TABLE II. Micrograms Growth Hormone per Milligram Pituitary and per Gram Rat.

Age, days	No. rats	A μg growth hormone/mg pituitary	B mg pituitary	C Body wt, g	$\frac{A \times B}{C}$ μg growth hormone/g rat
10	20	79.8	1.39	24.10	4.60
42	19	127.0	4.78	99.00	6.13
"	"	165.0	4.78	99.00	7.97
56	15	94.0	5.96	119.66	4.68
70	20	99.9	7.79	143.00	5.44
"	"	116.0	7.79	143.00	6.32
168	16	44.6	20.56	326.81	2.81
308	15	77.6	19.38	351.60	4.28
378	33	96.1	20.28	358.10	5.44
630	15	102.0	25.06	427.80	5.97

1. Armstrong, D. T., Hansel, W., *J. Anim. Sci.*, 1956, v15, 640.

2. Baker, B., Hollandbeck, R., Norton, N. W., *ibid.*, 1956, v15, 407.

3. Contopoulos, A. N., Simpson, M. E., *Fed. Proc.*, 1957, v16, 24.

4. Evans, H. M., Simpson, M. E., Marx, W., Kibrick, E. A., *Endocrinology*, 1943, v32, 13.

Received September 5, 1958. P.S.E.B.M., 1958, v99.

Use of Glass Electrode for Measuring Sodium in Biological Systems.*† (24480)

SYDNEY M. FRIEDMAN, JAMES D. JAMIESON, J. A. M. HINKE AND CONSTANCE L. FRIEDMAN

Dept. of Anatomy, University of British Columbia, Vancouver, Canada

In seeking to trace the meaning of the relationship between sodium and blood pressure we have been hampered by difficulties inherent in estimation of small quantities of this cation. While it is true that sodium movements regularly accompany changes in blood pressure(1) the magnitude of the shifts lies just on the borderline of what can be determined without resort to statistical analysis of small differences. Similarly, while we have shown that pitressin has extrarenal actions on sodium, we were forced to demonstrate these with relatively large doses in order to produce effects measurable by existing methods (2). A further major problem in these studies has been the fact that the time course of ion shifts can only be crudely estimated by blood sampling procedures. Recently, Eisenman, Rudin and Casby(3) reported on preparation of cation sensitive glasses and indicated their potential usefulness as electrodes for biological studies. The important implications of this work led us to explore, with the cooperation of Dr. Eisenman, the technical problem of adapting the electrodes for use in biological systems. The present report concerns only the sodium electrode.

Methods. Amplifying and recording circuitry. The basic electronic problem involves suitable high gain amplification from a high impedance source. Since a change of 1 meq/l concentration from a base of 150 meq/l should ideally yield a potential change of approximately 160 microvolts it is evident that the drift and noise characteristics of the instrumentation are the limiting factors in sensitivity. The Cary vibrating reed electrometer is a very satisfactory basic unit to meet these requirements, having a limiting sensitivity of about 20 microvolts and a very low drift rate.

* This work was carried out with the aid of a grant from the Muscular Dystrophy Assn. of Canada.

† We are grateful to Mr. John Lees for his skilled help in manipulating the glass.

The output of the electrometer is read directly on its panel meter. Additionally, it is fed into a direct writing oscillograph. A calibrated bucking potential is applied between electrode and amplifier so that net input potential can be limited to the most sensitive ranges of the instrument. In practice, we use a Grass Model 5 Polygraph as recording unit, receiving the electrometer output through a low-level DC preamplifier. The balance voltage circuit of the latter unit is externalized to serve conveniently as the source of the bucking potential. *Glass electrodes.* NAS_{11-18} glass, generously supplied by Eisenman, Rudin and Casby, was used throughout for fabrication of electrodes. In all cases, a standard calomel reference electrode was used. *A. Haber bulb electrode.* Measurements were made using Haber type bulbs filled with 0.1 M NaCl and containing a central Ag/AgCl lead according to the procedure of Eisenman *et al.* These proved suitable for estimation of large differences of Na concentration where relatively low amplifications are required. Fine measurements were not possible with this arrangement since solutions cannot be changed without considerable electrical interference. The bulb was then incorporated into a cannula into which solutions were fed from burettes at a distance. The cannula itself was shielded by a grounded box. This arrangement permitted work at high ranges of the amplifier and several versions of the assembly were tested. We were able to confirm the fact that the sodium sensitive glass responds only to Na^+ within the usual biological ranges for H^+ and K^+ background. We further observed that the response remains linear at high amplifications over small changes in Na concentration. We were, however, unable to provide any long-lived insulation for the stems of the bulbs despite trials with a wide variety of materials. This meant that we were continually plagued with fabrication of new electrodes until one of us (J. A. M. H.) simplified the problem by constructing the cannula itself of NAS_{11-18} glass. *B. Glass-to-silver electrode.* A glass-to-silver electrode in tubular form is shown in Fig. 1. NAS_{11-18} glass is blown into a thin-walled fusiform bubble which is silvered on the outside by the Rochelle method. The silver in turn is heavily copper plated with a lead wire incorporated directly into the copper deposit. Polyethylene tubing is attached to both ends, the outside of the assembly is heavily coated with a non-hardening bakelite for insulation, then housed in a plastic box for protection. The lead wire is connected to a screw terminal since hot solder connections cannot be used. These glass-to-silver electrodes have proved rugged and long-lasting. For unknown reasons they have not yielded the ideal 58 mv slope but have settled at approximately 48 mv with remarkable day to day stability. The results described below have all been obtained with the tubular glass-to-silver electrode, measuring 0.5 x 2.0 cm, although the size does not appear to be critical.

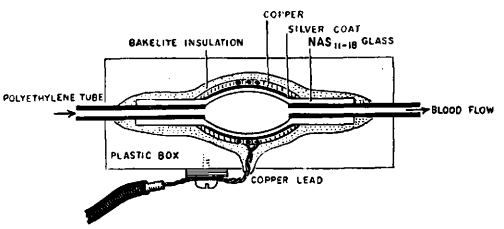

FIG. 1. Diagrammatic section through a tubular type of glass-to-silver sodium electrode. Measurements are approximately 0.5 × 2.0 cm.

Results. In vitro. The potentials developed by the electrode in static and flowing unbuffered systems differ by a constant (Fig. 2) which is itself a function of the rate of flow. The constant for a flow rate of 45 ml/min. compared with zero flow is almost 1 mv. Although this is a rather extreme situation, it is evident that rate of flow must be evaluated in any attempt to measure small changes in Na concentration.

Rate of change of flow, or acceleration, is another factor which may cloud the interpretation of electrode potentials in unbuffered systems. This factor can be demonstrated easily in start-stop experiments where an overshoot occurs on starting the flow through the tube; height of the overshoot can be reduced almost to the vanishing point by reducing the rate at which flow starts.

Changes in flow rate are less important with buffered solutions. Thus, with Na phosphate at pH 7.3, changing the flow rate from zero to 4, 6, 8, 10, 15 or 24 ml/min. gave a po-

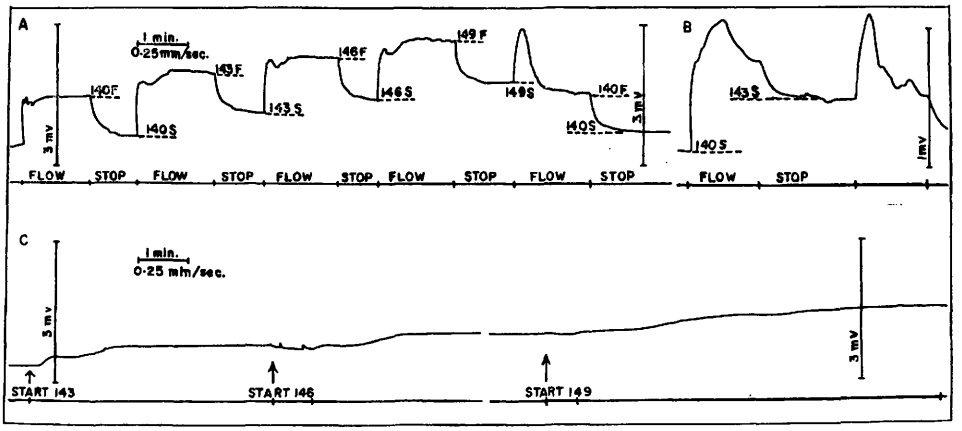

FIG. 2. Effect of altering Na (acetate) concentration in steps of 3 meq/l, between 140 and 149 meq/l, on sodium electrode potential. *A*. Potentials under static (S) and flowing (F) conditions. Flow rate is standard at 45 ml/min. *B*. Potential difference for a 3 meq/l step in concentration, amplified 3-fold in comparison with tracing in *A*. *C*. Electrode potentials recorded for 3 meq/l steps under continuous flow conditions. The increment for each step is the same as in *A*.

tential change of 50 to 100 microvolts while no change occurred once the system was flowing when the rate was changed from 4 to 18 ml/min.

Within the limitations noted above, fractional changes in Na concentration can be measured reproducibly. Thus, without serious attempt to reduce noise, changes of 50 microvolts, *i.e.*, of 0.3 meq/l out of 150 meq/l of Na can readily be distinguished. This sensitivity is considerably better than the 1% limit of the most careful flame determination and it is evidently possible to reduce this towards the 20 microvolt sensitivity limit of the instrument. A big advantage of the electrode is its ability to follow cyclic changes reproducibly without noticeable lag. Drift is ordinarily negligible for short term measurements and is easily compensated in longer procedures. The electrode can be accidentally polarized by the potential from the bucking source and this must be carefully avoided in switching. A stabilization period of at least 15 minutes is required after any interruption of the circuit.

In vivo. For this study, adult female rabbits, anesthetized with barbitone-urethane and fully heparinized to eliminate the possibility of clotting in the cannula, were used. The aim was to determine whether the electrode was able to follow cyclic changes in Na concentration in blood. The tube electrode was implanted as a by pass into the femoral artery. Blood pressure was recorded simultaneously from the opposite femoral artery using a Statham P23AA transducer. Infusions were given through polyethylene tubing implanted in the femoral vein. The calomel reference electrode was firmly anchored into the wound ooze.

The ability of the electrode to follow cyclic changes of Na concentration in whole blood was clearly demonstrated by infusion of measured amounts of Na as acetate or phosphate. Electrode response is a direct function of the amount of Na infused (Fig. 3). With each infusion there was a brief latent period, apparently corresponding to circulation time, followed by a rapid increase in electrode potential reflecting the initial small volume of distribution of the infused Na, and a gradual subsidence again as the Na became uniformly distributed. With each injection base line Na concentration rose in proportion to amount administered. Thus, the electrode is measuring Na concentration or, more accurately, activity.

In previous studies we have shown that Na concentration in the blood is related to the blood pressure. The electrode should follow these changes more faithfully than the crude sampling method originally used to demonstrate this relation. In Fig. 4 the typical coordinated response of blood pressure and Na

level to acute injection of norepinephrine is shown. Not only are the changes in Na concentration grossly similar to those shown chemically but they are here followed with a detail not previously possible.

Discussion. The NAS_{11-18} glass developed by Eisenman, Rudin and Casby responds only to sodium at a pH greater than 4 and in presence of a K^+ concentration less than 30 meq/l. This means that if high amplification is possible, this glass can be used for specific measurement of Na in ordinary biological systems. The present report has demonstrated that such amplification is feasible and that glass-to-silver electrodes are particularly suitable for this purpose. We have used the electrode in the form of a tube or cannula but evidently other arrangements are equally possible. Thus, the tube could be closed at one end and silvered either on the inside to form an immersion tip or on the outside to form a containing tube. Enlargement of the cannula to a bath in which muscle strips might be studied, or its contraction to microelectrodes should be equally feasible.

Thus far, we have been concerned only with ability of the electrode to measure relative changes in sodium activity. Additional problems require solution if the electrode is to be used for measurement of absolute quantities of sodium involved in such dynamic changes. Such studies are underway. Further, the usefulness and range of the glass-to-silver sodium electrode would be enhanced if simultaneous measurements of potassium could also be made. Preliminary studies indicate that the K^+ electrode may also be applicable to biological work.

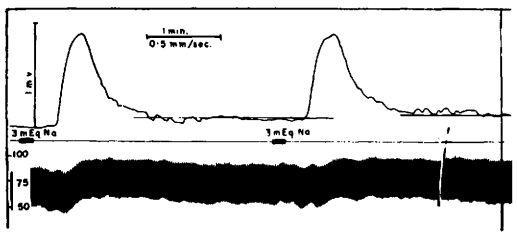

FIG. 3. Blood pressure (lower trace) and sodium electrode potential recorded simultaneously from femoral artery of a female rabbit of 3.5 kg. Sodium acetate (3 meq in 0.75 ml) was inj. rapidly as indicated by the bar. (Electrode potential, 48 mv/log unit.)

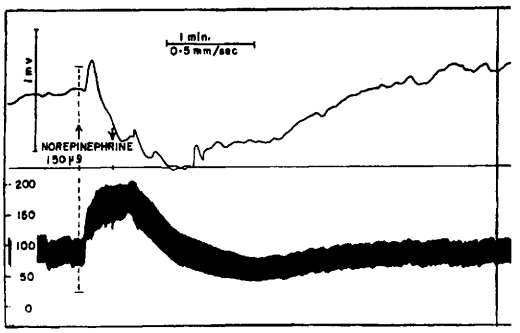

FIG. 4. Blood pressure and sodium electrode potential recorded simultaneously from femoral artery of a female rabbit of 3.5 kg. In the interval indicated by the arrows, 150 μg of norepinephrine (bitartrate) was inj. intrav.

Summary. Development of a sodium glass-to-silver electrode for biological use has been described. The instrument is simple and particularly useful for continuous recording of small changes in sodium concentration, both in static and in flowing systems. As such, it should be applicable to a wide variety of studies.

1. Friedman, S. M., Butt, R. M., Friedman, C. L., *Am. J. Physiol.*, 1957, v190, 507.

2. Friedman, S. M., Friedman, C. L., Nakashima, M., *Circulation Research,* 1957, v5, 261.

3. Eisenman, G., Rudin, D. O., Casby, J. U., *Science,* 1957, v126, 831.

Received September 11, 1958. P.S.E.B.M., 1958, v99.