AN ELECTROCHEMICAL METHOD OF RECORDING THE PATTERN OF OXYGEN METABOLISM IN THE TISSUES IN VIVO

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Insufficient attention is paid in the literature to methods of measurement of the oxygen metabolism in the organs and tissues of the living animal. The polarographic method is the most suitable for this purpose, using any form of solid polarized cathode. The cathode must make contact with the tissues only through a definite part of its metallic surface of strictly constant area (~1-3 mm^2); the rest of the cathode must be carefully insulated electrically. The material of the cathode must be chosen so that it is fully polarizing and, at the same time, enables reproduction of the measurements. The electrical circuit of the apparatus must be simple. We recommend the use of a modified visual polarograph, in which, in contrast to the usual circuits of polarographs with a solid cathode [1, 3], the four-volt battery, potentiometer, voltmeter and calomel element are absent. The suggested circuit is illustrated in Fig. 1. It consists of two electrodes, a galvanometer G and a shunt R.

The resistance of R must be equal to the external critical resistance of the galvanometer, the necessary sensitivity of which is obtained by means of the rider D.

A similar circuit, but with Au—Zn electrodes, has been used in the construction of the "oxygen lead" for measurement of the dissolved oxygen in the depths of reservoirs [4]. The polarized cathode was gold plate and the anode a zinc plate. The necessity of having an external source of tension and of regulating and controlling it was eliminated, because the zinc, dissolving in the water, maintains a constant potential difference required in the Au—Zn electrode couple to reduce the oxygen on the gold to hydrogen peroxide.

We tested the applicability of this electrode couple to the recording of the oxygen metabolism in the muscle of the rabbit (see Fig. 3). In the recording, difficulty was experienced in insulating the gold electrode. We therefore looked for another electrode couple which would be more readily prepared. The most suitable couple was found to be copper amalgam—iron. As polarized cathode we used ordinary lacquered copper armature wire PEL with a diameter of 0.5-0.9 mm. The insulating coating provided on this wire, when applied by the method used in the factory, usually possesses high mechanical and electrically insulating properties. The tip of the wire, sharpened to a point, is carefully amalgamated with mercury. The second electrode is an ordinary carbon steel needle. Stainless or any other type of steel with a passive surface is unsuitable for this purpose. The surface area of the steel electrode is not of significance: it should be at least 2-3 times as large as the active surface of the amalgamated electrode. Flexible leads with vinyl chloride insulation, soldered to the electrodes, are connected to the shunt of a mirror galvanometer, possessing a sensitivity of not less than 10^{-8} a/mm of the scale and an internal resistance of not less than 1000 ohm.

As shown by a calibration experiment in vitro (Fig. 2), the electrode couple Cu(Hg)—Fe which we selected gives a linear relationship between the strength of the surging depolarization current and the oxygen concentration in the solution. The surging current when working with a fixed cathode in polarography is defined as the

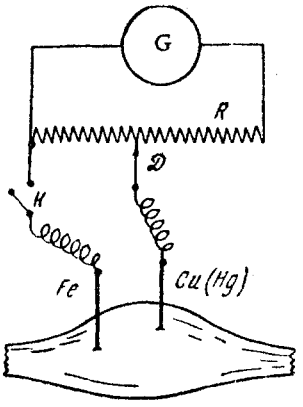

Fig. 1. Electrical circuit of the apparatus.

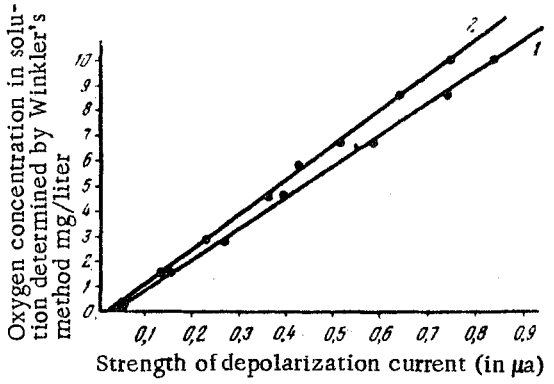

Fig. 2. Calibration chart of an electrode couple copper amalgam–iron. Area of amalgam surface 1.6 mm^2. Ringer's solution, T = 20°. 1) Exposure 30 sec; 2) exposure 60 sec.

current recorded during a short, conventionally chosen and accurately reproducible time of exposure (for example, 30 or 60 sec) after the closure of the circuit, before the current is established and while it still continues to fall [2].

The Au–Zn electrode couple with a gold cathode of equal surface area gives precisely the same calibration curve. Consequently, in accordance with the changes in the strength of the depolarization current it is possible to estimate the changes in the oxygen metabolism in the tissue in which the amalgam electrode is implanted.

We reproduce two experiments on rabbits using a gold (Fig. 3) and amalgam (Fig. 4) electrodes. Both experiments were carried out in the laboratory of the physiology and pathology of respiration and circulation of the blood (Head–Prof. M. E. Marshak) of the Institute of Normal and Pathological Physiology (Director–Active Member AMN SSSR V. N. Chernigovskii). In both cases the gas mixtures of different composition were supplied for respiration into the dissected trachea. The active electrodes–amalgam and gold–were implanted in the thigh muscle, the other electrodes anywhere at a distance not less than 1 cm from the first. The strength of the polarization current was recorded by means of a mirror galvanometer (sensitivity 460 mm/μa, internal resistance 535 ohm, external critical resistance 4200 ohm, t 2.5 sec). The true value of the depolarization current of the electrode was calculated from the ratio between the resistance of the shunts and the galvanometer. At the initial moment of closure of the switch K the galvanometer gives a brisk surge of current, and usually after 2-5 min, in the course of which an approximately stationary process of diffusion of oxygen molecules can be established at the cathode, the light indicator of the galvanometer begins to oscillate slowly near some mean value in accordance with the existing state of the oxygen metabolism in the area of tissue that is being investigated.

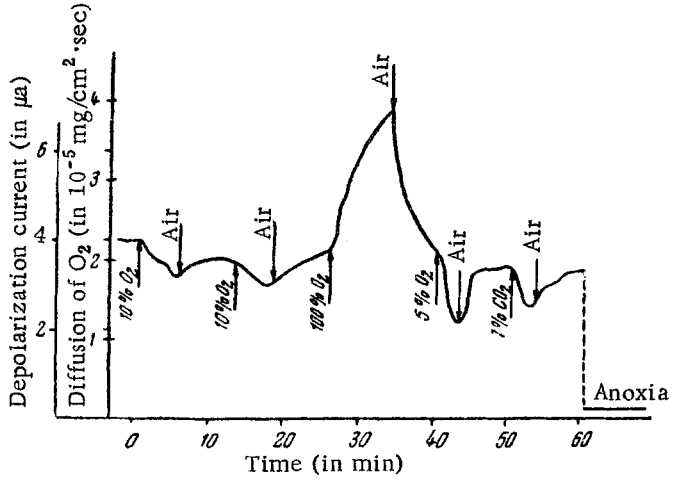

Fig. 3. Changes in oxygen metabolism in the thigh muscle of rabbit no. 1 depending on the composition of the inspired gas mixture. Electrode couple gold–zinc. Area of gold electrode 3 mm^2. Visual recording of mean values of current after every minute (April, 1956).

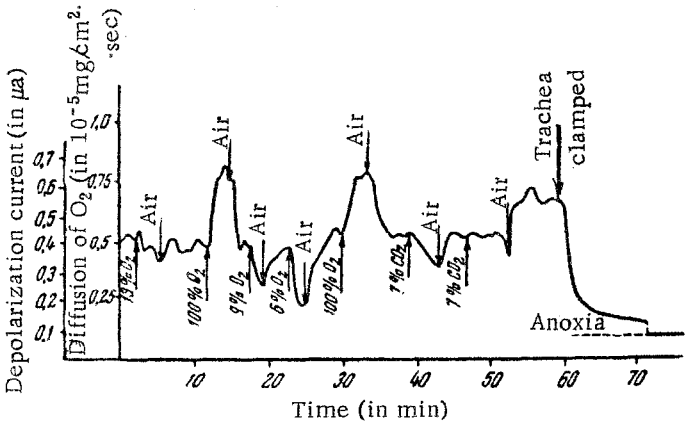

Fig. 4. Changes in oxygen metabolism in the thigh muscle of rabbit no. 2 depending on the composition of the inspired mixture. Electrode couple copper amalgam–iron. Area of amalgam surface 1.3 mm^2. Visual recording of mean values of current after every 15 sec (November, 1956).

As the curves show, besides the small, continuous spontaneous changes in the oxygen metabolism, which are of intrinsic interest, induced variations in metabolism rapidly become established, depending on the composition of the inspired mixture; the inspiration of pure oxygen increases the diffusion of oxygen into the tissues approximately twofold.

The oxygen metabolism in the tissue can be expressed quantitatively in milligrams of oxygen diffusing into the tissues and reduced on 1 cm^2 area of the polarized cathode in 1 sec. This value is proportional to the current density on the surface of the cathode and is calculated by Faraday's second law; the gram-equivalent of oxygen is taken to be 16 g, for under these conditions hydrogen peroxide is formed.

The strength of current remaining after complete anoxia of the tissue should be regarded as the oxygen zero; in the tissues there are other substances which are reduced along with oxygen on the cathode, and which form a small algebraic item which must be subtracted from the galvanometer readings. It is curious that the density of this current was found to be small and equal at the end of the two experiments, although the oxygen metabolism differed very considerably during the experiments.

The polarized cathode, when implanted in a tissue, may imitate the respiring cell; the oxygen utilized by the cathode from the tissue forms an equivalent electrochemical depolarization current, which may be recorded by the galvanometer. The number of molecules of oxygen diffusing to the polarized cathode in unit time, and the equivalent electric current are dependent on many factors: the partial pressure of oxygen in the blood, its coefficient of diffusion, the velocity and volume of the blood flow in the capillaries, the temperature, and the intensity of the oxygen demand by the surrounding cells.

These factors, however, taken as a whole, determine the pattern of the oxygen metabolism in the tissue. The method described is therefore an integrating indicator of the oxygen metabolism in the tissue.

SUMMARY

The author suggests a very simple method for the measurement of oxygen metabolism in animal tissues, which is based on a simplified polarograph system and electrodes with internal galvanic effect. Electrodes, used for the above purpose, consist of a copper cathode with amalgamated active surface, a needle from ordinary carbon steel acting as an anode. The cathode is made from lacquered copper winding PEL, 0.5–0.9 mm in diameter by amalgamating the cone-shaped tip with mercury. The above pair of electrodes, connected through a galvanometer, is inserted into tissues and oxygen metabolism is assessed through galvanometer readings.

LITERATURE CITED

1. I. M. Kolthoff and J. J. Lingane, Polarography [Russian translation] (Moscow, 1948) p. 412.
2. E. M. Skobets and N. S. Kavetskii, Zavododskaya Lab. 28, 1, 39(1952).

3. A. D. Snezhko, Biofizika 1, 6, 585 (1956).
4. W. Ohle, Die chemische und die electrochemische Bestimmung des molekular gelösten Sauerstoffs der Binnengewässer (Stuttgart, 1953).