INVESTIGATION OF THE RESPIRATORY METABOLISM OF WORKING ORGANS BY A FLUORESCENCE METHOD

V. L. Vodolazskii and F. F. Litvin

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Apparatus for measuring fluorescence of respiratory carriers (NAD·H_2 and flavoprotein) in functioning organs (spleen and heart) of animals is described. By means of a portable unit the organs of larger animals (dog's heart, rabbit's brain) can be investigated without being placed in a lightproof chamber. To abolish effects due to displacement of the heart and instability of the light flux, a differential circuit and a device with interchangeable filters were used. The results of measurement of the NAD·H_2 level of the working heart during changes in the gas composition of the inspired air and of investigation of the effect of Ca ions demonstrate the possibilities of the fluorescence method as a means of studying the respiratory metabolism of working organs.

Modern optical methods can be used to investigate the respiratory metabolism of tissues and organs in vivo even without direct mechanical contact [1, 2, 4]. These methods include measurement of the level of the reduced form of nicotinamide-adenine dinucleotide (NAD·H_2) and the oxidized form of flavoprotein by means of their fluorescence [3, 6]. Measurement of the fluorescence of one form of the respiratory carriers shows changes in oxidation-reduction processes in the terminal oxidation system. During excitation of NAD·H_2 in the ultraviolet region (absorption maximum about 340 nm) the reduced form of NAD flouresces in the region 467 nm [5], whereas the oxidized form does not. Only the oxidized form of the flavoproteins fluoresces in the region of 570 nm when excited at about 450 nm [6].

Since quantitative changes in the ratio between the oxidized and reduced forms of the respiratory carriers usually do not exceed a few percent, this makes particularly high demands on the sensitivity and accuracy of recording of the fluorescence. It is made more difficult to meet these demands because the intensity of the exciting light must not exceed the threshold at which injury to the cells or photochemical changes in the substances investigated can take place.

The object of the investigation described below was to develop a suitable method of fluorometric measurement of NAD·H_2 in the isolated heart and in the various organs of animals after laparotomy or thoracotomy (spleen, liver, heart).

The single-beam fluorometer is shown schematically in Fig. 1. The apparatus is based on a source of exciting light with absorption and interference filters (the spectral region of 330 nm was selected), a chamber for the object, interference filters (selecting fluorescence with a maximum at 467 nm, half-width 6-8 nm), and a low-noise photomultiplier with a photocathode of large area (FÉU-38). The LPU-01 pH-meter was used as amplifier. The signal was recorded continuously by a self-recording ÉPP-09 potentiometer or by a type USCh 8-03 8-channel automatic writer. The chambers for the objects were made in several modifications suitable for investigation of the isolated part and other organs of different species of animals.

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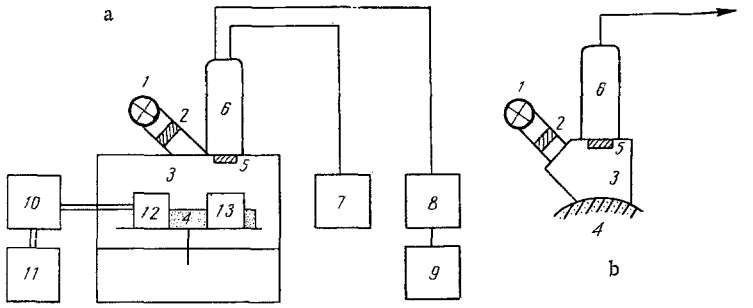

Fig. 1. Block diagram of fluorescence apparatus for investigating working organs of small animals by placing them in the chamber (a) and portable unit of the apparatus for investigating the organs of large animals without placing them in the chamber (b). Arrow indicates direction to measuring unit: 1) source of exciting light; 2) filters selecting exciting light; 3) lightproof chamber; 4) biological object; 5) filters selecting fluorescence from object; 6) photoelectronic multiplier; 7) high-voltage rectifier-stabilizer; 8) amplifier; 9) automatic writer; 10) artificial respiration apparatus; 11) gas mixer; 12) respiratory chamber; 13) constant temperature chamber.

The fluorescence apparatus with the chamber designed for investigation of the organs of small animals is shown schematically in Fig. 1a. The animal is fixed to a movable bench in the chamber so that the exciting light falls on the part of the organ to be tested. The gas composition of the air in the chamber and breathed by the animal can be changed during the experiment, an important fact if it is intended to study how oxidative processes depend on direct contact between the cells of the tested organ and the atmosphere. Parallel with the fluorescence, the ECG and respiration are recorded. The modification of this apparatus with the portable unit, including a source of light, photomultiplier, filters, and lightproof hood (Fig. 1b) can be used to measure fluorescence of individual organs of larger animals (the dog's heart or rabbit's brain) without placing them in the lightproof chamber. The accuracy of the method in the case of the single-beam design is limited chiefly by instability of the light flux of the excitation source and by movements of the object (in the case of the working heart). To eliminate these disturbances, it is best to use a two-beam method.

In the apparatus developed by the writers, one of the two photomultipliers records the fluorescence of the object while the other records the exciting light reflected by the object (or light directly from the source of excitation). The beams fall on the photocathodes of the two photomultipliers, the signals from which are amplified by two LPU-01 instruments and led to a differential dc amplifier. The differential signal from the output of the dc amplifier is led to an eight-channel USCh 8-03 automatic writer. Instability of the exciting light is largely abolished by the use of an incandescent lamp with iodine cycle (KIM-9-75) as the source of light. Instability of the light flux of this lamp, when powered from a rectifier through a battery, does not exceed 1-1.5%.

To minimize effects connected with movement of the object, the isolated heart is placed in a quartz chamber so that the area of myocardium for study is pressed against the anterior wall of the cell through which the fluorescence is measured. For work on the organs of larger animals using the portable measuring unit, the organ is immobilized by covering it with a thin quartz slab.

To record the reduced form of NAD and the oxidized form of flavoprotein simultaneously [6] a method of recording several signals phased in time was used. The filters in front of the objective and photomultiplier were fixed to a rotating disk. Interchange of the filters was carried out automatically; in this way signals proportional to the intensity of fluorescence of the two carriers and to the intensity of the exciting and scattered light falling on the photocathode because of incomplete crossing of the filters could be recorded alternately. After analyses of the data the true signal of fluorescence of the two carriers from the same area of the tested organ could be assessed.

By way of illustration of the potential usefulness of the method the results of a number of preliminary investigations of various objects using the instrument described above are given.

In an investigation of the effect of temporary interruption of artificial respiration (for between 20 and 50 sec) on the intensity of fluorescence of the rat heart in situ it was found that the NAD·H_2 concentration is reduced for a short time by 13-15%. This indicates an increase in the concentration of the oxidized form of NAD and, correspondingly, an increase in the relative velocity of oxidative processes. A similar increase in the relative velocity of oxidative processes is also observed in the spleen if the air is temporarily replaced by nitrogen (containing traces of oxygen). The NAD·H_2 concentration fell under these circumstances on the average by 11%. Changes in the level of fluorescence were investigated in the same organ during local heating of the spleen. Raising the temperature of the spleen by 10°C caused a decrease in the NAD·H_2 concentration by 60-70%. In experiments with the isolated frog's heart, the action of calcium ions led to a decrease of 10-12% in the NAD·H_2 concentration.

The results of these and other experiments confirm the potential value of the fluorescence method in the investigation of respiratory metabolism in experiments on whole animals and on individual functioning organs, including the working heart. It is considered that further development of the technique will enable small changes in the level of metabolism in physiological experiments and, perhaps, during operations on animals and human subjects to be recorded quantitatively.

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