REPORT DOCUMENTATION PAGE

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 5 JUNE 1995	3. REPORT TYPE AND DATES COVERED Technical Report

4. TITLE AND SUBTITLE

In vivo measurement of Na+ and K+ ions using

ion-selective electrodes

5. FUNDING NUMBERS

6. AUTHOR(S)

A.I. Osagie, C.B. Matthew, S.P. Mullen

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

U.S. Army Research Institute of Environmental Medicine
Natick, MA, 01760-5007

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Same as 7 above

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

Same as 8 above

DTIC
S ELECTE D
JUN 21 1995
B

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; Distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT Measurement of circulating levels of the electrolytes Na^+ and K^+ is integral to physiological studies of hyperthermia, dehydration, and nutrition. Current methods require removal of blood samples for later analysis. In small animals such as rats the volume removed can alter experimental results. In this study, cannulae were surgically implanted in a jugular vein and carotid artery of rats. Using ion selective electrodes and a flow-through sampling chamber of our own design, in vivo measurements of circulating Na^+ and K^+ concentrations were made by connecting the arterial cannula to the inflow port and the jugular cannula to the outflow port of the sampling chamber. The sampling chamber, electrode apparatus and the cannulae require only 0.8 ml of extracorporeal blood. Although measurement of K^+ concentration was abandoned because of continuous electrode drift, measurements of Na^+ concentrations accurately reflected expected values following dilution of in vitro blood samples with solutions of known electrolyte concentrations and expected changes in vivo following administration of hypertonic solutions to the animals. Use of this apparatus should allow continuous measurement of Na^+ concentration with minimal loss of blood volume in future studies.

DTIC QUALITY INSPECTED 3

14. SUBJECT TERMS

Electrolytes, sodium, ion-selective electrodes, in vivo measurements, extra-corporeal sample chamber, hypertonic saline in dextran

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT

TECHNICAL REPORT

<u>IN VIVO</u> MEASUREMENT OF Na$^+$ AND K$^+$ IONS USING

ION-SELECTIVE ELECTRODES

by

Anthony I. Osagie
Candace B. Matthew
Stephen P. Mullen

Environmental Pathophysiology Directorate

U.S. ARMY RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE

Natick, Massachusetts 01760-5007

19950619 053

CONTENTS

REFERENCES

DISTRIBUTION LIST

Accession For

NTIS GRA&I	☑
DTIC TAB	☐
Unannounced	☑
Justification	

By_____
Distribution/
Availability Codes

Dist	Avail and/or Special
A-1	

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

DISCLAIMER STATEMENT

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy or decision, unless so designated by other official documentation. In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", Department of Health and Human Services, NIH Publication No.86-23, revised 1985, as prepared by the committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources Commission on Life Sciences, National Research Council. United States Army Research Institute of Environmental Medicine is an AAALAC approved facility and will continue to adhere to the standards and requirements thereof. Citations of commercial organizations and trade names do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

EXECUTIVE SUMMARY

Measurement of circulating levels of the electrolytes Na^+ and K^+ is integral to physiological studies of hyperthermia, dehydration, and nutrition. Current methods require removal of blood samples for later analysis. In small animals such as rats, the volume removed can alter experimental results. In this study, cannulae were surgically implanted in a jugular vein and carotid artery of rats. Using ion selective electrodes and a flow-through sampling chamber of our own design, in vivo measurements of circulating Na^+ and K^+ concentrations were made by connecting the arterial cannula to the inflow port and the jugular cannula to the outflow port of the sampling chamber. The sampling chamber, electrode apparatus and the cannulae require only 0.8 ml of extracorporeal blood. Although measurement of K^+ concentration was abandoned because of continuous electrode drift, measurements of Na^+ concentrations accurately reflected expected values following dilution of in vitro blood samples with solutions of known electrolyte concentrations and expected changes in vivo following administration of hypertonic solutions to the animals. Use of this apparatus should allow continuous measurement of Na^+ concentration with minimal loss of blood volume in future studies.

1

INTRODUCTION

Measurements of circulating levels of the electrolytes Na^+ and K^+ are integral to studies of hyperthermia. Leakage from cells has been reported to be one of the earliest signs of hyperthermic injury (Shibolet, Lancaster et al., 1976). Measurements of these electrolytes are equally important in studies of hypertonic resuscitation fluids, such as prior studies with hypertonic saline in dextran (HSD), to determine changes following administration of the concentrated Na^+-containing solution (Matthew, 1994; Matthew et al., 1993; Zeigler et al., 1991). Also, studies of dehydration and nutrition would benefit from in vivo monitoring of electrolyte levels.

Bodily fluids (blood, cerebral spinal fluid, urine, intercellular fluid, etc.) are complex solutions containing electrolytes (mainly Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+}, and HCO_3^-) as well as proteins and other organic compounds. As these electrolytes are of significant diagnostic and physiological importance, they are frequently measured (Veselý et al., 1978). Current measurements of these electrolytes are based on an in vitro procedure. A sample of blood is removed and set aside for later analysis. The flame photometric method of measuring the concentration of the major cations in blood plasma is the most commonly used. However, a technique that requires removal of blood reduces the circulating volume, which may lead to altered experimental results in small animals.

The concept of ion-selective electrodes (ISE) is evolving into a new specialty area in biology. The advantages of this method lie in its directness, simplicity, sample conservation, and non-interference of measurements by other ions. ISEs specifically measure ion activities, on which many biological processes depend. Furthermore, ISEs have the additional unique potential of making continuous measurements of ionic species possible in vivo under conditions that approach normalcy (Berman, Hebert, 1973). This method would allow the continuous monitoring of circulating electrolytes without loss of blood from the animal.

A review of the literature did not reveal any information about the use of electrodes to monitor circulating levels of blood electrolytes. However, an electrode

system has been used to monitor Na$^+$ concentration in urine output of dogs with urinary catheters (Andersen, Bie, 1990). This indicates that measurement in a continuous-flow system is feasible. Therefore, this study was undertaken to set up a system for monitoring the circulating levels of Na$^+$ and K$^+$ using ISEs in rats.

MATERIALS AND METHODS

ANIMALS The following experimental procedures were approved by our Institute Animal Care and Use Committee and carried out with strict adherence to the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 86-23, Revised 1985.

Male Sprague Dawley rats (n = 12, 450-500g) were supplied by Charles River Laboratories. The rats were housed in the animal colony and caged individually. Purina rat chow (Purina, St. Louis, MO) and water were made available ad libitum, except during experimental intervals. Prior to use, they were moved to an environmental chamber maintained at 26°C and 50% rh, with lighting controlled automatically (on, 0600-1800 hrs).

One week prior to the experiment, rats were anesthestized with sodium pentobarbital (50 mg/kg intraperitoneally), and cannulae were inserted into the right atrium through the right jugular vein and into the aortic arch through the left common carotid artery as previously described (Kelly, Hubbard et al., 1979; Piascik, Sparks et al., 1992; Zeigler, Patel, 1991).

EQUIPMENT An Electro-Chemical Analyzer (ECA, Diamond General Development Corp., Ann Arbor, MI 48108) was used in this study. The ECA (Fig. 1) measures the potential difference of ISE and reads directly in concentration or mV. The ISEs are made from pieces of fine glass tubing that are pulled to a tip diameter of 1.5 mm providing 1 mm immersion depths and filled with a conducting solution (KCl solution). The ISEs consist of a barrier, permeable only to the selected ion, that separates a solution of known concentration of that ion from the sample being tested. The potential difference across the barrier is a measure of the ratio of the concentrations on either side (Alberts, Bray et al., 1989).

3

The sodium electrode possesses a sodium-containing glass membrane, across which a concentration-dependent potential develops when in contact with a solution containing sodium ion (Fig. 2). The potential is measured against a built-in reference electrode. The electrodes respond to sodium ion concentrations from 10^{-6} M to saturation at temperatures from -5° to 70°C (Handbook of Electrode Technology, 1982[b]).

The potassium electrode uses a replaceable module with a solid plastic membrane containing a potassium-selective ion exchanger (Fig. 3). When this membrane is in contact with a solution containing potassium ion, a concentration-dependent potential develops across the membrane. The potassium electrode is used with a reference electrode against which its potential is measured (Handbook of Electrode Technology, 1982[a]). The electrode responds to potassium ion concentrations from 10^{-6} to 1 M at temperatures from 0° to 40°C.

The FLM3 flame photometer (Rainin Instrument Co., Inc., Woburn, MA 01801) was used to determine the sodium and potassium content of diluted serum. A Multi-Osmette model 2430 automatic osmometer (Precision Systems Inc., Natick, MA 01760) was employed to determine the osmolality of calibration standards.

The extra-corporeal sample chamber (ECSC) was designed in our laboratory for use in conjunction with the ISE (Fig. 4 & 5). The sample chamber was constructed from materials readily at hand using a straight-forward, flow-through design. Its body was fashioned from a solid block of polytetrafluoroethylene (Teflon[TM]). Electrode seals were made by boring holes in standard resilient rubber stoppers taken from unused "red top" blood sample tubes. The other parts were machined from 1/4-inch thick transparent acrylic sheet (Plexiglass[TM]).

The chamber provides three ports for the ISEs. Different sized electrodes are accommodated by using seals with different sized holes. The seals are held in place by a clamping plate screwed onto the body of the device. Blood enters and leaves the chamber via hypodermic tubing brazed into threaded brass plugs (1/4-in NPT) in either end. Every effort was made to minimize the interior volume of the sample chamber (0.8 ml) while providing adequate interface between the electrodes and the blood.

4

EXPERIMENTAL DESIGN AND PROCEDURES

Electrode calibration Standard curves for the electrodes were derived by using solutions of various normal concentrations (0.1 N, 0.01 N, 0.001 N). Electrode potential was plotted as a function of ion concentration (Fig. 6).

Calibration solutions were made from NaCl in distilled/ deionized water (220, 180, 160, 140, 120, and 80 mEq/L). Calibration solutions were also prepared from KCl salt in concentrations of 10, 8, 6, 4 and 2 mEq/L and stored in the freezer. The osmolality, Na^+ and K^+ concentrations of calibration solutions were determined using the Multi-Osmette and flame photometer, respectively. These solutions were then used to calibrate the ISE. A calibration curve was generated (Fig. 7).

Serum, plasma, and whole blood concentrations of Na^+ and K^+ Blood samples were drawn from the arterial cannulae of previously cannulated rats and immediately divided into three portions. One portion was collected in a plain tube, allowed to clot, and spun down to yield serum. The two remaining portions were put in separate heparinized tubes. Lithium heparin was used to prevent changes in Na^+ concentration obtained with the use of sodium heparin (Shek, Swaminathan, 1985). One portion was used as whole blood while the other was centrifuged and plasma was separated from the formed elements. The Na^+ and K^+ concentrations of serum and plasma samples from the same rats were measured using both ISEs and the flame photometer (Table 1). For other samples, serum, plasma and whole blood were analyzed for Na^+ concentration using the ISE (Table 2).

Dilution test For this test, blood was collected in a heparinized tube from a cannulated rat. The Na^+ concentration of the whole blood was measured and then portions of the whole blood sample were diluted 1:1 with calibration solutions. The Na^+ concentrations of the diluted solutions were then calculated (predicted) and measured

using the ISE (Table 3). The predicted concentration was calculated as 0.5 x (whole blood concentration + concentration in calibration solution).

In vivo HSD (7.5% NaCl in 6% dextran 70) Calibration solutions were flushed through the ECSC for calibration prior to the in vivo measurements (Table 4). The ECSC (Fig. 4) was connected between the arterial and venous cannulae to shunt the flow of blood. Heparin (100iμ) was injected through the jugular cannula to prevent clot formation during the movement of blood through the ECSC. Hematocrit samples were obtained prior to and after the passage of blood through the lumen of the ECSC. Since the ECSC shunted the blood by the tips of the electrodes, these measurements should reflect the circulating ion concentrations. Readings were taken for 10 min, 2.0 mls of HSD were injected through the jugular cannula, and readings were taken for another 25 min (Table 5).

RESULTS

This study was planned to measure circulating concentrations of both Na^+ and K^+ ions using the ISEs. However, due to the continuous drift of the K^+-selective electrode, measurement of the K^+ concentration was abandoned, except for a comparison between the ISEs and the flame photometer (Table 1).

Standardization of the ISE was done using 0.1, 0.01, and 0.001 N solutions of NaCl (Fig. 6). Calibration of the electrodes was accomplished by using solutions containing 220-120 mEq/L of Na (Fig. 7).

The Na^+ and K^+ concentrations of the same samples of serum or plasma were determined first with the ISEs and then with the flame photometer (Table 1). There was no significant difference (paired "t" test) between the values from the flame photometer and the ISEs.

The Na^+ concentration (Table 2) of whole blood, serum and plasma from the same blood samples were measured. The whole blood values were found to be

significantly different from those of plasma (p=0.017) and serum (p=0.02); there was no significant difference between serum and plasma (p=0.999).

The Na^+ concentration of samples of whole blood was measured before and after dilution with calibration solutions of known Na^+ concentration (Table 3). A paired "t" test done on the measured versus the predicted concentrations indicated no significant difference between the measured and predicted values.

Prior to in vivo testing, calibration solutions were slowly flushed through the lumen of the ECSC (Table 4). No changes in values were noticed when the solutions flowed through the ECSC. During the first 10 min of in vivo measurement (Table 5), moderate drift in values of circulating ion were seen. However, after the HSD administration, there was an abrupt increase and subsequent gradual decline in concentration of Na^+.

Table 1. Comparison between flame photometer and ISE
values for Na$^+$ and K$^+$(mEq/L)

Method	Flame photometer		ISE	
Samples	Na$^+$	K$^+$	Na$^+$	K$^+$
Control	140	5.0	-	-
Serum	128	5.1	137	5.5
Serum	128	5.6	139	6.1
Serum	142	4.4	140	4.5
Plasma	135	4.5	140	4.7
Mean ± SD	133±6	4.9±0.5	139±1	5.2±0.6
Cal.sol.[a]	120	2.0	120	2.0
Cal.sol.[b]	220	10.0	219	10.0

a. 120 mEq Na$^+$, 2 mEq K$^+$
b. 220 mEq Na$^+$, 10 mEq K$^+$

Table 2. Serum, plasma and whole blood Na$^+$ using ISE

Test samples	Na$^+$ (mEq/L)		Mean
Serum	130	124	127
Plasma	129	125	127
Whole blood	81	91	86

Table 3. Na$^+$ dilution test results

Calibration sol.(mEq/L)	Diluted samples : ISE measured (calculated)$^+$				
220	162 (169)	169 (172)	187 (186)	177 (175)	179 (175)
180	142 (149)	-	-	-	-
160	131 (140)	142 (142)	-	145 (145)	146 (145)
120	107 (120)	130 (122)	-	-	-
80	-	96 (102)	115 (116)	103 (105)	102 (105)
Whole blood	119	125	153	130	130

* Diluted sample = 0.5 ml of whole blood mixed with 0.5 ml of cal. solution.
$^+$ Calculated value: 0.5 (whole blood mEq + Cal. sol. mEq).

Table 4. Ion concentration (mEq/L) of calibration solutions flushed through the ECSC

Exp. #	Cal.Sol (220)	Cal.Sol (180)	Cal.Sol (160)	Cal.Sol (140)	Cal.Sol (120)	Cal.Sol (80)
1	225	187	161	138	120	80
2	220	180	160	140	120	80
3	220	180	160	140	120	80
4	221	178	156	138	120	76
5	220	178	155	138	120	75
6	220	184	164	144	121	81
7	217	180	162	141	120	80
x±SD	220±2	181±3	160±3	140±2	120±1	79±2

Table 5. Result of <u>in vivo</u> measurement of Na^+ in rats treated with HSD

Drug		Pre HSD		POST HSD			
Time (min)		-10-5	-5-0	0-5	5-10	10-15	15-25
Exp.	1	121	127	148	148	145	-
	2	121	127	145	145	144	144
	3	136	142	151	151	148	147
	4	110	126	144	144	142	142
	5	128	131	143	140	139	139
	6	131	138	147	148	148	147
	7	133	143	155	153	154	155
	x±SD	126±9	133±7	148±4	147±4	146±5	146±6

All values are means within time frame.

Time 0 min = start of HSD administration.

10

DISCUSSION

It has been suggested that dilution of serum or plasma as required for flame photometry may change the active or "free" molal concentration of sodium and that ISE may give a more accurate value (Kissel, Sandifer et al., 1982; Külpmann, 1990). However, a comparison of Na^+ measurements in the same samples using an ISE and a flame photometer in this study revealed no significant differences (Table 1), and Na^+ values were as previously reported for the flame photometer (Lum, Gamboni, 1974). The lower Na^+ concentrations observed with whole blood (Table 2) are expected, because the volume of red blood cells is diluting the Na^+ concentration. Earlier measurement of Na^+ in whole blood involved removal of blood samples and lysing the cells prior to measurement (Bugyi, Magnier et al., 1969). An additional advantage of using ISEs for determinations in samples is that the sample is not consumed and could then be used for another assay.

The dilution studies (Table 3) resulted in no significant difference between measured and calculated Na^+ concentrations following dilution with solutions of known concentration. This demonstrates the linearity of measurements with ISEs over the range of potential physiological values.

Flushing calibration solutions through the ECSC (Table 4) demonstrated that the flow of the solutions by the electrode did not alter the values. This is consistent with earlier work (Andersen, Bie, 1990) indicating that the Na^+ concentration could be measured accurately by an ISE in a stream of urine flowing at 2.2 to 9.7 ml/min, and K^+ could be measured in venous effluent from an in situ muscle preparation (Hńik, Holas et al., 1976). Following baseline measurements in the in vivo studies (Table 5), a hypertonic solution was administered intervenously. The increase in Na^+ was consistent with that observed previously when blood samples were removed for analysis (Matthew, 1994; Walsh, Kramer, 1991).

Drift in the readings from the K^+ electrode never stabilized, but was continuous even in a static measurement with no flow; therefore, measurement with this electrode

was abandoned. Some drift was evident with the blood flowing by the Na^+ electrode, but the readings stabilized after 10 min of flow (Table 5). Alternative electrodes such as those suggested for use with automated analyzers (Telting-Diaz, Regan et al., 1990) may be evaluated for use in this system.

With some modification to the electrodes, the combination of ISEs and the ECSC described in this report could enable in vivo measurement of a variety of electrolytes to more closely link changes in ion concentration to other physiological parameters. This system could be used to determine the time course of changes in ion concentrations during the following: hyperthermia, dehydration, administration of various resuscitation fluids, and absorption, to determine rates following oral or various parenteral routes of fluid administration. Thus, this system allows in vivo measurement of electrolyte concentrations in real time with minimal loss of blood in a variety of experimental situations.

REFERENCES

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J.D. Probing chemical conditions in the interior of living cells. In: Molecular Biology of the Cell. (Chap. 4) Garland Publishing, Inc., New York, NY: 155-156, 1989.

Andersen, S.E. and Bie, P. Continuous servo-controlled replacement of urinary sodium loss in conscious dogs. Am J Physiol, 259: R313-R316, 1990.

Berman, H.G. and Hebert, N.C. Ion-selective electrodes. Adv Exp Med Bio, 50: 3-5, 1973.

Bugyi, H.I., Magnier, E., Joseph, W. and Frank G. A method for measurement of sodium and potassium in erythrocytes and whole blood. Clin Chem, 15: 712-719, 1969.

Handbook of Electrode Technology. Orion Research, Inc., Cambridge, MA P: 1-4; 1982[a].

Handbook of Electrode Technology. Orion Research, Inc., Cambridge, MA S: 1-6, 1982[b].

Hník, P., Holas, M., Krekule, I., et al. Work-induced potassium changes in skeletal muscle and effluent venous blood assessed by liquid ion-exchanger microelectrodes. Pflügers Arch, 362: 85-94, 1976.

Kelly, C.B., Hubbard, R.W. and Hamlet, M.P. A method for the chronic cannulation of the superior vena cava and the aortic arch in the rat using cannulas made of silicone elastomer rather than polyethylene. Natick, MA: U.S. Army Research Institute of Environmental Medicine, Technical Report T4-79, 1979.

Kissel, T.R., Sandifer, J.R. and Zumbulyadis, N. Sodium ion binding in human serum. Clin Chem, 28: 449-452, 1982.

Külpmann, W.R. Determination of sodium with ion-selective electrodes: a new method or a new quantity? J Clin Chem Clin Biochem, 28: 813-815, 1990.

Lum, G., and Gamboni, S.R. A comparison of serum versus heparinized plasma for routine chemistry tests. Am J Clin Pathol, 61: 108-113, 1974.

Matthew, C.B. Treatment of hyperthermia and dehydration with hypertonic saline in dextran. Shock, 2: 216-221, 1994.

Matthew, C.B., Durkot, M.J. and Patterson, D.R. Fluid shifts induced by the administration of 7.5% sodium chloride in dextran 70 (HSD) in dehydrated swine. Cir Shock, 41: 150-155, 1993.

13

Piascik, M.T., Sparks, M.S. and Pruitt, T.A. Effect of chlorethylclonidine on arterial blood pressure and heart rate in the conscious rat. J Pharmacol Exp Ther, 262: 901-906, 1992.

Shek, C.C. and Swaminathan, R. Errors due to heparin in the estimation of plasma sodium and potassium concentrations. Intensive Care Med, 11: 309-311, 1985.

Shibolet, S., Lancaster, M.C. and Danon, Y. Heat stroke: A review. Aviat Space Environ Med, 47: 280-301, 1976.

Telting-Diaz, M., Regan, F., Diamond, D. and Smyth, M.R. Comparison of a calixarene-based ion-selective electrode with two automated analyzers for the clinical determination of sodium in blood plasma. J Pharm Biomed Anal, 8: 695-700, 1990.

Veselý, J., Weiss, D. and Štulík, K. Ion-selective electrode measurements in biochemistry, biology and medicine. In: Analysis with ion-selective electrodes. (Chap. 3) Halsted Press, New York, NY: 119-123, 1978.

Walsh, J.C. and Kramer, G.C. Resuscitation of hypovolemic sheep with hypertonic saline/dextran: The role of dextran. Circ Shock, 34: 336-343, 1991.

Zeigler, D.W and Patel, K.P. Reduced renal responses to an acute saline load in obese Zucker rats. Am J Physiol, 261: R712-R718, 1991.
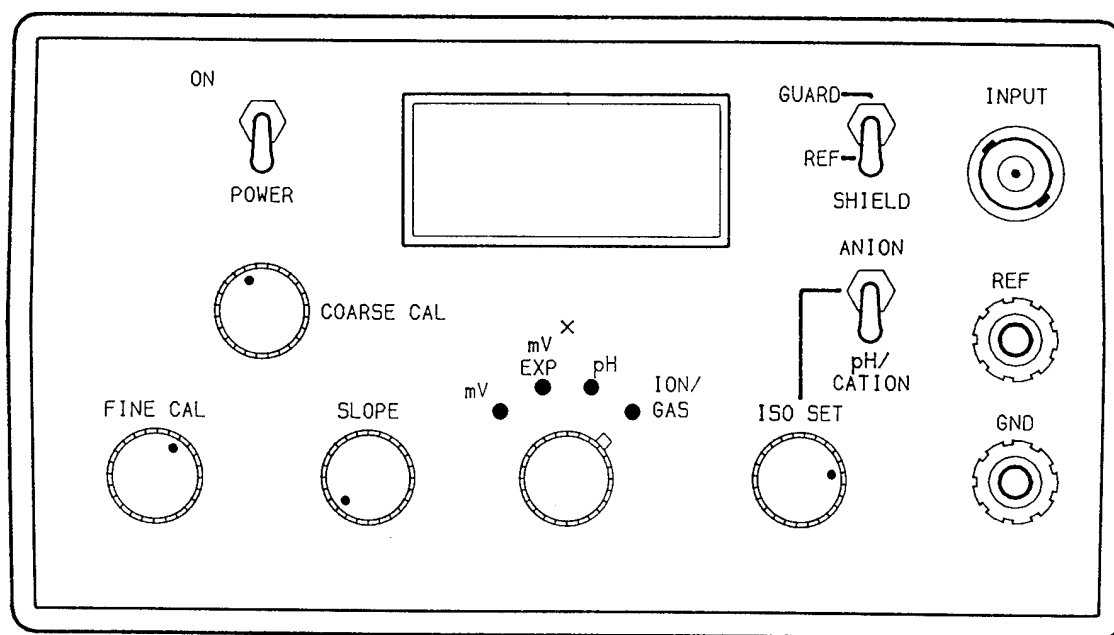
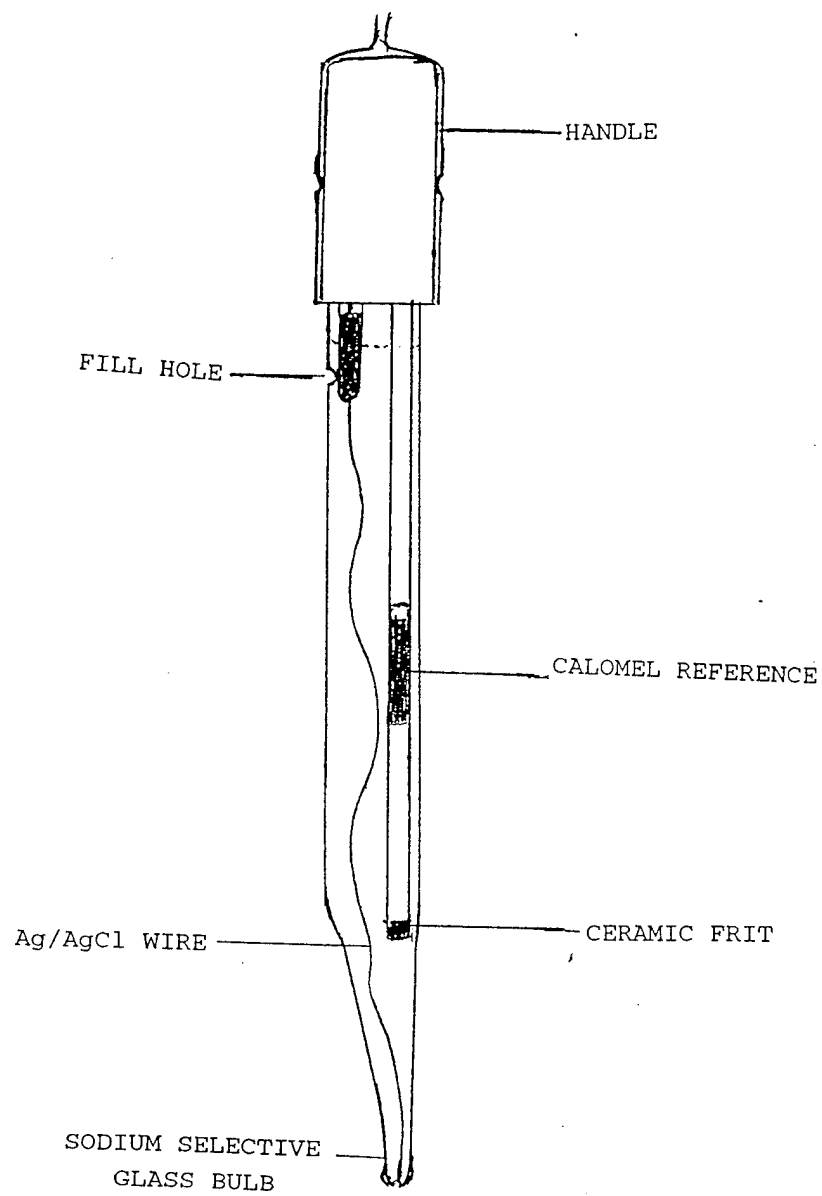14

Fig. 1   Electro-Chemical Analizer
Front Panel

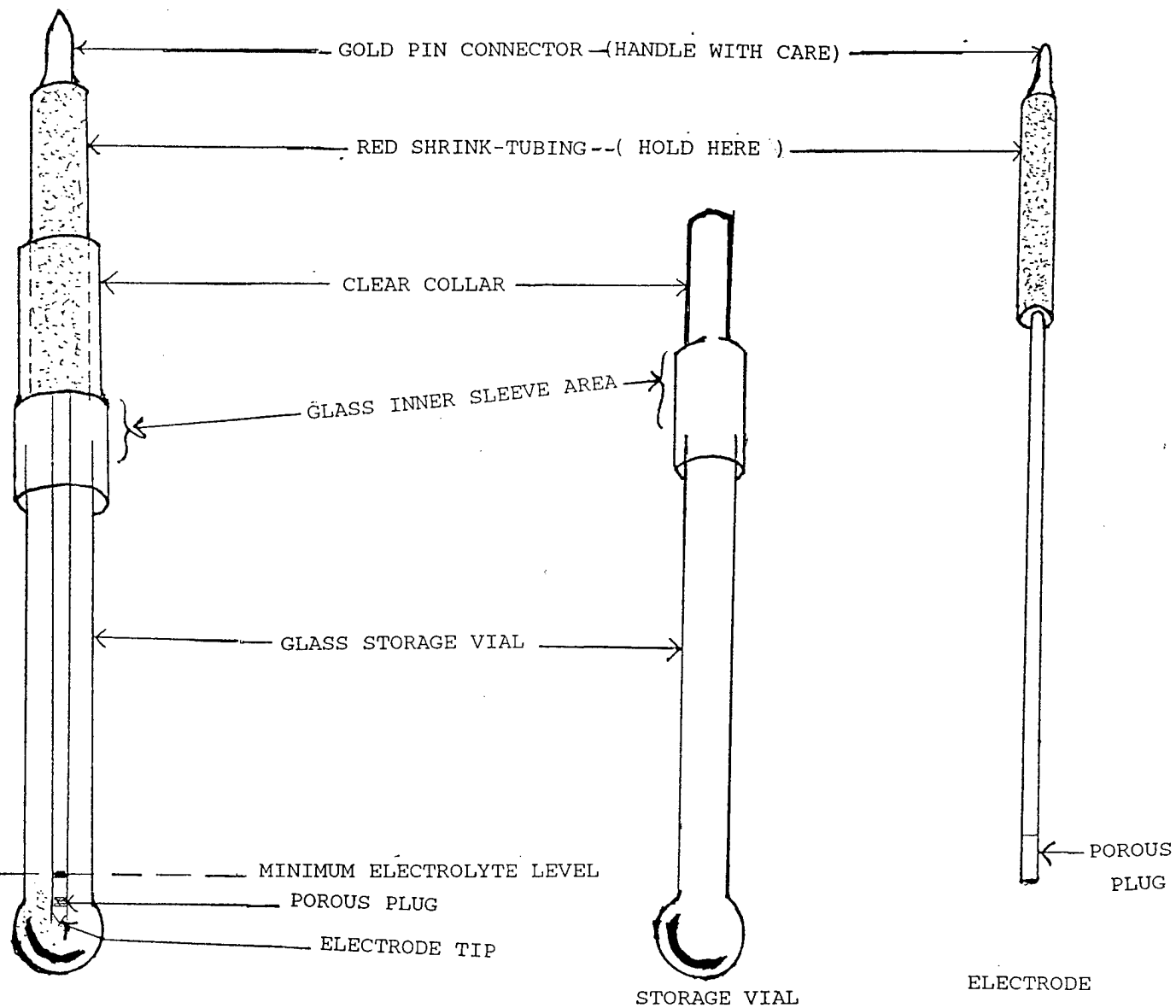Fig. 2   Glass combination electrode for sodium

GOLD PIN CONNECTOR —(HANDLE WITH CARE) ——

RED SHRINK-TUBING --(HOLD HERE) ——

CLEAR COLLAR ——

GLASS INNER SLEEVE AREA →

GLASS STORAGE VIAL ——

MINIMUM ELECTROLYTE LEVEL

POROUS PLUG

ELECTRODE TIP

POROUS
PLUG

STORAGE VIAL

ELECTRODE

Fig. 3   Potassium electrode

Acrylic Back

Electrode Support Clamp

Electrode Ports

Electrode Seal Clamp

Outlet

Inlet

Brass Plug w/hypodermic tube

Teflon Chamber Block
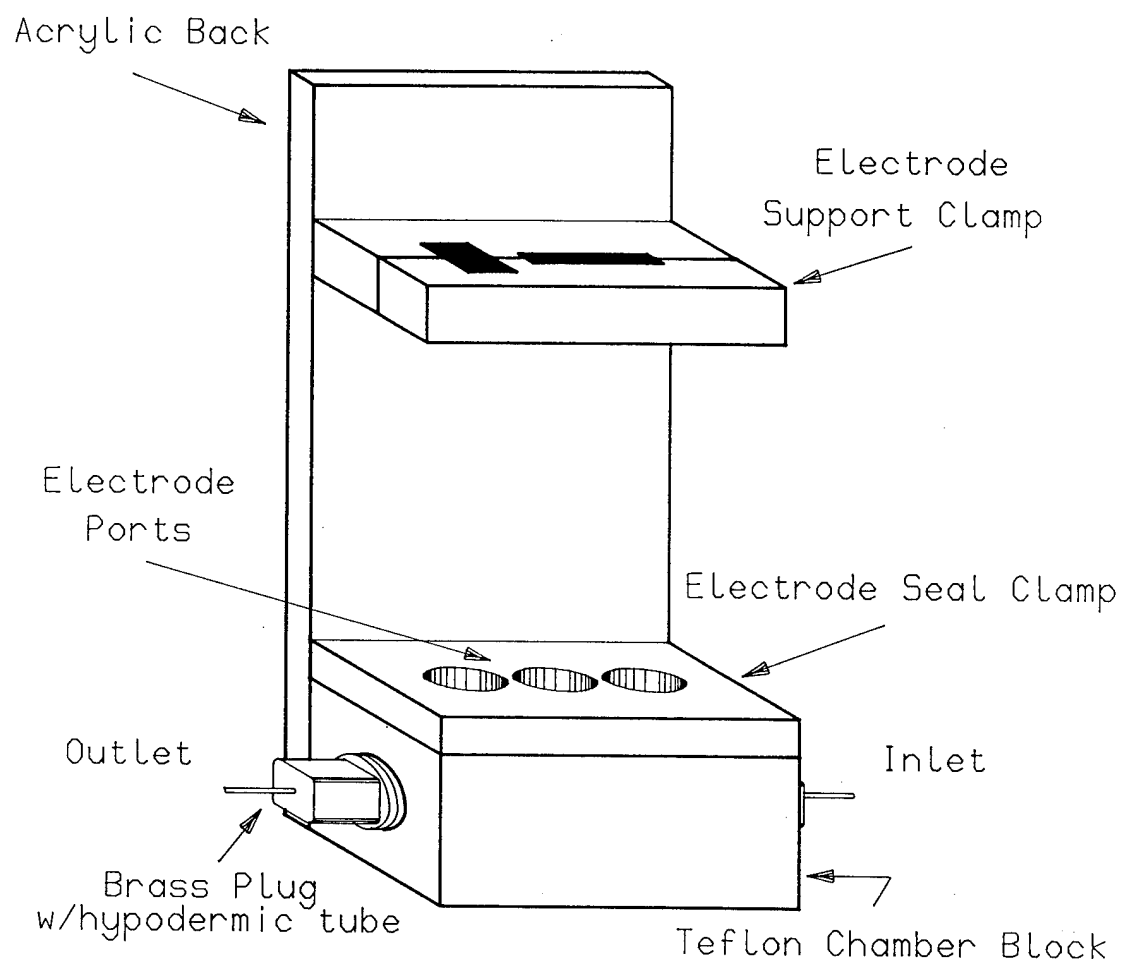
Fig. 4   Extra-Corporeal Blood Sample Chamber
Whole View

To Electronics

Electrode Clamp

Na⁺ Electrode

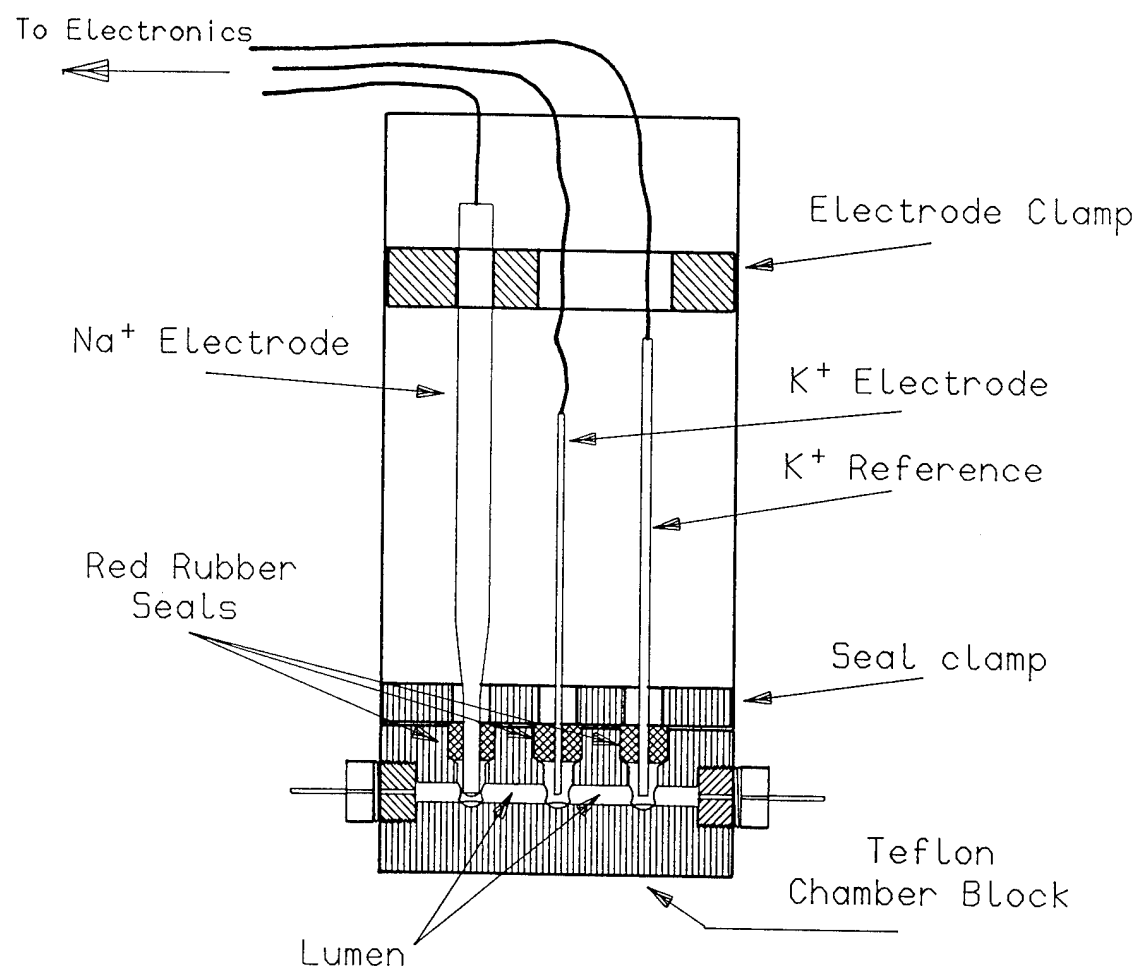K⁺ Electrode

K⁺ Reference

Red Rubber
Seals

Seal clamp

Teflon
Chamber Block

Lumen

Fig. 5 Extra-corporeal Blood Sample Chamber
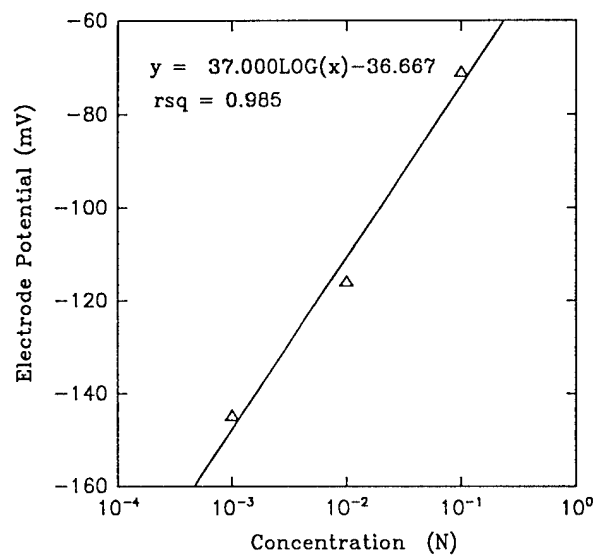Section view showing lumen and seals.

Fig. 6 Standard curve of NaCl concentration
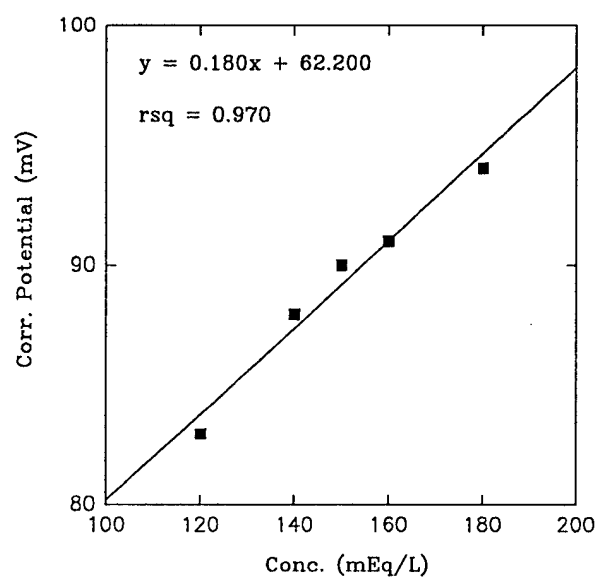vs. electrode potential.

Fig. 7   Calibration curve of calibration solution
concentration vs. electrode potential.