}{2}} \exp\left[-\{\ln (r/r_m)\}^2/2\lambda^2\right], \tag{10}$$

where r_m is the mode and also the geometric mean, and λ is the standard deviation of $\ln r$. Hence if a similar condition to that stated in eqn. (4) is followed, we have

$$f(r) = r^{-1}(2\pi\lambda^2)^{-\frac{1}{2}} \exp\left[-\{\ln (r/r_m)\}^2/2\lambda^2\right]. \tag{11}$$

It can be shown (see Finney, 1941) that the nth moment is given by

$$E(r^n) = \int_0^\infty r^n f(r)\,\mathrm{d}r = \bar{r}_m^n \exp (n^2\lambda^2/2). \tag{12}$$

In particular the mean $\bar{r}$ and the S.D. σ are given by

$$\bar{r} = \int_0^\infty rf(r)\,\mathrm{d}r = r_m \exp (\lambda^2/2) \tag{13}$$

and

$$\sigma^2 = \int_0^\infty r^2 f(r)\,\mathrm{d}r - (\bar{r})^2 = (\bar{r})^2\{(\exp \lambda^2) - 1\}. \tag{14}$$

These results have been stated by Finney (1941).

The parameters of the population of semichords which is found experimentally is now deduced. The probability of encountering a cell of given radius is proportional to $rf(r)$. The distribution of means α is also proportional to $rf(r)$, and $f(\alpha)$ follows a similar condition to that of eqn. (4). The proportionality factor is found as follows. By eqn. (7), $\mathrm{d}\alpha$ equals $(\pi/4)\,\mathrm{d}r$. Now

$$\int_0^\infty rf(r)\,\mathrm{d}\alpha = (\pi/4)\int_0^\infty rf(r)\,\mathrm{d}r = (\pi/4) r_m \exp (\lambda^2/2). \tag{15}$$

Hence

$$f(\alpha) = (4/\pi)\{r_m \exp (\lambda^2/2)\}^{-1}\, rf(r). \tag{16}$$

Then $\bar{l}_a$, which is the grand mean of the observed population of semichords, is given by the first moment of α where

$$\bar{l}_a = \int_0^\infty \alpha f(\alpha)\,\mathrm{d}\alpha = (\pi/4) r_m \exp (3\lambda^2/2). \tag{17}$$

The distribution of β is also proportional to $rf(r)$, and in a similar manner it follows that

$$f(\beta) = (3/4)\{r_m^2 \exp(2\lambda^2)\}^{-1} rf(r). \tag{18}$$

The mean of the β values is given by the first moment of β, and hence σ_a^2, the variance of the observed population, is

$$\int_0^\infty \beta f(\beta)\, d\beta - (\bar{l}_a)^2 = (2/3)\, r_m^2 \exp(6\lambda^2) - (\bar{l}_a)^2. \tag{19}$$

The distribution of the mean cubed semichord γ is also proportional to $rf(r)$, and similarly

$$f(\gamma) = (16/9\pi)\{r_m^3 \exp(9\lambda^2/2)\}^{-1} rf(r). \tag{20}$$

The mean cubed semichord of the observed population is given by the first moment of γ, and hence the mean cubed deviation G of the observed population is

$$\int_0^\infty \gamma f(\gamma)\, d\gamma - (\bar{l}_a)^3 - 3\bar{l}_a\sigma_a^2 = (3\pi/16)\, r_m^3 \exp(27\lambda^2/2) - (\bar{l}_a)^3 - 3\bar{l}_a\sigma_a^2. \tag{21}$$

Since both $\bar{l}_a$ and σ_a have been determined experimentally, it is of value to express r_m and λ in terms of them. Let the term $\{(\bar{l}_a)^2 + \sigma_a^2\}/(\bar{l}_a)^2$ be designated by t. Then from eqn. (17) and eqn. (19) we have

$$\exp(3\lambda^2) = 3\pi^2 t/32 \tag{22}$$

and

$$r_m^2 = 512\,(\bar{l}_a)^2/(3\pi^4 t), \tag{23}$$

also

$$G = 81\,\pi^4 t^3 (\bar{l}_a)^3/8192 - (\bar{l}_a)^3 - 3\bar{l}_a\,\sigma_a^2. \tag{24}$$

The experimental value of the mean chord thickness, which is $2\bar{l}_a$ in the above nomenclature, is $26 \cdot 7\,\mu$. The corrected mean diameter, which is $2\bar{r}$, is $31 \cdot 6\,\mu$. These fibres are from stretched diaphragm muscles from rats of 200–300 g.

Creese (1954) obtained a value of about $20\,\mu$ by histological methods in the case of diaphragm muscles from rats of 120 g.

The observed S.D. of chord thickness is $\pm 15 \cdot 7\,\mu$, and this corresponds to $2\sigma_a$. The corrected value, given by 2σ, is $\pm 8 \cdot 7\,\mu$. The value of λ is 0·27 $\log_e$ units, or 0·12 $\log_{10}$ units. This may be compared with the value of 0·076 $\log_{10}$ units found by histological methods (Creese *et al.* 1956). The greater scatter in the present study may be attributed to the larger range in the weights of the rats.

The experimental value of g, the skewness statistic, is +1·1. The value computed with the aid of eqn. (24) is +1·5.