

METHODS

APPARATUS FOR ONE-STAGE DETERMINATION OF REDOX POTENTIAL, FREE OXYGEN CONCENTRATION, AND LOCAL CIRCULATION IN A TISSUE

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An apparatus for the combined investigation of tissue bioenergetics is described: the redox potential, free oxygen concentration, and local blood flow can be determined.

KEY WORDS: *Redox potential; partial pressure of free oxygen; tissue circulation.*

Some of the principal methods used in the study of the tissue bioenergetics are oxygen polarography [1-3, 9], hydrogen polarography [8], by means of which the partial pressure of free oxygen (pO_2) in a tissue and the magnitude of the local circulation can be determined from the rate of elimination of inhaled hydrogen, and redoxometry [7], used to measure the redox potential (RP) of a tissue.

The RP is determined [7] on an electrode composed of an inert metal, such as platinum, relative to a certain comparison electrode, such as Ag-AgCl. The same pair of electrodes is usually used also for polarography [6, 9]. Meanwhile high-ohmic millivoltmeters with which the RP is usually measured can also be used for polarography if a simple attachment is added. Schemes for such attachments for oxygen polarography have been described [4-6].

The special features of techniques of polarography and redoxometry described above have been used by the writers to develop the instrument suggested in this paper for the rapid and consecutive (virtually simultaneous) determination and graphic recording of the redox potential, pO_2 , and the local blood flow in a tissue.

A scheme of the apparatus is shown in Fig. 1. Electrodes (17) are connected to the input socket (16) of the apparatus (1). The active electrode is a platinum electrode 0.1 mm in diameter and the reference electrode a chlorided silver plate or needle, which must not be more than 1000 times larger in area than the active electrode [4]. The active electrode is inserted into the tissue for testing and the reference electrode is placed on the surface of the subject's body (plate) or inserted beneath the skin of the experimental animal (needle). A laboratory pH meter, the pH-340 (2) is connected to the output socket (9) of the apparatus, and an ÉPP-09M electronic writer is connected to the output (up to 20 mV) of the pH meter.

During measurement of RP, the double-pole switch of the apparatus (15) is put in the "off" position and the tumbler switch (10) in position α . In this case the electrodes are connected directly to the input of the pH meter, on the scale of which the value of RP can be read.

To record pO_2 the tumbler switch (15) is put in the "on" position and the tumbler switch (10) and the polarity-reversing switch in position b. In that case, when switch (5) is on, a negative potential is applied to the active electrode from the 1 V battery (4), and its magnitude can be set by means of the 1 k Ω potentiometer (6) and the voltmeter (7) at -0.65 V. With this value of the cathode potential the current reaches a maximum (the plateau of the polarogram), the value of which is proportional to pO_2 . The current passing through the cell (in this case the "electrode-tissue" system) is measured by the millivoltmeter of the

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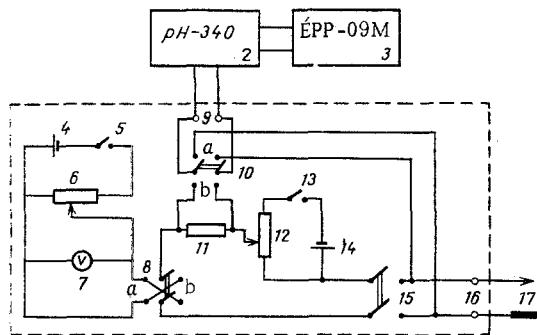


Fig. 1.

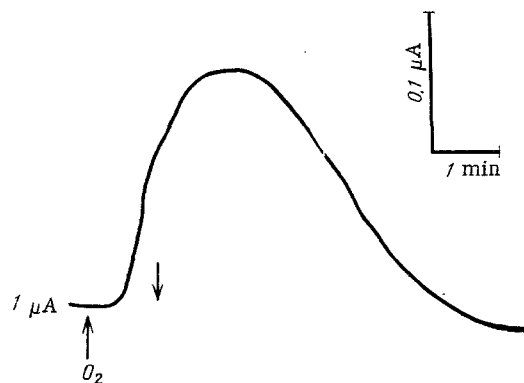


Fig. 2

Fig. 1. Scheme of apparatus for combined investigation of tissue bioenergetics (explanation in text).

Fig. 2. Dynamics of pO_2 in muscle tissue of healthy subject after inhalation for 1 min. Arrows indicate beginning and end of inhalation.

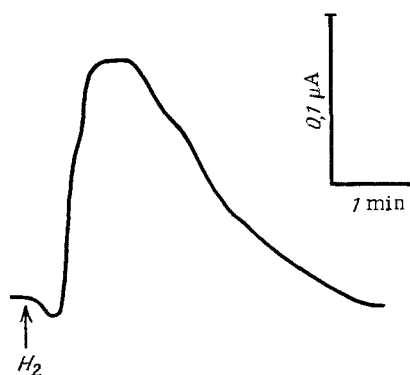


Fig. 3. Dynamics of hydrogen, after a single inhalation, in muscle tissue of healthy subject. Arrow marks time of inhalation.

pH-340 instrument from the voltage drop across a standard resistor (11). Under these circumstances the millivoltmeter scale is graduated in units of current. The standard resistor is chosen experimentally and must be about 5 times less than the resistance of the cell [5, 6]. In our investigations it was of the order of 10 kΩ.

The 1 V battery (14), switch (13), and 1 kΩ potentiometer (12) form a background current compensator, by means of which the zero current can be set in the absence of an external voltage on the electrodes.

The dynamics of pO_2 in the muscle of a healthy person after inhalation of oxygen for 1 min, obtained by means of the apparatus described above, is shown in Fig. 2.

Hydrogen polarography is carried out with the active electrode at a potential of +0.3 V [8]. The polarity of the voltage on the electrodes is reversed by the tumbler switch (8), in position a. The remaining switches are left in the same positions as for oxygen polarography. The dynamics of hydrogen in the tissue after a single inhalation of the gas, represented graphically, has the appearance of a wave (Fig. 3), from which the local blood flow can be determined also [8]. It is expressed in ml/100 g tissue/min and is calculated by the equation:

$$K = \frac{69.3}{T_{1/2}},$$

where K is the local blood flow; $T_{1/2}$ the time (in min) for the amplitude of the hydrogen wave to be reduced by half (the half-elimination period of the inhaled hydrogen).

Trials of the apparatus in practice have shown its reliability in operation and the good reproducibility of the results.

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QUANTITATIVE ANALYSIS OF STIMULATION FREQUENCY TRANSFORMATION IN THE NEUROMUSCULAR APPARATUS

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The dependence of the parameters of neuromuscular transmission on the frequency of stimulation was determined. This dependence was analyzed in relation to "fatigue" of the synapse arising during prolonged repetitive stimulation of muscle. The proposed mathematical model and the method of statistical analysis of the records of evoked responses of the muscle derived from it permit approximate estimates to be made from the experimental data of parameters quantitatively reflecting frequency (transmission) properties of the neuromuscular apparatus.

KEY WORDS: *Electrical activity of muscle; models of neuromuscular transmission; electromyogram.*

Investigation of the dependence of parameters of neuromuscular transmission on the frequency of stimulation is of great importance both to the analysis of the experimental data and to the diagnosis of diseases associated with the disturbance of this transmission. This dependence is studied in the investigation described below as it applies to the development of "fatigue" processes in the synapse during prolonged repetitive indirect stimulation of a muscle, although the approach to be considered can also be used to analyze other work regimes of the neuromuscular apparatus (NMA).

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