NAMI die von uns präparativ durchgeführte Ablösung der ribosomalen Ferritinfraktion, die nach den Gradientenbildern¹ im Mittel mit 70 S sedimentiert, 10 Jahre früher erstmals im analytischen Maßstab gelungen ist.

Die für die Nucleoprotein-Komponente mit 49 S bestimmte Sedimentations-Konstante²¹ entspricht der Ferritin-freien größeren ribosomalen Untereinheit, von uns mit 47 S angegeben¹, während die nach Ferritinablösung in kleinere Partikel von 8 S¹

zerfallende kleine ribosomale Untereinheit von TAKANAMI unbeachtet blieb, obwohl die Aufnahme seines Sedimentationslaufes²¹ deutlich eine startnahe, jedoch nicht ausgemessene Bande erkennen läßt.

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Potential Transducing Properties of Microelectrodes Filled with Electrolyte or Metal

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Glasmikropipetten mit Spitzendurchmessern von $2,5\ \mu\text{m}$, d. h. eine Größenordnung größer als die gewöhnlichen electrophysiologischen Elektroden, werden dazu benutzt, in $0,1\ \text{M}$ KCl die Potential-Übertragungseigenschaften (*PTP*) für Sinus- und Stufenpotential von Elektroden zu vergleichen, die gefüllt sind a) mit $0,1\ \text{M}$ KCl (EM) und b) mit Metall (MM). Die *PTP* sind abhängig von drei Elektrodenparametern, R_s , R , C und von der Verstärkerstreukapazität C_a . Die Parameter werden durch Messung des bei Anlegen einer Dreiecksspannung entstehenden Strom-Zeitverlaufs bestimmt, Fig. 3. R_s , der Lösungs-Serienwiderstand, ist vor der Elektroden Spitze lokalisiert, Fig. 1, table 1. R_s bestimmt zusammen mit C_a die obere Grenzfrequenz der Messung, eqs. (11) und (12). Der Elektrodenwiderstand R (table 2) und die Elektrodenkapazität C sind bei EM kleiner als bei MM. Das führt — bei unkompensiertem C_a — zu komplementären *PTP* von EM und MM: EM sind besser für Niederfrequenzmessungen ($\omega RC \leq 1$, d. h. 0–10 KHz oder lange Stufendauer $t/RC \geq 1$), während MM — wegen größerem C — besser verwendbar sind zur Hochfrequenzmessung ($\omega RC \geq 1$, $f \geq 10\ \text{KHz}$ oder kurze Stufendauer $t/RC \leq 1$), Figs. 4 und 5. Der Spannungsmeßverstärker, Fig. 2, gestattet elektronische Kompensation von C_a . Die resultierende Verbesserung der *PTP* beträgt zwei Größenordnungen, Figs. 4, 5 und 6. MM mit Ag/AgCl- oder Sb-Füllung können zur Messung von pCl bzw. pH in anorganischen Elektrolyten verwendet werden, Fig. 7. Die unvermeidlichen Probleme von MM (sehr kritisch für MM kleiner als die Modellelektroden) werden im Appendix behandelt.

Of eminent importance for electrophysiology is the accurate measurement of *ac* and *dc membrane potential changes*. For analytical purposes, the measurement of ion specific ($\text{H}^\oplus$, $\text{Na}^\oplus$, $\text{Cl}^\oplus$ etc.) *dc electrode potentials*¹ is needed. HODGKIN and HUXLEY² carried out the precise electrical characterization of the Loligo giant axon (diameter $\cong 1\ \text{mm}$). They showed that in this nerve potential changes occur which last only fractions of a millisecond. It is also known that cells smaller than the

Loligo axon undergo similar potential changes³. By using very small microelectrodes ($\leq 0,5\ \mu\text{m}$ tips) for the investigation of such small cells one inevitably falsifies the result more than with measurements in a macroscopic scale. Attempts to control the disturbances by microelectrodes have been made by studying the electrical properties of two types of microelectrodes a) electrolyte and b) metal filled (FRANK and BECKER⁴ and LETTVIN et al.⁵). The potential transducing properties of the two types of electrodes

¹ P. C. CALDWELL, J. Physiology **142**, 22 [1958].

² A. L. HODKIN and A. F. HUXLEY, J. Physiology **116**, 449 [1952].

³ J. C. ECCLES, The Physiology of Synapses, Springer-Verlag, Berlin-Göttingen-Heidelberg 1964.

⁴ K. FRANK and M. BECKER, Physical Techniques in Biological Research, Vol. V, Part A, Editor W. L. NASTUK, Academic Press, New York-London 1964.

⁵ R. C. GESTELAND, B. HOWLAND, J. Y. LETTVIN, and W. H. PITTS, Proc. I.R.E. **47** (11), 1856 [1959].

are, however, difficult to compare because the investigated electrodes had different shapes and insulation.

This paper has the aim to present a manufacture of reproducible model electrodes which differ only in their electrolytic or metallic filling. The potential transducing properties are to be compared. With the results one should be able to extrapolate potential transducing properties of electrodes which differ in shape and nature from the used model electrodes.

Characterization of electrode parameters

The electrical analog for intracellular potential measurements with electrolyte filled micropipettes (EM) and metal filled micropipettes (MM) is seen in Fig. 1. The input impedance of the amplifier is represented by the resistance R_a and the capacitance C_a , respectively.

Fig. 1. Schematic drawing of an electrical equivalent circuit of micropipettes filled with electrolyte (EM) or metal (MM) which are impaled into a biological cell, symbolized by the dashed lines. The input impedance of the amplifier is represented by R_a and C_a . The impedance of the counter electrode outside the cell is assumed to be zero and is therefore omitted in the figure. For further details see text.

The *resistive components* of the electrode impedance are for both types of electrodes represented by two resistances: a) R_s is located outside of the tip and is due to the concentration of electrical field lines in the immediate vicinity of the tip. For both types of electrodes R_s is assumed to be the same. Its value is obtained by integrating the resistance of a sphere of electrolyte with the resistivity ρ [Vcm/A]

of the surrounding fluid from the radius r of the tip of the opening to $r = \infty$:

$$R_s = \frac{\rho}{4\pi r s} \text{ [V/A]}. \quad (1)$$

The factor s corrects for the screening which is exerted by the glass insulation, $0 < s < 1$. b) The resistive components which are located within the tip. For EM R_e is calculated by integrating the resistance of a cone filled with an electrolyte of the resistivity ρ_t from the radius r of the opening to $r = \infty$.

$$R_e = \frac{\rho_t}{\pi r \tan \alpha} \text{ [V/A]}, \quad (2)$$

where α is the angle between the axis and the mantle. Eq. (2) is only valid for electrodes with not too small tip diameters. Below a critical radius, which depends on the glass type and the electrolyte concentration the electrode rectifies and the electrode resistance becomes a function of the surface charge of the glass^{6,7}.

The electrodes used in electrophysiological experiments are usually filled with 3 M KCl. The specific resistivity of 3 M KCl can, however, not be used to calculate R_e since mixing of 3 M KCl and cell fluid takes place. If diffusion is rate controlling the initial transient mixing of electrolyte requires

$$\tau_{\text{diff}} \approx \frac{r^2}{D} \text{ [sec]}^8. \quad (3)$$

Where D [cm²/sec] is the average diffusion constant. For the model electrodes $r = 1.25 \mu\text{m}$ and with the usual value of $D = 10^{-5} \text{ cm}^2/\text{sec}$ τ_{diff} is calculated to 1.5 msec; for the usual fine tips ($\phi \cong 0.2 \mu\text{m}$) $\tau_{\text{diff}} \cong 10 \mu\text{sec}$.

The order of magnitude of the contaminating *steady state diffusion current* i_0 can be estimated by using the following relation:

$$i_0 = \frac{\Phi}{R_e} \text{ [A]}, \quad (4)$$

where $\Phi = RT/F$ [V] is the thermal voltage equivalent (at room temperature = 0.025 V) with R [Vasec/°K equiv] the gas constant, T [°K] the temperature, and F [Asec/quiv] the Faraday constant. In eq. (4) R_e is the resistance of the electrode after allowance of equilibration (i.e. the tip is filled with a mixture of outside and inside electrolyte).

For MM R_{dl} is the resistance of the interphase metal-electrolyte. The value of R_{dl} is inversely proportional to the surface area. For a plain circular

⁶ C. C. KRISCHER, Z. Naturforschg. **24b**, 151 [1969].

⁷ LAVALLÉE, SCHANNE, and HÉBERT, Glass Microelectrodes, J. Wiley & Sons, New York-London-Sydney-Toronto 1969.

⁸ J. C. JAEGER, Studies in Physiology, Editor: D. R. CURTIS and A. K. MCINTYRE, Springer-Verlag, Berlin-Heidelberg-New York 1965.

surface

$$R_{dl} = \frac{R_{dl}^*}{\pi r^2} [V/A], \quad (5)$$

where R [Vcm²/A] is the specific interphase resistance.

R_{dl} is the resistance equivalent of reversible or irreversible faradaic reactions which occur at the interphase metal-electrolyte. This reaction corresponds to the mixing in the tip of EM. Pioneering works in correlating the heterogeneous reaction kinetics with the electrical characteristics of electrodes are of WAGNER and TRAUD⁹, the Bonhoeffer school (see VETTER¹⁰), DELAHAY¹¹ and others. For a reversible reaction the rate, resistance and concentration c of reacting species, are correlated by the following expression^{10, 11}

$$i_0 = \frac{\Phi}{R_{dl}} = \pi r^2 \cdot k \cdot c \cdot F [A]. \quad (6)$$

Many values for the rate constant k [cm/sec] or $i_0/\pi r^2$, the exchange current density¹⁰ can be obtained from electrochemical tables^{10, 11}. By inserting these values into eq. (6) one can calculate the interphase resistance or the flux.

The resistance R_{dl} is, however, not truly ohmic in character. For electrode polarizations exceeding 25 mV R_{dl} usually changes. An exponential decrease of R_{dl} with increasing polarization is observed for reversible (and many irreversible) reactions¹⁰. For some electrodes such as Germanium¹² or metals covered with insoluble oxides i. e. Ta¹³ or chlorides, Ag/AgCl (own observations) R_{dl} increases with positive polarization and decreases on current reversal. Such rectifying current voltage relations are also typical for solid state P-N junctions (diodes)¹⁴.

The ratio of the resistances of EM and MM with equal radii is with eqs. (2) and (5) given by:

$$\frac{R_e}{R_{dl}} = \frac{\varrho_f}{\varrho R_{dl}^* \tan \alpha} r \equiv \frac{r}{\varepsilon}. \quad (7)$$

The constant $\varepsilon = \varrho R_{dl}^* \tan \alpha / \varrho_f$ has the dimension of a length. If this length constant exceeds r the resistance R_e is smaller than R_{dl} . Inserting usual values of ϱR_{dl}^* , ϱ and $\tan \alpha$ in eq. (7) one obtains for ε values between 5 μ m and 1 cm.

The capacitive components C of the electrode impedances arise at the phase boundaries which separate the conducting phases of the filling and the surrounding fluid. One capacity, C_{gl} , fig. 1, is due

to the glass mantle of the electrode. If differences in the fillings of EM and MM are neglected and if the thickness of the glass wall is assumed to increase linearly with increasing axial distance l , C_{gl} is for both types given by:

$$C_{gl} = C_{gl}^* \cdot l [\text{Asec/V}], \quad (8)$$

where C_{gl}^* [Asec/Vcm] is a constant. Observed values for C_{gl} range between 4 and 10 pF/cm^{15, 4}.

MM have in parallel to C_{gl} the capacitor C_{dl} of the interphase metal-electrolyte, fig. 1:

$$C_{dl} = C_{dl}^* \cdot \pi \cdot r^2 [\text{Asec/V}], \quad (9)$$

where C_{dl}^* [Asec/Vcm²] is the double layer capacitor referred to unit area. In the region of small polarizations C_{dl}^* has a constant value of 20–100 μ F/cm²^{16, 10}. It is seen from eqs. (8) and (9) that MM have always a larger capacitor than EM. The ratio is:

$$\frac{C(\text{EM})}{C(\text{MM})} = \frac{C_{gl}}{C_{gl} + C_{dl}}. \quad (10)$$

The potential transducing properties of EM and MM to sine wave potentials arising at E in fig. 1 are described by:

$$\frac{E_{out}}{E} = \sqrt{\frac{1 + \omega^2 (RC)^2}{(1 - \omega^2 R_s C_a RC)^2 + \omega^2 (R_s C_a + RC + RC_a)^2}}, \quad (11)$$

where $\omega = 2\pi f$; f = frequency (Hz); R is R_e for EM and R_{dl} for MM; C is C_{gl} for EM and $(C_{gl} + C_{dl})$ for MM. In the classical derivation of eq (11) it was assumed that the cell membrane is a potential source of zero internal impedance and $R_a \gg R$ i. e. R_a was neglected.

For potential steps arising at E , fig. 1 the response is given by:

$$\frac{E_{out}}{E} = 1 - \gamma \exp\left(-\frac{t}{\gamma R_s C_a}\right) + (\gamma - 1) \exp\left(-\frac{t}{R \Sigma C}\right), \quad (12)$$

where $\Sigma C = C_{gl} + C_a$ for EM and $\Sigma C = C_{gl} + C_{dl} + C_a$ for MM and $\gamma = C_{gl}/\Sigma C$ for EM and $\gamma = (C_{gl} + C_{dl})/\Sigma C$ for MM.

⁹ C. WAGNER and W. TRAUD, Z. Elektrochem. angew. physik. Chem. **44**, 391 [1938].

¹⁰ K. J. VETTER, Elektrochemische Kinetik, Springer-Verlag, Berlin-Göttingen-Heidelberg 1961.

¹¹ P. DELAHAY, New Instrumental Methods in Electrochemistry, Interscience, New York 1954.

¹² H. GERISCHER, in: Advances in Electrochemistry and Electrochemical Engineering, P. DELAHAY, Interscience, New York 1962.

¹³ A. GÜNTHERSCHULZE and H. BETZ, Z. Elektrochem. angew. physik. Chem. **37**, 726 [1931].

¹⁴ W. SHOCKLEY, Electrons and Holes in Semiconductors, van Nostrand Co., New York 1950.

¹⁵ G. FALK and P. FATT, Proc. Roy. Soc. **160**, 69 [1964].

¹⁶ D. C. GRAHAME, Chem. Rev. **41**, 441 [1947].

Eq. (12) has been derived with the simplifying assumption that the time course of E_{out} is determined by the sum of two exponentials, one containing the time constant $\gamma R_s C_a$ and reaching the potential level $E_{\text{out}}/E = \gamma$, and another exponential containing the time constant $R \cdot \Sigma C$ which leads to the final output potential $E_{\text{out}}/E = 1$. R_a was neglected.

Steady state *dc* potentials of both types of electrodes have to be taken into account for *dc* potential measurements. The fluxes of eqs. (4) and (6) cause for EM, eq. (4), a potential difference due to different concentrations and mobilities of the ions outside and in the interior of the electrode. In case of very narrow openings⁷ with rectifying properties⁶ the ionic double layer glass electrolyte can lead to ion specificity¹⁷. For MM, eq. (6), the flux at the interphase metal-electrolyte is potential determining (i. e. redox reactions). Coverings with sparingly soluble chlorides or oxides may lead to ion specificity in regard to $\text{Cl}^\ominus$ or $\text{H}^\ominus$, respectively. For both, EM and MM, the potential usually is proportional to $n \Phi \log c$ where c is the concentration of the potential determining ion and n a factor which commonly has values between 0 and 2.

Methods

Electrode preparation was done for EM and MM by breaking — prior to filling — the tips under the microscope to an outer diameter of $\cong 2,5 \mu\text{m}$; EM were then filled by the action of capillary forces¹⁸ and MM by a method similar to the one described by LETTVIN⁵: Molten woods metal was forced into the tip by the pressure of hot CH_2Cl_2 vapor which was sealed into the glass tube behind the zone of liquid metal. The metal in the outmost tip was then etched away chemically for a few μm and refilled electrolytically by silver or antimony. Care had to be taken not to exceed current densities of 20 mA/cm^2 .

For the measurement of electrode parameters a triangle voltage which changes linearly with time was employed. For ohmic components (i. e. R_s and R_e) the current i is proportional to the potential, i. e. $(i)_t = (E)_t/R$. For capacitive components (i. e. C_{gl} and $C_{gl} + C_{dl}$) the current i_c is proportional to the first derivative of the potential with respect to the time, i. e. $i_c = C_{gl}(dE/dt)$. For details see fig. 3. To avoid *dc* polarization of MM during the capacity measurements the triangle voltage was delivered via a $0,01 \mu\text{F}$ condenser to an Ag/AgCl wire as counter electrode. The latter was contained in a grounded metal box which had a

5 mm hole for the insertion of the microelectrode. This provision suppressed unwanted potential pickup. The *dc* resistance of MM was measured by taking the slope of the *dc* current voltage curves at small polarizations ($\leq 25 \text{ mV}$)¹⁰. The current was measured with a current to voltage amplifier based on an operational amplifier P25A^{18,19}. In this current measurement no additional current monitoring resistor is introduced in series with the electrode resistance.

The potential transducing properties of EM and MM were measured with an operational amplifier in the configuration shown in fig. 2. C_f serves to compensate the stray capacity C_a at the input, fig. 1. C_a is compensated if $(A-1) C_f = C_a$ where A is the gain of the amplifier⁴. In fig. 2 the gain $A = 1,5$. For most ac measurements a commercial amplifier Philbrick type P25A¹⁹ was employed. Its approximate input characteristics are: $R_a = 10^{11} \text{ Ohm}$, $C_a = 8 \text{ pF}$ and the offset current $i_{\text{off}} = 10^{-11} \text{ A}$. For *dc* measurements with MM a Philbrick module P2AU with offset current $\leq 10^{-12} \text{ A}$ was employed.

Fig. 2. Circuit diagram of a potential measuring amplifier with provision for the compensation of stray capacity. The operational amplifier (triangle) was a PHILBRICK¹⁹ model P25A or P2AU. The trim condenser C_f serves to compensate the stray capacity.

Results

Fig. 3 demonstrates how electrode parameters can be obtained from a current pattern which results when EM of tip diameter $\cong 2,5 \mu\text{m}$ is subjected to a triangle voltage clamp. For better comparison the negative current peaks are taken as reference current and labelled zero. It is seen that the different penetration of the electrode into the agar gel mainly yields a change of the capacitive current level i_c . The straight portions of the curves which are due to the resistive components of the electrode are relatively independent of the immersion as it is expected

¹⁷ A. A. LEV, in: Glass Microelectrodes, Editors: LAVALLEE, SCHANNE, and HEBERT, John Wiley & Sons, New York 1969.

¹⁸ C. C. KRISCHER, Z. Naturforschg. **24b**, 156 [1969].

¹⁹ PHILBRICK, Applications Manual, Philbrick Researches Inc., Nimrod Press, Boston 1966.

Fig. 3. Current flow obtained by applying a triangle voltage to EM of tip diameter $\approx 2.5 \mu\text{m}$. Depth of immersion into 1% agar gel in 0.1 M KCl is indicated at each curve. dE/dt of the input signal was $\pm 185 \text{ V/sec}$. EM is filled with 0.1 M KCl. The current was measured with an operational amplifier type P25A¹⁹ with 1 MOhm as feedback resistor. The values are: $R_s = 700 \text{ KOhm}$, $R_e = 10 \text{ MOhm}$. Inlet: Values of two measurements of C_{gl} as a function of the depth of immersion. The slope of the curve corresponds to the parameter C_{gl}^* .

theoretically. The resistances evaluated from the slopes of the straight portions are $R_s = 700 \text{ KOhm}$ and $R_e = 10 \text{ MOhm}$.

Table 1 contains a collection of measurements of R_s , the resistance of the solution in front of the tip.

electrode type	$\varnothing$ tip μm	filling	R_s [MOhm]	total number of electrodes
EM	2.5	0.1 MKCl	0.85 ± 0.1	5
EM	0.2	0.1 MKCl	4.80 ± 1.4	5
MM	2.5	Ag/AgCl	0.22 ± 0.045	12

Table 1. Series Solution Resistances.

The measurement of the *electrode resistance* (i. e. within the tip) yielded the values shown in table 2.

electrode type	$\varnothing$ tip μm	filling	surrounding solution	R_e or R_{dl} [MOhm]	total number of electrodes
EM	0.2	0.1 MKCl	0.1 MKCl	50.6 ± 14.6	5
EM	2.5	0.1 MKCl	0.1 MKCl	9.4 ± 0.1	5
MM	2.5	Ag	0.1 MKCl	280.0 ± 54.0	6
MM	2.5	Ag/AgCl	0.1 MKCl	45.0 ± 3.0	4
MM	2.5	Sb	0.1 MKCl	83.0 ± 16.0	3

Table 2. Electrode Resistances.

The resistances of table 2 were only for EM independent of polarization up to 50 mA/cm^2 . For MM in 0.1 M KCl the resistance of polarized electrodes, R_i , differed from the resistance of unpolarized electrodes, R_0 . The bare Ag electrodes had the resting potential at

-400 mV vs saturated calomel electrode (S.C.E.). At cathodic current densities of 5 mA/cm^2 ($E = -550 \text{ mV}$) R was $0.7 \cdot R_0$, whereas at 5 mA/cm^2 anodic polarization R was $6.5 \cdot R_0$ at 0 mV vs S.C.E. The antimony electrode had the resting potential at -300 mV vs S.C.E. The current increased in the cathodic and anodic direction exponentially with increasing polarization. At cathodic current densities of 50 mA/cm^2 ($E = -600 \text{ mV}$) R was $0.25 \cdot R_0$, at 50 mA/cm^2 anodic polarization ($E = 0 \text{ V}$) R was $0.40 \cdot R_0$ vs S.C.E. Ag/AgCl electrodes were obtained by anodic charging of Ag with $\approx 5 \cdot 10^{-10} \text{ Asec}$. The resting potential was at $+50 \text{ mV}$ vs S.C.E. The resistance of polarized electrodes differed from the resistance of the unpolarized electrodes. In the cathodic direction it was $0.7 \cdot R_0$ and in the anodic direction it was $1.2 \cdot R_0$.

The *capacitive components* of the electrode impedance were obtained as described in connection with Fig. 3. For EM the slope of the curve in the inset $dC_{gl}/d(d)$ is the parameter C_{gl}^* . At small penetrations of the electrode into the agar gel $C_{gl}^* = 0.7 \text{ pF/mm}$. 12 other EM yielded for C_{gl}^* $0.8 \pm 0.1 \text{ pF/mm}$.

For MM (Ag/AgCl) the electrode capacity $C = C_{gl} + C_{dl}$ was $18.6 \pm 2.1 \text{ pF}$ (12 measurements) for $dE/dt = 50 \text{ V/sec}$. Test measurements at $dE/dt = 5 \text{ V/sec}$ and 500 V/sec yielded values of C which differed not more than 15% from this value.

The *ac potential transducing properties* of EM and MM for *sine wave* of 20 mV amplitude (peak to peak) are shown in fig. 4. It is seen that for *uncompensated* C_a (curves to the left) the output potential begins to decrease for MM at lower frequencies

Fig. 4. Sine wave frequency response of microelectrodes of a tip diameter of $\approx 2.5 \mu\text{m}$, dipping 1.5 mm into 1% agar gel in 0.1 M KCl. Each bar represents the standard deviation of measurements obtained with three different electrodes. The sine wave (20 mV peak to peak) was applied via an Ag/AgCl-wire to the microelectrode. The potential measuring circuit of fig. 2 containing a Philbrick P25A amplifier was used. The curves are calculated with eq. (11). The assumed parameters were: MM, Ag/AgCl filling: $C_a = 16 \text{ pF}$, $C = 8 \text{ pF}$, $R_s = 0.2 \text{ MOhm}$, $R_{dl} = 70 \text{ MOhm}$. EM, 0.1 M KCl filling: $C_a = 12 \text{ pF}$, $C = 2 \text{ pF}$, $R_s = 1.0 \text{ MOhm}$, $R_{dl} = 10 \text{ MOhm}$.

than for EM. MM maintain, however, at higher frequencies a higher level of the output potential. For optimally compensated stray capacity (upper curves) MM maintain full amplitude up to 100 KHz whereas for EM E_{out} has dropped at 100 KHz to 70% of the input potential.

The response curves of EM and MM to applied step potentials of 20 mV amplitude are shown in fig. 5. C_f of fig. 2, was zero. Analogous to the response to sine wave MM have lower amplitude than EM at long durations whereas for short durations the reverse is true: $E_{out}(MM) > E_{out}(EM)$. The mentioned potential levels $\gamma = C/C + C_a$ are marked by double arrows in figs. 4 and 5.

Fig. 5. Response of microelectrodes to applied potential steps of 20 mV amplitude. The measurements were carried out as indicated in fig. 4. C_f of fig. 2 was zero. The markings $\times$, $\circ$, $\triangle$ are values calculated with eq. (12). The chosen parameters were: EM, 0.1 M KCl filling, ϕ 2.5 μ m: $C_a = 12$ pF, $C = 2$ pF, $R_e = 7$ MOhm, EM, 0.1 M KCl filling, $\phi \leq 1.0$ μ m: $C_a = 12$ pF, $C = 2$ pF, $R_e = 40$ MOhm, MM, Ag/AgCl filling, ϕ 2.5 μ m: $C_a = 12$ pF, $C = 12$ pF, $R_{dl} = 25$ MOhm, R_s for all electrodes = 1 MOhm.

The effect of electronic compensation of C_a , fig. 1, via C_f , fig. 2, on the potential transduction of step functions is demonstrated in fig. 6. The response of MM closely resembles the input signal. The delay of 2 μ sec could be reduced to 1 μ sec by using an especially wideband amplifier, Philbrick, type P25AH¹⁹. EM of tip diameter ≈ 2.5 μ m are seen to have a much slower rise to the final potential level $E_{out}/E = 1$. For the included curve of EM with the usual fine tip of diameter ≤ 0.5 μ m the final potential level could not be reached in the first 15 μ sec because the amplifier began to oscillate in the used configuration if C_f was increased more.

Measurements of dc potentials of MM reported in fig. 7. It is seen that MM filled with Sb or Ag/AgCl have ion specific potentials depending on pH or pCl,

Fig. 6. Response of microelectrodes to applied potential steps of 20 mV amplitude. Same electrodes and measuring conditions as in fig. 5 with the exception that C_f (see fig. 2) was for each electrode adjusted to yield optimal response. The response of the measurement with the fine electrode ($\phi \leq 1$ μ m) could not be improved because of the onset of oscillations.

Fig. 7. Potential E_0 measured versus saturated calomel electrode (S.C.E.) of three different metal microelectrodes (tip diameter ≈ 2.5 μ m) as a function of the logarithm of ion concentration (the bars represent the standard deviation). Upper curves: a) Ag/AgCl microelectrodes in 0.1 M KCl/KNO₃ mixtures of varying Cl⁻ content. b) Ag/AgCl-wire in the same mixtures. Lower curves: Sb microelectrode and Sb rod (0.5 $\times$ 1.0 cm), respectively, in universal buffer (0.1 M H₃BO₃ H₃PO₄ CH₃CO₂H + KOH) of varying pH. The measuring amplifier was Philbrick P2AU in the configuration of fig. 2. Prior to the measurement the offset trim was adjusted to have an offset current ≈ 0.2 pA (measured with a resistor of 500 MOhm). Note: the scales to the two curves at the top are at the right side, the scales to the bottom curves are at the left side.

respectively. The curves measured at MM and at the corresponding macroscopic electrodes are parallel. With a pretreatment of the Sb rod by concentrated HNO₃ the curve could be shifted 100 mV towards positive potentials. The use of the amplifier with offset current ≤ 1 pA was especially needed for MM filled with Sb. Larger offset currents sometimes

destroyed the electrode quickly by anodic dissolution. At Ag/AgCl electrodes anodic offset currents increased the resistance and decreased the capacity but the steady state potential remained unaffected.

Discussion

The experiments showed that the *measurement of all microelectrode parameters* is possible by employing a linearly changing voltage. A simple current to voltage converter based on operational amplifiers serves as a convenient current measuring device¹⁸. The *series solution resistance* — which is responsible for the high frequency limit of recording, see below — was characterized as being located in front of the tip, fig. 1. Because the pipettes and the electrolyte of EM and MM were identical, the R_s values should have been the same. Table 1 shows, however, that R_s (EM) is $\cong 4 \cdot R_s$ (MM). This discrepancy can be explained by assuming that for EM part of R_e has to be included into R_s ; the capacity of the glass wall, C_{gl} , shunts R_e incompletely. The shunt of the electrode resistance R_{dl} by C_{dl} is evidently perfect for MM.

If an estimate of R_s is required (for high frequency measurements, see below) the approximation $R_s(\text{EM}) \cong 0.1 \cdot R_e$ can be used. This means that in eq. (1) s has to be taken 0.25 [if $\alpha = 6^\circ$, eq. (2)]. For MM the estimate $R_s \cong 0.025 \cdot R_e$ would be adequate (R_e is the *dc* resistance of a micropipette which has the same radius as the metal electrode and which is filled with the liquid that surrounds the metal electrode).

The *electrode resistances* R , (i. e. R_e and R_{dl}) were for EM smaller than for MM. From table 2 one obtains for R_e/R_{dl} 0.034; 0.21; 0.11 for Ag, Ag/AgCl, and Sb, respectively. With eq. (7) one calculates for the constant $\varepsilon[\mu\text{m}]$ the values of 36 μm , 5.6 μm , 11 μm for Ag, Ag/AgCl and Sb, respectively. Metal electrodes with such radii would have identical resistances as EM of the same radius.

The *steady state flux* of ions diffusing through the tip of EM is, with eq. (4), calculated 0.27 nA or $i_0/F = 2.8 \cdot 10^{-14}$ eq/sec in fair agreement with a measured value of $6 \cdot 10^{-14}$ eq/sec for a 0.5 μm tip, 3 M KCl, quoted in reference²⁰. The corresponding corrosion fluxes of MM are much smaller, i. e.

0.09 nA, 0.056 nA, 0.029 nA or $i_0/F = 0.094 \cdot 10^{-14}$ eq/sec, $0.058 \cdot 10^{-14}$ eq/sec, $0.031 \cdot 10^{-14}$ eq/sec, for Ag, Ag/AgCl, and Sb, respectively. It has, however, to be remembered that the corrosion products might be poisonous to biological cells.

For the *capacitive components* C of the electrode impedance, eqs. (8) and (9), the agreement of derived and measured values is satisfactory: For EM $C = C_{gl}$ depends on the length of the wetted part of the electrode, eq. (8), fig. 3. C_{gl}^* was found in the same order of magnitude as the values which were reported by other authors^{4, 15}. In intracellular measurements C_{gl} is divided by the membrane into one part inside the cell and another part outside. The latter contribution of C_{gl} has to be considered as stray capacity and becomes part of C_a .

The capacity of MM is mainly the double layer capacity of the interphase metal-electrolyte. Insertion of the common value $C_{dl}^* \cong 100 \mu\text{F}/\text{cm}^2$ into eq. (9) yields for MM (1.5 mm immersion) a capacity of $\cong 12$ pF in fair agreement with the measurements.

The electrode capacitances of the used model electrodes are therefore with 2 to 20 pF in the same order of magnitude as the specified input capacitors of usual amplifiers.

The discussion of the *ac potential transducing properties of EM and MM* can be restricted to the more familiar sine wave because this wave form and step function are correlated via Fourier transform. For most experiments step functions are to be preferred because a single step response — appropriately displayed — contains the required information. The discussion therefore includes in brackets step durations which correspond to certain frequency ranges.

The response curves of EM and MM with *uncompensated stray capacity* (i. e. C_a of similar size as C) are mainly determined by two time constants, $R \cdot C = \tau$ (i. e. electrode resistance times electrode capacitance) and $R_s \cdot C_a = \tau_s$ (i. e. series solution resistance times stray capacity), see eqs. (11) and (12). Two different frequency ranges (step durations) of the response curves can be distinguished:

1. at low frequencies (long step durations t) i. e. $\omega \cong 1/\tau$ ($t \cong \tau$) the terms $\omega \tau$ (t/τ) and $\omega R \Sigma C$ are, in eq. (11), responsible for the decrease of E_{out}/E with increasing frequency (decreasing step duration). The terms containing R_s in eqs. (11) and (12) can be neglected. It is seen in fig. 4 (5) that for the used model electrodes this frequency is near

²⁰ BURES, PETRAN, and ZACHAR, *Electrophysiological Methods in Biological Research*, Academic Press, New York-London 1967.

100 Hz (1 msec) for MM and near 1 KHz (100 μ sec) for EM, respectively.

2. at high frequencies (short step durations) i. e. $\omega \cong 1/\tau_s$ the term $\omega \tau_s$ in eq. (11), $[t/\tau_s, \text{eq. (12)}]$, predominates the frequency response. The terms containing R can be neglected and E_{out}/E approaches zero with $1/\omega \tau_s (1 - \exp - t/\tau_s)$. The curves in fig. 4 indicate that this frequency is near 100 KHz for EM and near 400 KHz for MM. The measured values do, however, not extend far enough into this frequency range.

The capacitive parameters $\gamma = C/C + C_a$ determine the magnitude of E_{out} in the frequency range (time span) in between the frequency ranges which were discussed above. for $1/\tau < \omega < 1/\tau_s$ ($\tau_s < t < \tau$) E_{out}/E is close to the potential level γ , eq. (12), figs. 4 and 5. It is seen in fig. 4 (5) that due to $C(\text{MM}) > C(\text{EM})$ metal electrodes have $E_{\text{out}}/E = \gamma(\text{MM}) = 0,33$ (0,5) whereas EM have $\gamma(\text{EM}) = 0,14$ (0,14). The bandwidth (time span) of this potential level is (for similar values of C and C_a) given by the values of R and R_s . For EM R/R_s was $\cong 10$ whereas for MM R/R_s was $\cong 300$. It can be extrapolated in fig. 4 that the bandwidth of γ is for EM indeed much smaller than for MM. The small divergence of the parameters used to compute the curves and the ones reported in the tables is probably due to polarization by offset currents. It was impossible to avoid this polarization because the wideband measurements excluded ac coupling.

The effect of *electronic compensation* of C_a is a reduction of the effective value of C_a ; γ in eq. (12) approaches one. In eqs. (11), [(12)] $E_{\text{out}}/E = 1$ for low frequency (long step duration). At high frequency (short step duration) $E_{\text{out}}/E_{\text{in}}$ decreases (increases) with $1/\omega R_s C_a (1 - \exp - t/R_s C_a)$, where C_a is the remaining rest of stray capacity. The improvement of the response curves by the neutralization of C_a is evident in fig. 4: For EM the recorded E_{out} is at $\cong 20$ KHz 80% of E whereas MM record the full signal up to 100 KHz. This superiority of MM for recording is seen in more detail in fig. 6: EM with tip diameter $\cong 2,5 \mu\text{m}$ reach 70% of the final amplitude in $\cong 10 \mu\text{sec}$. For MM the recorded signal is – apart from the mentioned $2 \mu\text{sec}$ delay – practically identical with the input signal.

The *dc potential measurements* of MM filled with Ag/AgCl and Sb, fig. 7, show that these MM had for more than three decades of concentration in the used inorganic electrolytes *dc* potentials proportional to pCl and pH. The constant of proportionality is with 50 mV/decade for Sb and 48 mV/decade for Ag/AgCl fairly close to the theoretical value of 58 mV/decade of the Nernst equation for monovalent reactions. The curves measured at Sb are in accord with measurements at somewhat larger Sb-microelectrodes²¹.

Under the conditions of the reported experiments EM have no *dc* potential drop across the opening. For different electrolytes inside and outside of the tip other investigators have observed the expected relationship: $E = E_0 \pm b \log c$, with b a constant usually smaller than 58 mV/decade^{17, 22, 23, 7}.

Appendix

The results of this paper allow a crude estimate of the potential transducing properties (*PTP*) of microelectrodes which have smaller tips than the model electrodes.

For the usual fine tipped micropipettes, EM_f, (tip diameter $\leq 0,5 \mu\text{m}$) filled with 3 M KCl and impaled 150 μm into a biological cell the electrode resistance R is probably one order of magnitude larger than the resistance of the model electrode, EM_m, (tip diameter 2,5 μm) 0,1 M KCl filling. The recording electrode capacity is much smaller, i. e. $C(\text{EM}_f) \cong 0,1 \cdot C(\text{EM}_m)$. The upper frequency limit f_{lim} for recording with an amplifier like the one in fig. 2 is with these EM_f parameters at least one order of magnitude lower than for EM_m; $f_{\text{lim}} = 1$ KHz is an optimistic estimate.

If appropriate MM were used for intracellular recording the frequency limit could be extended. The extrapolation of MM electrode parameters to a tip diameter $\cong 1 \mu\text{m}$ may be taken as example for MM with very small tips. For Ag/AgCl the parameters are estimated as follows:

$$R_s \text{ (extrapolated for 200 Ohmcm cytoplasma)} \cong 1 \text{ MOhm,}$$

$$R_{\text{dl}} \text{ (extrapolated in 0,1 M KCl)} \cong 500 \text{ MOhm,}$$

$$C_{\text{dl}} \text{ (extrapolated in 0,1 M KCl)} \cong 1 \text{ pF.}$$

A crude extrapolation of the upper frequency limit f_{lim} of recording with compensated C_a is $f_{\text{lim}} = 20$ KHz (like the upper curve "EM" in fig. 4).

It has to be pointed out that such small MM are extremely sensitive to offset currents or transient charging. The electrochemical formation of a monolayer of sparingly soluble substances such as AgCl would re-

²¹ I. LEVIN, Chemist-Analyst **41**, 89 [1952].

²² G. KÜCHLER, H. BEYER, M. HIMMEL, and B. MERREM, Pflügers Arch. ges. Physiol. Menschen Tiere **280**, 210 [1964].

²³ H. KRUEGER, Elektrochemische Eigenschaften von Kapillarmikroelektroden, Diplomarbeit, Universität München 1963.

quire the charge $q = p \cdot e_0 \cdot f \cong 1,6 \cdot 10^{-12}$ Asec, where: $p = 10^{15}$ Atoms/cm², the population of atoms on one square centimeter of surface, $e_0 = 1,6 \cdot 10^{-19}$ Asec/atom, the elementary charge and $f \approx 10^{-8}$ cm² the surface area. A common offset current of 10^{-11} A could thus produce AgCl at a rate of 300 layers/minute.

Other metals, such as tantalum or tungsten, have also been used to make microelectrodes^{24, 1}. The hardness of these metals permits making very fine tips which have a relatively large surface area. (The surface ratio of a fine cone to a half sphere is l/d with l = length of cone and d = diameter of half sphere = diameter

of cone base.) The layer of oxide on these metals causes unfavourable surface properties. C_{dl}^* is smaller than for bare metal surfaces¹³ and R_{dl}^* is considerably larger than the observed value of Ag/AgCl^{13, 10}. The mentioned gain in surface area could therefore be negligibly small for ordinary W or Ta electrodes. Special plating techniques are needed to reduce R_{dl}^* and increase C_{dl}^* ⁵.

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²⁴ H. A. BALDWIN, S. FRANK, and J. Y. LETTVIN, Science [Washington] **148**, 3676, 1965, 1462.

Some Heterocyclic Nitrogenous Compounds as Radioprotectants for Hematological Levels in X-Irradiated Mice

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In normal controls, the levels of hemoglobin concentration and hematocrit values remained almost unchanged throughout the experimentation period.

Intraperitoneal administration of imidazole and benzimidazole affected rapid pharmacodynamic incidences which were not detected with cysteamine. Administration of imidazole did not induce detectable change in the level of hemoglobin concentration but slightly decreased the hematocrit value. Benzimidazole created slight depression in hemoglobin concentration and exerted no effect on hematocrit value. Cysteamine caused slight depression of both hemoglobin concentration and hematocrit value. Those changes were readily restorable with imidazole and benzimidazole while those with cysteamine lasted for more than three days.

Irradiation caused progressive decrease in hemoglobin concentration and hematocrit values which continued till the death of the animals. A significant increase in the sedimentation rate of red blood cells was recorded.

Irradiation of chemically protected animals caused initial decrease in hemoglobin concentration and hematocrit values which lasted till the fifth post-exposure day. Later, gradual restoration of normal levels of hematological values was recorded. Restoration was more rapid with benzimidazole than with imidazole, while cysteamine lied half way in its action. The increase in sedimentation rate of red blood cells was less pronounced and gradual restoration of the normal sedimentation rate was maintained during the post-irradiation time.

It seems likely that the radioprotectants act on cellular and subcellular levels in the protected tissues but the actual role played by such compounds still awaits further investigation.

Imidazole and benzimidazole proved to be effective radioprotectants of the hematopoietic tissue. Benzimidazole proved to exert the best radioprotective characters amongst the tested compounds in this concern.

Since 1949, when the chemical protection against ionizing radiation has been recorded for the first time, excessive studies have been carried out on enormous numbers of chemical compounds that have been suspected to exhibit radioprotective properties (BACQ^{1a}, TIUNOV et al.², etc.).

In order to give a comprehensive explanation for the phenomenon of chemical radioprotection, repeated investigations have been made to study the chemical structural formation of different radioprotectants and its correlation with their radioprotective properties (BACQ^{1a}). Moreover, extended

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^{1a} Z. M. BACQ, Chemical Protection against Ionizing Radiations, Charles and Thomas, American Lecture Service, 1965.

² L. A. TIUNOV et al., Foreign Technology Division, Wright Patterson Air Force Base, Ohio, A.D. 633268 (1966).