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# Neutral Carrier Electrode for Continuous Measurement of Blood $Ca^{2+}$ in the Extracorporeal Circulation

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A neutral-carrier-based $Ca^{2+}$-selective electrode exposed to whole blood exhibited an EMF-stability better than 0.13 mV (~1% change of the initial activity) during 3.7 to 6.6 h. It has been used successfully to monitor ionic $Ca^{2+}$ in the extracorporeal circulation during administration of calcitonin to dogs. Data simulation shows that the apparent concentration of ionic $Ca^{2+}$ is affected by the concentration of $Na^+$. A variation in $[Na^+]$ by ±5 mmol/L from a value of 143 mmol/L used for the calibration leads to uncertainties of ±0.8% in the $Ca^{2+}$ concentration and ±0.2% in the $Ca^{2+}$ activity if 3 mol/L KCl is used as the reference electrode solution. When an isotonic solution was used as reference electrolyte, errors of ±0.8% and ±1.7%, respectively, were observed.

Both the classical ion-exchanger membranes based on organo-phosphates (*1–5*) and the neutral carrier membranes (*6–10*) have been optimized for use in clinical analysis. Ion-exchanger membranes are now used successfully in clinics (*11–14*), although the ion-selective membrane must be covered with a dialysis membrane to avoid changes in the reference potential after the first direct contact with serum (*12, 14*). This covering somewhat prolongs the response time of the electrode (*12*).

The carrier membrane we describe here does not seem to be contaminated on the surface by serum components, so no protective dialysis membrane is needed and the related technical problems, including the membrane's unfavorable influence on response time, are obviated.

A further distinct difference between the two types of membranes is their $Ca^{2+}/H^+$ selectivity, which differs by about five orders of magnitude in favor of the carrier membrane (*8*). Consequently, only the carrier membrane can be used for concurrent measurements of ionized $Ca^{2+}$ in blood at physiological pH values and of the total calcium in strongly acidified samples (*8*).

Here we report on the use of such a carrier membrane in continuous $Ca^{2+}$ measurements in whole blood of dogs during calcitonin administration.

## Materials and Methods

### Extracorporeal Bypass

Venous extracorporeal circulation of the blood of a living dog (young beagle) was effected, from the cephalic to the saphenous vein. The blood flow was diverted and re-injected through silicone rubber catheters, which were connected to the flow-through electrode system with poly(tetrafluoroethylene) tubes (see Figure 1 and ref. *8*). As anticoagulant, about 1250 int. units of heparin per kilogram body weight was administered intravenously just before we began the measurement and then 250 int. units/kg every 2 h. The volume of blood in the extracorporeal bypass was 2.5 mL and the blood temperature in the flow-through electrode system was 25 °C.

### Flow-Through Electrode System and Voltage Measurements

For these measurements, we used cells of the type Ag, AgCl; reference electrolyte | sample ‖ membrane ‖ 1 mmol/L $CaCl_2$; AgCl, Ag. The reference and the $Ca^{2+}$-selective electrodes were provided with FET operational amplifiers and the analog signal of the difference amplifier (input impedance: 2pF ‖ $10^{15}\Omega$) was sampled with a digital voltmeter (0.1 mV resolution). The electromotive force (EMF) was continuously registered on a recorder (Recorder Series 310; W+W Electronic, Basle, Switzerland) as a function of time. Further details on the flow-through electrode system are found in ref. *8*.

An electrolyte solution isotonic with beagle serum was chosen as the reference electrode solution (see Figure 1). The membrane being examined consisted of, per 100 mg, 3.6 mg of $Ca^{2+}$ ionophore, 2.2 mg of potassium tetrakis(*p*-chlorophenyl) borate, 62.3 mg of bis(2-ethylhexyl) sebacate, and 31.9 mg of polyvinyl chloride. The preparation of such flow-through ion-selective electrodes is described in ref. *15*. Selectivity factors ($K^{Pot}_{CaJ}$) were obtained according to the separate-solution method (*16*).

### Measuring Procedure and Evaluation

Before we inserted the flow-through electrode system into the extracorporeal blood flow, we measured three $Ca^{2+}$ calibration solutions—0.5, 1.0, and 5.0 mmol/L—each containing, per liter, 143.0 mmol of $Na^+$, 4.6 mmol of $K^+$, and

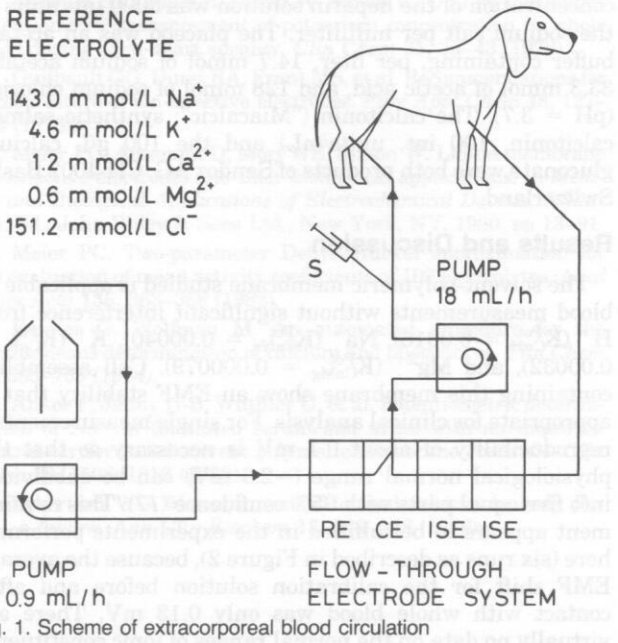


Fig. 1. Scheme of extracorporeal blood circulation

*RE*, reference electrode; *CE*, common electrode; *ISE*, ion-selective electrode; *S*, syringe for drug administration or blood collection

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0.6 mmol of $Mg^{2+}$. These experimental EMF values were corrected for changes in the liquid junction potential by use of the Henderson formalism (*17*). The activity coefficients were calculated according to the Debye–Hückel formalism and the parameters given in ref. *18*. The corrected EMF values were plotted vs the logarithm of the calcium concentration. This is permissible because the activity coefficient of calcium in the calibration solutions is virtually constant, owing to the high ionic strength. The actual (local) slope, s, of the electrode was then determined by a linear regression. The 1 mmol/L $Ca^{2+}$ calibration solution was aspirated until the extracorporeal bypass was ready, then connected to the flow-through electrode system. After the blood-$Ca^{2+}$ measurement (3.7 to 6.6 h duration) a second calibration was performed. The concentration was obtained with the following (derived from the Nernst equation):

$$c_{Ca^{2+}}(t) = 10^{-\Delta E(t)/s}$$

where $c_{Ca^{2+}}(t)$ is the ionized calcium concentration (in mmol/L) in the blood at the time t, $\Delta E(t)$ is the EMF difference (in mV) of the cell assembly between the 1.0 mmol/L $Ca^{2+}$ calibration solution and blood at the time t, and s is the local slope (in mV). Assuming the ionic background in the sample and the calibration solution to be identical and therefore a constant activity coefficient ($\gamma = 0.336$), we calculated the sample activity with the following relation:

$$a_{Ca^{2+}} = \gamma \cdot c_{Ca^{2+}}$$

A 2-mL blood sample was taken about every 30 min for continuous-flow colorimetry of total calcium in serum (AutoAnalyzer; Technicon Instruments Corp., Tarrytown, NY) by a method adapted for such analyzers (*19*).

## Reagents

For all aqueous electrolyte solutions we used doubly quartz-distilled water and salts of the highest available purity. From Fluka AG, CH-9470 Buchs, Switzerland, we obtained the following membrane components: (−)-(R,R)-*N,N'*-bis[(11-ethoxycarbonyl)undecyl]-*N,N'*,4,5-tetramethyl-3,6-dioxaoctane-diamide, $Ca^{2+}$ ionophore (ETH 1001); bis(2-ethylhexyl) sebacate (DOS); potassium tetrakis(*p*-chlorophenyl) borate (KTpClPB); high-molecular-mass polyvinyl chloride (PVC S 704); and tetrahydrofuran (THF). The concentration of the heparin solution was 5000 int. units of the sodium salt per milliliter. The placebo was an acetate buffer containing, per liter, 14.7 mmol of sodium acetate, 33.3 mmol of acetic acid, and 128 mmol of sodium chloride (pH = 3.7). The calcitonin ("Miacalcic"; synthetic salmon calcitonin, 100 int. units/mL) and the 100 g/L calcium gluconate were both products of Sandoz AG, CH-4002 Basle, Switzerland.

## Results and Discussion

The solvent-polymeric membrane studied is applicable to blood measurements without significant interference from $H^+$ ($K^{Pot}_{CaH} = 0.0016$), $Na^+$ ($K^{Pot}_{CaNa} = 0.00040$), $K^+$ ($K^{Pot}_{CaK} = 0.00032$), and $Mg^{2+}$ ($K^{Pot}_{CaMg} = 0.000079$). Cell assemblies containing this membrane show an EMF stability that is appropriate for clinical analysis. For single measurements a reproducibility of about 0.1 mV is necessary so that the physiological normal range (≈2.3 mV) can be subdivided into five equal parts with 95% confidence (*17*). This requirement appears to be fulfilled in the experiments performed here (six runs as described in Figure 2), because the average EMF shift for the calibration solution before and after contact with whole blood was only 0.13 mV. There are virtually no data on the normal ranges of ionic constituents of beagle blood serum, so the above statement is based on the assumption that beagle and human blood serum composition is very similar.

Figure 2 shows the results of a typical experiment (one of six such runs) in which calcium was measured in the extracorporeal blood circulation. The total calcium as measured by an independent procedure (colorimetry) as well as the potentiometrically determined concentration and activity, respectively, of the ionized calcium are given as a function of time. The similarity between the profiles is evident, even though three physiologically different variables are being compared. Obviously, ion-selective electrode devices can be used for continuous pharmacokinetic studies in whole blood.

Even if standards for $Ca^{2+}$ activity were available, there would still be uncertainties arising from voltage instability and voltage measurement with the digital voltmeter, the liquid junction potential $E_D$, and the interference of other ions. For a 3 mol/L KCl bridge electrolyte the liquid junction potential varies less than 0.01 mV over the physiological $Ca^{2+}$ range (1.0 to 1.2 mmol/L $Ca^{2+}$; 143 mmol/L $Na^+$ background). For the selectivity factors given above, the contribution of sodium ($K^{Pot}_{CaNa} \cdot a^2_{Na}$) is 1.03% of $a_{Ca^{2+}}$ for a sodium concentration of 143 mmol/L. For a given $Na^+$ concentration used as background in the calibration solutions, this error is completely compensated by the evaluation procedure. Such errors are negligible if the $Na^+$ concentration of the sample and of the calibration solutions remains within the physiological range. Thus, the only other serious source of error would be the EMF quantification error, here taken to be ±0.1 mV. Therefore, an uncertainty of ±0.0034 log $a_{Ca^{2+}}$ (±2.9 μmol/L at a typical $a_{Ca^{2+}}$ of 370

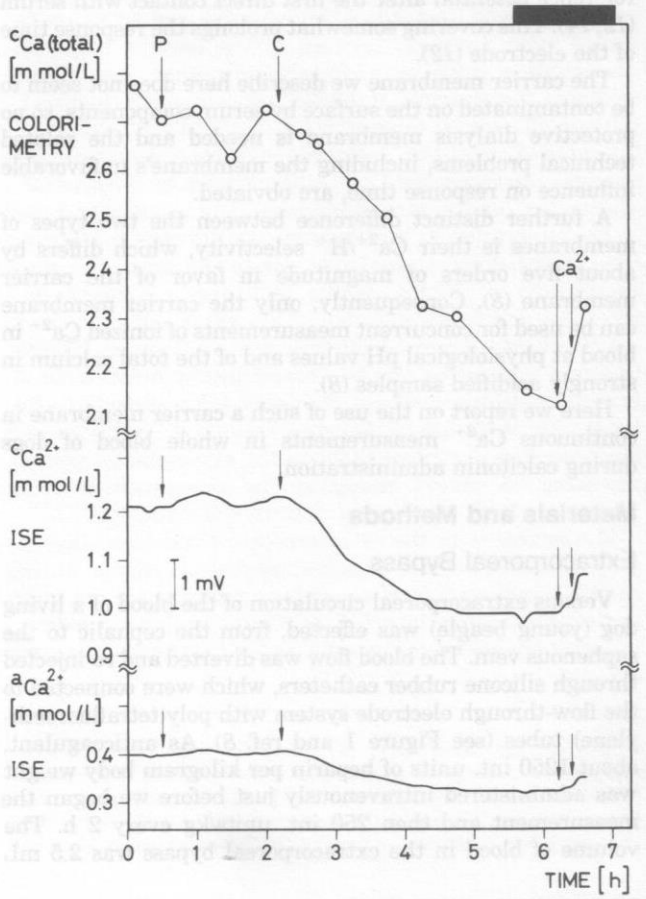


Fig. 2. Profiles of total calcium concentration, ionized calcium concentration, and activity measured in the extracorporeal blood circulation

*P*, addition of 5.4 mL of placebo; *C*, addition of 540 int. units of calcitonin; $Ca^{2+}$, two additions each of 232 μmol of calcium gluconate

$\mu$mol/L) is found, which corresponds to 4.4% of the physiological normal $a_{Ca^{2+}}$ range, 67 $\mu$mol/L. This obviously would satisfy the stipulated 20-fold subdivision of the range. But the problem remains that as yet there are no standards for calcium activity. A way out of this dilemma is to have calibration solutions contain an isotonic background concentration of 143 mmol of NaCl, 4.6 mmol of KCl, and 0.6 mmol of $MgCl_2$ per liter. For such a solution the apparent calcium activities can be estimated. This implies correspondence between the concentration and electrochemical properties of all ionic species in the calibration solution and the sample, so further errors are introduced insofar as this approximation of the activity coefficients is incorrect (for a discussion, see ref. *17*). For example, if the calibration solution and the blood sample differ in their sodium concentrations—e.g., 143 and 148 mmol/L, respectively—an error in the $Ca^{2+}$ activity of $-0.0011$ log $a_{Ca^{2+}}$ units ($-1$ $\mu$mol/L at a typical $a_{Ca^{2+}}$ of 370 $\mu$mol/L) would be read off the 143 mmol/L $Na^+$ calibration line for 3 mol/L KCl as reference electrolyte solution and the experimental procedure described above. In the case considered, a change from 143 to 148 mmol/L $Na^+$ results in a change in $E_D$ of about $-0.03$ mV.

In the experiments described, the blood sample was reintroduced into the dog's circulation. The 3 mol/L KCl bridge electrolyte had to be replaced by an isotonic reference electrode solution to prevent $K^+$ stress to the dog (0.9 mL of 3 mol/L KCl per hour would double the concentration of $K^+$ in the serum in an hour if uniform mixing is assumed). With such an arrangement a change from 143 to 148 mmol/L $Na^+$ at a constant $Ca^{2+}$ concentration of 1.1 mmol/L yields a change in $E_D$ of about $-0.15$ mV.

If information on the concentration of ionized $Ca^{2+}$ is what is sought (instead of the activity), the situation becomes more complex. Because of the specific numerical constellation, errors may compensate in some cases but not in others. For example, if the $Na^+$ concentration changes by $\pm 5$ mmol/L (initial concentration: $Na^+$, 143 mmol/L; $Ca^{2+}$, 1.1 mmol/L; isotonic reference electrode solution), this changes the measured $Ca^{2+}$ concentration by $\pm 8.6$ $\mu$mol/L when a $Na^+$ background of 143 mmol/L is assumed and used in the calibration in the experimental procedure described (due to the different contributions to the error, the same uncertainty of $\pm 0.8\%$ results on using a 3 mol/L KCl reference electrode solution). This error corresponds to $\pm 4.3\%$ of the 0.2 mmol/L physiological $Ca^{2+}$ range. If better accuracy is required, the $Na^+$ concentration must be simultaneously determined, e.g., by using a $Na^+$-selective electrode (*20*). With use of the same calibration solutions and the procedure described above in the experimental section, the same $\pm 5$ mmol/L change in $Na^+$ concentration would lead to a $\pm 1.7\%$ error in the determined activity of $Ca^{2+}$.

Measurement in the extracorporeal blood circulation as presented here circumvents problems of pH change during sample handling (*21*). If anaerobic conditions are not guaranteed, controlling the pH of the blood sample to a predetermined value has been suggested as the next-best alternative (*13*, *14*).

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