ULTRAMICRO GLASS ELECTRODE FOR SODIUM ANALYSIS IN BIOLOGICAL FLUIDS

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Based upon the specific ion sensitivities of glasses of different composition, as reported by EISENMAN et al.³⁾, use of ion sensitive glass electrodes for the determination of cations such as sodium and potassium in biological fluids was reported by FRIEDMAN and FRIEDMAN⁴⁾, HINKE⁵⁾, and MOORE WILSON⁶⁾. Further studies have been directed toward the measurement of intracellular ion concentrations and of very small amounts of sample solutions (CALDWELL^{1,2)}, HINKE⁵⁾, HIRAKAWA and YOSHIMURA⁶⁾, and KHURI et al.⁷⁾. The present report describes the construction and use of a sodium micro-electrode designed to measure the sodium concentration of sweat in different parts of a single sweat gland and its duct. The electrode should be useful in investigation of the mechanisms of secretion and possible reabsorption of electrolytes in the sweat glands. Since the amount of fluid available for estimation is extremely small in this type of study, methodological studies were the first problem to be solved.

CONSTRUCTION OF THE ELECTRODE

The electrodes were made from sodium sensitive glass tubing (MAS₁₁₋₁₈ sodium sensitive-Eisenman, Corning Glass Works) 1 mm o. d. and 200 μ i. d. As illustrated in FIG. 1, in making the electrode a sodium glass capillary tube is pulled manually to an o. d. 500 μ by using a small gas flame of 5 $\times$ 2 mm (FIG. 1). A 22 ga hypodermic needle is used as the burner. A 5 mm length of high resistance Pyrex glass capillary (Corning Glass Works) whose inner diameter would just permit it to be fitted over the sodium sensitive capillary is fused to the latter by the gas flame. After the fusing is completed the exposed part of the capillary is pulled further into a finer capillary (o. d. 150 μ , i. d. 100 μ). A mantle is made of a larger Pyrex glass (o. d. 1.8 mm, i. d. 1.5 mm and about 15 mm in length) fused glass to-glass at one end to the previously fused portion

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of the capillary (FIG. 1 and 2). The exposed capillary protruding out of the mantle glass tubing is now pulled further to form a capillary pipette tip of appropriate size. The innerspace between the mantle tubing and the capillary is now filled with the electrode solution (0.1 N NaCl solution). An Ag-AgCl electrode (dia. 200μ) is put in the electrode solution and the opening of the mantle tubing is sealed by rubber cement to avoid evaporative changes of the electrode solution. The completed electrode is illustrated in FIG. 2. The capillary portion between A and B in the figure serves as the exposed glass membrane of the electrode. The whole electrode except for the capillary tip is enameled (Cashew enamel, Cashew Co., Tokyo). This sealing with enamel greatly increases the stability of the readings by assuring good insulation of the lead wire from the electrode glass tubing. The other end of the electrode

FIG. 1. Steps in making the electrode from Na sensitive and pyrex glass tubing.

FIG. 2. Micro-electrode detail approximate dimensions.

capillary (opposite the tip) is connected by polyethylene tubing to a suction syringe by which a negative pressure is applied to draw the test solution into the electrode capillary, as illustrated in FIG. 3. The electrical resistance of the electrode was found to be as high as 200 to 300 megohms when the capillary was filled with 0.1 N NaCl.

CALIBRATION EXPERIMENTS

The electrical potential developed between the internal test solution and the external electrode solution across the exposed glass membrane is measured by an electrometer (Keithley Instr. Inc., Model 603, in some experiments by Model 610B) with a calomel reference electrode which is in electrical connection with the internal test solution through a saturated KCl-agar-agar bridge. During the measurement of an unknown solution the electrode capillary is first filled with a standard solution of known Na ion concentration (0.1 NaCl solution was usually used) before the capillary tip is inserted in the test solution. After the electrical circuit is completed by means of the agar bridge, the electrometer is adjusted to give a 0 reading. The test solution is then drawn into the capillary and as it reaches the exposed ion sensitive glass membrane a potential is produced which varies with the ratio of the ion concentration (or more exactly, ion activity) of the external electrode solution to that of the internal test solution. A semilogarithmic plot of the output potential against Na ion concentration in the test solution is demonstrated in FIG. 4. For concentrations higher than about 0.01 N a straight line relationship is obtained, but for lower concentrations the linearity of the calibration curve is lost. The reasons for this will be discussed later.

Interelectrode as well as intraelectrode reproducibility was examined in the following manner: Eight electrodes constructed in the same manner were tested with 0.1, 0.08, 0.06, 0.04, 0.04, 0.02 and 0.01 N NaCl test solutions. Four independent determinations were made at each concentration. The variations of the output potential in the eight electrodes were 61.68 ± 0.534 mV for tenfold difference of Na ion concentration. The deviations in the readings with each electrode for each concentration were in the range of a standard error of 0.231 to 0.166 mV (mean 0.179 mV).

EXPERIMENTS ON A SINGLE SWEAT GLAND

The electrode was used to measure directly the Na concentration in sweat as it was secreted by an individual sweat gland. As shown in FIG. 3 a sweat gland of the nailbed was utilized for this purpose. Vital staining of the sweat duct was accomplished by iontophoresis of 0.1% methylene blue solution (0.2 mA/cm², 5 min) whereby the duct became easily visible under a dissecting

FIG. 3. Diagram of measurement of sweat sodium concentration in a single sweat gland.

microscope. The electrode capillary was filled prior to the puncture with 0.1 N NaCl solution. After the capillary was successfully inserted in the duct and the electrical circuit completed the electrometer was balanced to 0 mV. Sweating was produced by intracutaneous injection of 10^{-3} pilocarpine. A slight negative pressure applied to the capillary drew the sweat into the capillary as it was secreted. As the sweat filled the electrode the 0.1 N NaCl solution contained within it was expelled and a potential developed dependent on the Na ion concentration of the sweat. Fluctuations in the output potential were observed as the incoming sweat flowed past the exposed ion sensitive glass membrane. These fluctuations appear to be caused by concentration changes following changes in sweat rate. Values of 19.2–42.5 meq/l Na concentration in sweat were obtained. Detailed studies will be reported later.

DISCUSSION

The amount of test solution required for an analysis is equal to the volume of the electrode capillary from the tip to point B in FIG. 2. For the electrode depicted in this figure the minimal amount was 0.044 microliter. Because the present electrode was made originally for insertion into a single sweat duct the mechanical strength of the electrode capillary had to be taken into consideration, lest it be broken during puncture. It is not difficult to construct an electrode in which the minimal amount required is much less than $0.044 \mu\text{l}$, by pulling an extremely fine electrode capillary. However, the introduction

of the "compensation method" described later in this paper increases to some extent the volume of solution required. A 2 to 3 fold increase in the minimal volume of test solution is required for stabilization of the electrometer.

The output potential of the coupled electrode is influenced by various factors, such as temperature, contact conditions of the electrode, and disturbances by potential sources in the circuit other than the ion potential in the measuring electrode. The latter is considered to be especially serious when

FIG. 4. Electrode potential for wide range variations in concentration of the test NaCl solution and demonstrating inter-electrode variation at low concentrations. Solution temperature 32°C. The solid line is for electrode (a) and the broken line is for another electrode (b). The effect of dilution of the test solution depends upon individual electrode at concentrations below about 0.01N.

the electrodes are used for direct sampling from a living body when an electrical circuit is made between the measuring electrode inserted in the living tissue and the reference electrode placed somewhere else on the body. The accuracy of estimation by the present electrode depends upon readjusting the electrometer with a standard solution of known ion concentration after the electrical circuit is completed (compensation method). In almost all of the electrodes hitherto reported there exists no possibility of compensation after the electrode is applied. With the electrode described continuous recording of the changes in ion concentration is possible as the test solution is drawn into the electrode capillary and flows past the exposed glass membrane.

FIG. 5. Concentration of test solution plotted against conductance, equivalent conductance and activity coefficients.

FIG. 6. Recording of changes in output potential while a test solution is being drawn into or forced out of the electrode capillary. Values given in mmHg are applied suction pressure (negative sign) or expulsion pressure (positive sign) in the capillary.

In spite of the small size of the electrode the reproducibility of determinations made with standard NaCl solutions was found to be satisfactory. The interelectrode fluctuations of the readings were 61.68 ± 0.534 mV for 8 electrodes for tenfold differences of Na ion concentration. The intraelectrode fluctuations for each electrode were in the range of standard errors of 0.231 to 0.166 mV.

Linearity of semilogarithmic plots of the output potential against Na concentration is valid only for test solutions of concentration higher than about 0.01 N (FIG. 3). Below that concentration the output potential deviated less than that which would be expected by Nernst's equation. The variations are due to the characteristic design or the measuring principle of the electrode. The electrical resistance of the electrode is due mainly to the glass membrane and the test solution in the narrow capillary, the former being a constant value, while the latter changes functionally with its Na ion concentration. As the NaCl concentration in the test solution is lowered its ion activity coefficient and the equivalent conductivity of the solution are increased (FIG. 5). On the other hand, the conductance of the solution, which is given by the product of the equivalent conductivity and the concentration of the solution, is decreased with decreasing concentration (FIG. 5). Internal resistance of the electrode system is increased in the capillary which results in decreases of the output potential. The effect of the ion concentration of the test solution on the output potential of the electrode therefore involves several factors. It varies somewhat with the individual electrode, and is dependent mainly upon the

FIG. 7. Effect of intracapillary pressure upon output potential of the electrode. Values on each line are the concentration of the tested NaCl solution.

thickness of the glass membrane and diameter of the electrode capillary. In spite of these facts the calibration experiments show that the electrode is accurate with solution with concentrations in the range of most biological fluids.

Any pressure, negative or positive, applied in filling or emptying the capillary with test solution causes changes in output potential (FIG. 6), presumably due to Dorn's phenomenon by causing the streaming potential. It may be possible that the changes in the asymmetry potential in the capillary due to mechanical deformation of the thin glass membrane are also involved in the pressure effect. The effect of the applied pressure depends greatly upon the ratio of the ion concentration across the membrane (FIG. 7). For a test solution of 10^{-4}N , for instance, a suction pressure of -10 mmHg caused about a $+40\text{ mV}$ change in the output potential, when compared with that measured at 0 mmHg , whereas for test solution of 10^{-2}N the same pressure changed the potential only by $+0.4\text{ mV}$. The effect of the intracapillary pressure upon the output potential of an individual electrode was expressed by the following formula;

$\log (\Delta) = -0.9420 \times \log (C) - 3.2356$ where Δ is potential change per unit pressure (mV/mmHg) and C is concentration of the test solution in meq/l. Since the Na concentration in biological fluids is usually higher than $10^{-2}N$ and since a suction pressure of only -10 to -20 mmHg is needed to fill the electrode the effect of the intercapillary pressure is very small and can be ignored in all but the most critical determinations. However, care must be taken not to measure the potential during a high suction pressure especially when the concentration of the fluid under test is very low.

SUMMARY

An ultramicro open end capillary glass electrode for sodium determination in biological fluids was developed based on a new design. The characteristics of the electrode are as follows:

- 1) The minimal amount of test solution required for an estimation was 0.044 of a microliter, but even smaller electrodes may be constructed without difficulty.
- 2) The open end capillary tubes were inserted into sweat ducts and glands and Na ion concentration of the sweat determined directly as it was being produced.
- 3) Continuous recording of Na ion content of the test solution is possible as the solution flows past the ion sensitive membrane in the capillary.
- 4) The accuracy of determination is greatly increased by readjusting the electrometer with a standard solution after the circuit is completed, especially when the electrode is applied to a living body for sampling and direct measurement.
- 5) In spite of the small size of the electrode the reproducibility of readings made on standard NaCl solutions in the range of concentrations of biological fluids was found to be satisfactory in respect to both intraelectrode and inter-electrode fluctuations.

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