DETERMINATION OF THE pH OF LIVING TISSUES

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The determination of the active hydrogen ion concentration of living tissue has been extensively used in solving certain problems in normal and pathological physiology. This index has acquired particular importance in pathology in connection with the study of the nature of the inflammatory reaction, as in H. Schade's [8] research. In spite of the fact that Schade's theory has been subjected to very serious and well-grounded criticism, the determination of the pH of inflammatory tissue is still a problem of the utmost urgency. No accurate and reliable methods of determination of the pH of the tissues of a living animal, exist yet, however.

In the research of Schade and his co-workers [13] the pH was determined by means of a hydrogen electrode. In this method, the hydrogen ion concentration is not measured directly in the tissues, but in the tissue fluid which is drawn from the tissue into the tip of the electrode. Because of this, the method has been found unsuitable for the determination of the pH of comparatively dry tissues as, for example, the normal uninflamed skin.

This last aspect is stressed by A. D. Ado [1, 2], who used this method to determine the pH of animal tissue in inflammation of allergic origin or due to turpentine. A fundamental defect of the hydrogen electrode is the very slow development of the potential (40 minutes to 2 hours). In such conditions it is nearly impossible to study the pH changes during the initial stages of development of inflammation. It should be added that the platinum black gradually comes away from the electrode when it is buried in the tissues; this may interfere with the working of the electrode. The method is very clumsy and requires a complicated apparatus for the continuous supply of hydrogen.

The theoretical importance of a solution of this problem necessitated a search for more adequate methods of estimation of the pH of living tissues. This provided the impetus for the development of various modifications of the hydrogen electrode and for the introduction of new methods. Nevertheless it has proved almost impossible to overcome the technical difficulties in measurement of the pH in vivo.

Lanz and Malyoth suggested a gold-hydrogen-iridium electrode, which had a number of advantages. However the variations in potential by this method were so great that, in order to obtain reliable data, it was necessary to take the mean values of a large number of successive measurements [11].

Many workers, among them A. F. Gol'dberg [4] and A. O. Voinar [3], used a quinhydrone electrode introduced directly into the tissues for determination of the pH. Subsequent verifying investigations showed that this method also was unsuitable. V. P. Grechanovskii and I. I. Matusis [5], in particular, showed that when the quinhydrone electrode was used the potential changed so quickly that it was difficult to settle on any particular figure. Another grave defect of the quinhydrone electrode is its irremediable error with regard to protein and salt. There is no guarantee that, when the electrode is inserted, sufficient quinhydrone enters the tissues to cause saturation.

In just the same way, the antimony electrode [9] and the platinum electrode coated with MnO_2 [10] and others failed to justify themselves in the measurement of the pH of living tissues.

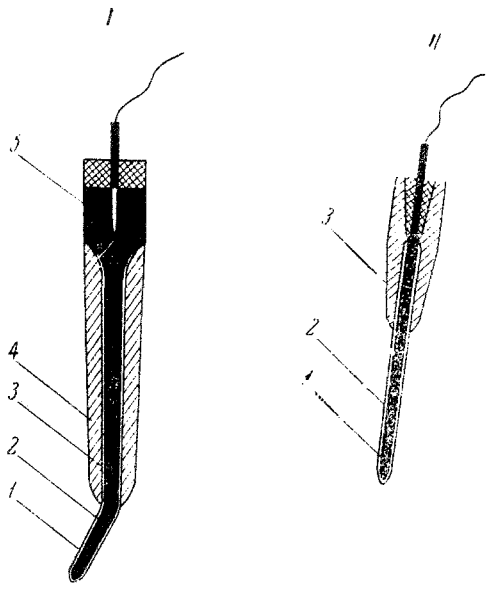

Fig. 1. Scheme of the constructions of the electrodes. I) Glass microelectrode, filled with mercury: 1) glass capillary tube; 2) mercury; 3) 0.1 N HCl solution; 4) mixture of resin and paraffin wax; 5) widened part of the body of the electrode with the wire immersed in mercury; II) glass electrode with platinum base: 1) platinum needle; 2) glass film; 3) mixture of resin and paraffin wax.

The best prospects for the determination of the pH of the tissues are given by the glass electrode, for it does not require the introduction of additional substances into the tissue and is not harmed by oxidizing and reducing substances in the tissue; its potential is quickly established. Attention was first drawn to this by Voegthlin and his co-workers [14], who suggested and used their variant of the glass capillary electrode in their experiments. With many undoubted advantages over the methods described above, this electrode has, nevertheless, certain defects – it is very fragile and has a high internal resistance; it is also necessary to use two additional semielectrodes of calomel.

We found no reports in the Soviet literature of investigations of the pH in vivo with a glass electrode.

The aim of our research was to study the pH of the tissues of a living animal in various pathological processes. Taking into consideration the defects of all the known methods used for this purpose, and also the fact that, in general, this problem is associated with technical difficulties, we started our work by attempting to produce a model electrode, free as far as possible from the defects inherent in the electrodes described above.

EXPERIMENTAL METHOD AND RESULTS

We prepared two types of glass electrode; their principle of action did not differ in its general outline from that of other electrodes of this type (this question is discussed in detail in the exhaustive monograph by V. A. Pchelin [6]).

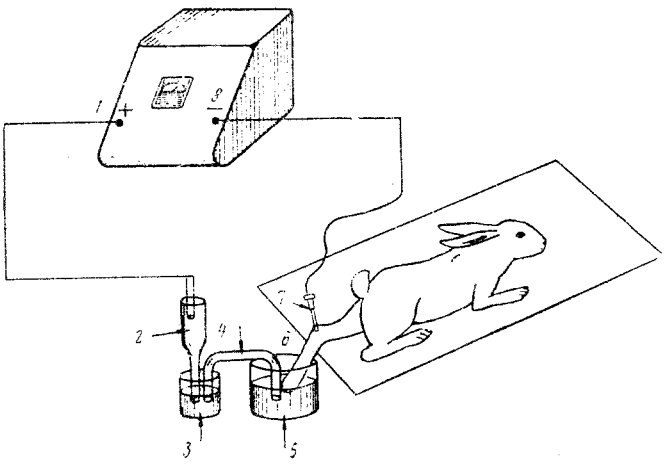

Fig. 2. Scheme of the measurement circuit. 1) Apparatus (positive terminal); 2) calomel semielectrode; 3) vessel containing a saturated solution of potassium chloride; 4) electrolytic bridge; 5) vessel containing physiological saline; 6) hind limb of a rabbit; 7) glass electrode buried under the skin; 8) negative terminal of the apparatus.

The first electrode consists of a platinum needle (Fig. II, 1) covered with a thin film of glass with a high sodium and magnesium content (É-S 2 glass). At the junction of glass and metal a reasonably stable potential is formed. The main conditions for the proper working of this electrode are an absolutely close covering of the metal with glass, without any space or air bubble, and very strict insulation of its outer surface (in contact with the test solution or tissue) from the lead connecting the platinum wire to the apparatus. The outer surface of the electrode above the buried portion (i. e.,the strictly working portion) is coated with a mixture of resin and paraffin wax. Firstly, this produces a working surface of the electrode of strictly constant size, and secondly, it prevents the formation of a hygroscopic film on the glass surface, and leakage of the current. After it has been made, the electrode must be immersed for a week in a 0.1 N solution of hydrochloric acid and then for not less than 2 weeks in distilled water. In the intervals between investigations the electrode must be kept in distilled water, for drying leads to a deterioration in the electrode properties of the glass.

When properly made, the electrode gives a stable and rapidly established (in 20-30 seconds) potential, and is very convenient to use — with a long enough wire it can be taken to an animal in any position. The necessity for a second calomel semielectrode is obviated, but the considerable length of the working part of the electrode (1. 5-2 cm) introduces difficulties into the work. In the second type of electrode we were able to shorten the working part. This electrode (see Fig. I, 1) consists of a capillary tube — the working part proper — , 9-10 cm long, and a body, widened at the top and sealed with an airtight cork. Through the cork passes a copper wire, one end of which is connected to a measuring apparatus and the other is immersed in the mercury which fills the whole electrode (in the manner of the macroelectrodes suggested by A. I. Rabinovich and V. A. Kargin [7]). In order to stabilize the potential at the mercury — glass junction, the electrode is at first filled with normal hydrochloric acid, and the acid is then gradually replaced by mercury. A thin film of hydrochloric acid is formed between the liquid mercury and the glass, thus ensuring constancy of the potential inside the electrode.

Immersion of the electrode and coating of its body with the mixture of resin and paraffin wax are done just as for the first type of electrode. In its construction, the necessity for using a second calomel semielectrode is obviated. In this electrode, the potential is established rather more slowly than in that described above (1-1½ minutes), but it is more stable. Before each experiment the electrodes must be calibrated in 2-3 buffer solutions of known pH. The internal resistance of both electrodes was high, but did not exceed the input resistance (100 meg) of the "RFT, TUR-190 pH-meter", with which the measurements were made.

When the measurement circuit is being assembled the following points are essential: 1) the glass electrode must be connected to the negative terminal of the apparatus and the calomel semielectrode to the positive terminal; 2) the lead from the electrodes, the terminal and the apparatus itself must be carefully screened; 3) all parts of the measuring circuit must be insulated from each other, for the potential is so small that the least leakage of current may cause essential distortion of the experimental results.

Experiments were carried out on rabbits, lightly anesthetized and fixed to an electrically insulated bench. The electrode, fixed in a special clamp, was introduced through a preliminary puncture wound into the part of the body selected for investigation (beneath the skin, into the muscle and so on). Care had to be taken to ensure that the puncture wound was not bleeding and that the thick portion of the electrode firmly prevented the access of air to the tissue.

The hair in the region of the talocrural joint of the rabbit was carefully shaved; the skin incision was then made, inevitably with bleeding, and the joint was immersed in the vessel containing physiological saline. This vessel connected through an electrolytic bridge to a vessel containing a saturated solution of KCl, into which was dipped the end of the calomel semielectrode. The scheme of the measuring circuit is shown in Fig. 2.

With accurate and delicate working, requiring the development of skill in certain directions, reasonably precise estimations of the pH of the tissue can be made.

Using this method, we determined the pH of healthy rabbits tissues. The subcutaneous cellular tissue and striated muscles were investigated. As a result we were able to show the hydrogen ion concentration was reasonably close in different rabbits. Under morphine anesthesia its average value was 7.27, with a mean square deviation of ±0.17. Individual variations in the pH of different rabbits lay within limits of 0.1-0.5 pH units.

The electrode may stay in the tissue for several hours and continue to show constant values of the pH; this enables accurate determinations of the pH to be made in the living animal under normal and pathological conditions at intervals over a period of time.

The characteristics of the pH changes in inflammatory tissues will be the subject of our next communication.

SUMMARY

Two types of glass electrodes for the electrometric determination of the pH of the tissues in the living body are suggested. The pH of the tissues of healthy rabbits and the pH shifts occurring in an inflammatory process were studied with the aid of these electrodes.

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