erences to the literature on hematologic mechanisms see (*1*-*3*). In Eq. 2, β_0, θ, and n are positive constants adjusted to fit experimental observations, and it is assumed here that $\beta_0 > a > 0$, $n\beta > 2$, and $6aB > \beta_0$, where $B = (\beta_0 - a)/\beta_0$. For the system given by Eqs. 1 and 2 there is a unique steady state $x_e = \theta(B\beta_0/a)^{1/n}$. About this equilibrium, the linear part of Eq. 1 takes the form

$$dz(t)/dt = -az(t) - bz(t - \tau)$$
$$z = x - x_e \qquad (3)$$

The solutions of this equation have a natural frequency $\omega = (b^2 - a^2)^{1/2}$ at a critical value of the lag given by

$$\tau_c = \frac{\arccos(-a/b)}{(b^2 - a^2)^{1/2}}$$
$$0 < \arccos(-a/b) < \pi \qquad (4)$$

where $b = a(nB - 1)$ for Λ given by Eq. 2. The steady state of the nonlinear system is stable for $\tau < \tau_c$. Considering the nonlinear terms and using the Hopf bifurcation algorithm developed in (*4*), we obtain the mathematical result that as τ increases above τ_c, this state gives rise to asymptotically orbitally stable periodic solutions. These have periods longer than $2\pi/\omega$ and exist for τ in some interval $\tau > \tau_c$. Realistic values of the parameters can be substituted in Eq. 4 to give a numerical value of τ_c; for example, the parameters $a = 0.1$ per day, $\beta_0 = 0.2$ per day, and $n = 10$ used in (*1*) for numerical simulations yield $\tau_c = 4.71$ days. For lags greater than this value oscillations occur with periods greater than 16.22 days. In (*1*) the simulations are compared with observed periodic fluctuations in patients suffering from chronic granulocytic leukemia. For the choice of Λ in Eq. 2 it can be seen, for example, that τ_c increases if β_0 or n is decreased (while the other parameters are held fixed).

Results qualitatively the same as those stated above for the function Λ given by Eq. 2 are obtained for a wide class of such functions that depend only on the density at a lagged time. In particular, the onset of stable oscillations is insensitive to the algebraic sign of the second derivative of Λ at x_c. The algorithm used to derive the results presented here (*4*, *5*) gives an exact asymptotic formula for the period and amplitude of the bifurcating periodic solutions and enables their stability (or instability) to be determined. No recourse to numerical methods is required. The theory is, however, a local one; thus the sequence of bifurcations and aperiodic (chaotic) behavior, found numerically in (*1*) for some Λ when $\tau >> \tau_c$, is outside the range of the analytical results reported here.

Equation 1 may not be sufficiently general to model the blood cell control mechanism accurately. Mackey (*2*) considers a more general Λ that includes dependence on the present state. Mackey and Glass (*1*, *3*) consider an arterial CO_2 control system that also includes this form of Λ, which is explicitly

$$\Lambda(x(t), x(t - \tau)) = \lambda - \frac{\alpha V_m x(t) x^n(t - \tau)}{\theta^n + x^n(t - \tau)} \qquad (5)$$

where λ, α, V_m, θ, and n are constants, x is the arterial CO_2 concentration, and τ is the time between oxygenation of blood in the lungs and stimulation of receptors in the brainstem. For this Λ, with $a = 0$, τ_c is given in (*1*), and the algorithm of (*4*) shows that for normal ranges of parameter values stable periodic solutions occur in a range of τ for $\tau > \tau_c$ with period $> 4\tau_c$. Thus the advice for regulation of respiratory disease modeled by Eqs. 1 and 5 is again to seek to increase the critical lag.

More realistic models may require multiple lags or weighted lags. Such models can be analyzed by the algorithm in (*4*) [see also (*5*, *6*)]. Experiments are needed to establish the connection between the physiology of control processes subject to diseases with oscillatory symptoms (for instance, hematopoiesis) and the various model parameters; these could lead to means for control of the critical lag to cause less likelihood of stable or unstable oscillations.

NICHOLAS D. KAZARINOFF
Department of Mathematics, State University of New York, Buffalo 14214
PAULINE VAN DENDRIESSCHE
Department of Mathematics, University of Victoria, Victoria, British Columbia, Canada V8W2Y2

References and Notes

1. M. C. Mackey and L. Glass, *Science* **197**, 287 (1977).
2. M. C. Mackey, in *Cellular Mechanisms of Disease and Aging*, in press.
3. L. Glass and M. C. Mackey, *Ann. N.Y. Acad. Sci.*, in press.
4. N. D. Kazarinoff, Y. H. Wan, P. van den-Driessche, *J. Inst. Math. Its. Appl.* **21**, 461 (1978).
5. S. N. Chow and J. Mallet-Paret, *J. Diff. Eqs.* **26**, 112 (1977).
6. J. Kaplan and J. A. Yorke, *ibid.* **23**, 293 (1977).
7. This research was partially supported by National Science Foundation and National Research Council of Canada grants MC-78-19647 and A-4645.

11 August 1978; revised 27 November 1978

Sodium-Selective Liquid Ion-Exchanger Microelectrodes for Intracellular Measurements

Abstract. *The sodium-selective ligand 1,1,1-tris[1-(2-oxa-4-oxo-5-aza-5-methyl) dodecanyl]propane dissolved in 3-nitro-o-xylene containing a small amount of the lipophilic anion tetrachlorophenyl borate was used as a liquid ion-exchanger in sodium-selective microelectrodes. The microelectrodes gave rapid, stable responses that were linear functions of the logarithm of sodium activity. They were tested under conditions approximating those to be expected in the cell interior, and the results indicated that they can be used to measure intracellular sodium activity without significant interference from intracellular potassium.*

Intracellular ionic activity measurements with ion-selective microelectrodes are important in many areas of biophysics and physiology (*1*). The regulatory role of Na^+ in cellular function (*2*) makes the development of simple, accurate methods for measuring intracellular Na^+ activity (a^i_{Na}) of special interest. For several years, liquid ion-exchanger solutions (477317 and 477315, Corning Glass Works, Corning, N.Y.) selective to K^+ and Cl^-, respectively, have been commercially available, and they are widely used to make microelectrodes for measuring intracellular K^+ and Cl^- activities (*1*, *3*). So far, the development of liquid ion-exchanger microelectrodes, capable of measuring a^i_{Na} without significant interference from cellular K^+, has been frustrated by the lack of a ligand that, in liquid ion-exchanger form, discriminates at a sufficiently high level between these ions.

Palmer and Civan (*4*) used a 1.5 percent solution of potassium tetrachlorophenylborate in trihexylphosphate as the liquid ion-exchanger solution in Na^+-selective microelectrodes. Recently, we modified the Na^+-selective microelectrodes developed for extracellular Na^+ activity measurements by Kraig and Nicholson (*5*) and used them to measure steady-state a^i_{Na} values in epithelial cells of *Necturus* and bullfrog small intestine (*6*). The Na^+-sensing element in our microelectrodes was a 10 percent solution of the antibiotic monensin (*7*) in 3-nitro-*o*-xylene containing a small amount

0036-8075/79/0330-1349$00.50/0

(~ 1.0 percent) of potassium tetrachlorophenylborate. These microelectrodes have one serious limitation, their relatively low Na^+/K^+ selectivity ratios (average value about 3/1). Thus it is necessary in determining a^i_{Na} to apply a correction for K^+ interference whose magnitude may equal or exceed that of a^i_{Na}. Although this can be done, under steady-state conditions (*4*, *6*), by measuring a^i_K independently (for instance, with a K^+-selective microelectrode containing Corning 477317), it is a potentially serious source of error in the estimate of a^i_{Na}. Further, any attempt to monitor experimentally induced changes in a^i_{Na} with Na^+-selective microelectrodes of this kind would be, at best, extremely uncertain.

Very recently (*8*), the gift of a sample of the neutral ionophore 1,1,1-tris[1'-(2'-oxa-4'-oxo-5'-aza-5'-methyl) dodecanyl] propane (*9*) enabled us to develop Na^+-selective liquid ion-exchanger microelectrodes that are essentially free from K^+ interference over a range of activity ratios (a_K/a_{Na}) corresponding to those one would expect to encounter in cytoplasm. When this compound was incorporated into polyvinyl chloride membrane electrodes (*9*), their response to 100 m*M* NaCl and KCl solutions indicated a Na^+/K^+ selectivity of about 250.

We used conventional methods (*3*, *6*) to construct liquid ion-exchanger microelectrodes containing this compound (*10*). Attempts to use the liquid ion-exchanging solution given to us (10 percent Na^+-selective ligand plus 90 percent *o*-nitrophenyl octyl ether) were frustrated by the excessive times (5 to 10 minutes) required for the resulting microelectrodes to attain a steady potential in NaCl solutions. Their electrical responses to KCl solutions were even slower. Such slow responses in liquid systems where a hydrophobic phase separates two aqueous solutions are frequently due to the poor electrical conductance of the nonaqueous layer. In these systems, the conductance of this layer is dramatically increased when certain large hydrophobic anions, such as tetraphenylborate, are dissolved in it (*11*). We found earlier (*6*) that the presence of tetrachlorophenylborate in the ion-exchanger solution significantly shortened the response time of Na^+-selective microelectrodes containing monensin. The neutral ionophore (*9*) was therefore synthesized. We used 10 percent (by volume) of this compound, dissolved in 3-nitro-*o*-xylene containing a small amount (~ 1 percent) of tetrachlorophenylborate, as the liquid ion-exchanger (*12*).

We calibrated the microelectrodes at 23°C in 5, 10, 20, 50, and 100 m*M* NaCl or KCl solutions. Steady-state microelectrode potentials were plotted as a function of log a_{Na} or log a_K, and lines of best fit to the data were calculated by linear regression analysis (Fig. 1A). The a_{Na} and a_K values were calculated from an extended form of the Debye-Hückel equation (*6*). In NaCl solutions, microelectrode responses were complete about 10 seconds after immersion. The average slope S_{Na} of the plot of potential versus log a_{Na} was 62.9 ± 1.3 mV (standard error of the mean; $N = 10$; $r > 0.998$ in every instance). Microelectrode resistances in 100 m*M* NaCl were about 2×10^9 ohms. The selectivity coefficient K_{NaK} (which may be equated to the reciprocal of the Na^+/K^+ selectivity ratio) in 100 m*M* NaCl versus 100 m*M* KCl was calculated (*1*) for seven microelectrodes. It ranged from 0.01 to 0.005 (mean value, 0.008). However, the slopes S_{Na} and S_K of the electrode potentials as a function of log a_{Na} and log a_K (Fig. 1A) differed significantly in every instance, so that K_{NaK} showed concentration (activity) dependence. Some microelectrodes failed to show a linear response to log a_K over the concentration range examined, and the potentials registered in dilute KCl solutions (5 to 20 m*M*) were often highly unstable. This instability suggests that a very small amount of K^+ was solubilized in the ion-exchanger solution. It was not further investigated.

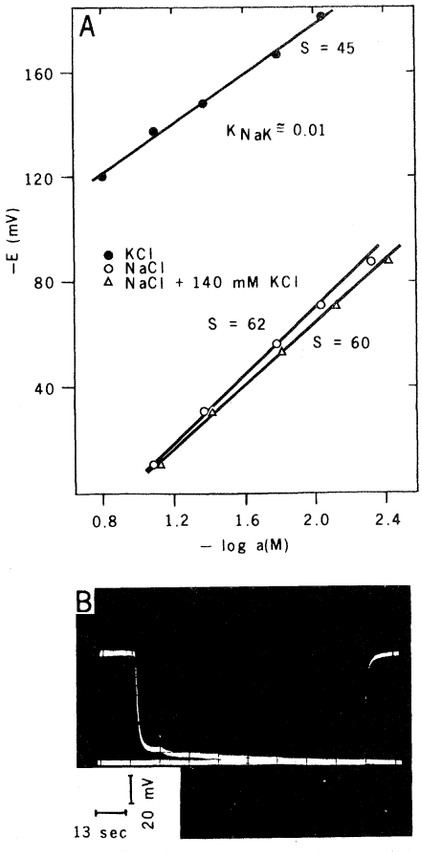

Fig. 1 (A). Steady-state electrical potentials (with respect to a grounded reference electrode in the test solution) registered by a Na^+-selective liquid ion-exchanger microelectrode plotted as a function of (●) log a_K or (○ and △) log a_{Na}. Lines were fitted to the data by least-squares analysis. (B) Impalement, through its mucosal membrane, of an epithelial cell in *Necturus* small intestine with a Na^+-selective microelectrode similar to the one represented in (A).

It seemed more appropriate for our purpose to determine the extent, if any, to which K^+ interfered with the Na^+ response of the microelectrodes under conditions approximating those to be expected in the cell interior. We therefore calibrated our microelectrodes in solutions containing 5, 10, 20, 50, and 100 m*M* NaCl together with 140 m*M* KCl. This KCl concentration corresponds to the mean a^i_K (108 m*M*) previously reported (*6*) for epithelial cells of *Necturus* small intestine. The electrical response of the microelectrodes to these solutions was rapid (~ 10 seconds), stable, and (Fig. 1A) closely paralleled their response to solutions containing the same concentrations of NaCl alone. Most significantly, ten microelectrodes calibrated in this way showed a linear relation between the electrode potential and log a_{Na} ($r > 0.995$ in every instance). Their average slope (S_{NaK}) was 57.4 ± 1.3 mV. This did not differ significantly ($P > .1$) from the calculated slope (58.7 mV) for a perfect Na^+ electrode at 23°C. Hence we conclude (*1*) that these microelectrodes can be used to measure a^i_{Na} in most cells without significant interference from intracellular K^+.

Epithelial cells of *Necturus* small intestine stripped of external muscles were mounted as flat sheets, under open-circuit conditions at 23°C, between identical oxygenated solutions containing 100 m*M* Na^+ and Cl^- ($a_{Na} = a_{Cl} = 77$ m*M*) and 5.4 m*M* K^+ (*p*H 7.2). The cells were impaled through their mucosal aspect with the Na^+-selective microelectrodes. The tissue chamber, methods of electrical recording, and criteria used to determine the acceptability of impalements are described in detail elsewhere (*6*). Microelectrodes were calibrated in NaCl solutions containing 140 m*M* KCl before and after each experiment. If S_{NaK} changed by more than ± 1 mV between calibrations, or if the microelectrode potential in the mucosal medium changed by more than ± 2 mV during an experiment, the results were discarded.

Figure 1B is an oscilloscope tracing of a cell impalement recorded in one of

these experiments. After impalement there was a rapid downward (negative) deflection of the microelectrode potential, which reached a minimum (− 87 mV) about 1 minute after impalement. The delay probably reflects local membrane damage followed by spontaneous repair at the site of impalement (*1, 3, 6*). On reaching its minimum value after impalement, the potential remained steady until the microelectrode was withdrawn 3 minutes after a steady state was established. After withdrawal, the potential returned rapidly to its initial value (7 mV) in the mucosal medium.

The equation $a^{i}_{Na}/a^{o}_{Na} = 2.303 \exp [(\Delta E - E_m)/S_{NaK}]$ was used to calculate a^{i}_{Na} from 14 recordings, similar to Fig. 1B, in tissues from six different animals. Here ΔE is the difference between the steady-state microelectrode potentials in the cell interior and mucosal medium (average value, − 88.2 mV), E_m is the mucosal membrane potential, and S_{NaK} is the slope of the microelectrode used to measure each value of ΔE. The average E_m (− 35 mV) found previously (*6*) in 104 impalements with tissues from 11 different animals was assumed in the present study.

The a^{i}_{Na} in these experiments was 9.1 ± 0.9 m*M*. This agrees reasonably well with the value previously found (*6*) with Na^+-selective microelectrodes containing monensin (6.3 m*M*). We believe, however, that this agreement reflects the capacity of poorly selective microelectrodes, when properly used, to yield reasonably accurate estimates of a^{i}_{Na} under steady-state conditions (*4, 6*), rather than the utility, for this purpose, of the highly Na^+-selective microelectrodes described in this report.

J. O'Doherty
J. F. Garcia-Diaz
W. McD. Armstrong
Department of Physiology,
Indiana University School of Medicine,
Indianapolis 46223

References and Notes

1. A. A. Lev and W. McD. Armstrong, *Curr. Top. Membr. Transp.* **6**, 59 (1975); W. McD. Armstrong, in *Water Relations in Membrane Transport in Plants and Animals*, A. Jungreis *et al.*, Eds. (Academic Press, New York, 1977), p. 215; J. L. Walker and A. M. Brown, *Physiol. Rev.* **57**, 729 (1977); M. M. Civan, *Am. J. Physiol.* **234**, F261 (1978).
2. I. M. Glynn and S. J. D. Karlish, *Annu. Rev. Physiol.* **37**, 13 (1975).
3. J. L. Walker, Jr., *Anal. Chem.* **43**, 89A (1971); in *Ion and Enzyme Electrodes in Biology and Medicine*, M. Kessler *et al.*, Eds. (University Park Press, Baltimore, 1976), p. 116; R. N. Khuri, J. J. Hajjar, S. K. Agulian, *J. Appl. Physiol.* **32**, 419 (1972); J. F. White, *Am. J. Physiol.* **231**, 1214 (1976).
4. L. G. Palmer and M. M. Civan, *J. Membr. Biol.* **33**, 41 (1977).
5. R. P. Kraig and C. Nicholson, *Science* **194**, 725 (1976).
6. J. F. Garcia-Diaz, J. O'Doherty, W. McD. Armstrong, *Physiologist* **21**, 41 (1978); W. McD. Armstrong, W. R. Bixenman, K. F. Frey, J. F. Garcia-Diaz, M. G. O'Regan, J. L. Owens, *Biochim. Biophys. Acta*, in press.
7. A. Agtarap, J. W. Chamberlain, M. Pinkerton, L. Steinrauf, *J. Am. Chem. Soc.* **89**, 5737 (1967).
8. J. O'Doherty, J. F. Garcia-Diaz, W. McD. Armstrong, *Physiologist* **21**, 86 (1978).
9. M. Güggi, M. Oehme, E. Pretsch, W. Simon, *Helv. Chim. Acta* **59**, 2417 (1976). W. Simon, Eidgenössische Technische Hochschule, Zurich, kindly supplied this compound.
10. We pulled micropipettes (tip diameters, ~1 μm) from Kwik-Fil borosilicate glass tubing (outer diameter, 1.2 mm; inner diameter, 0.68 mm; W-P Instruments, New Haven, Conn.), silanized their inside surfaces with dimethylchlorosilane (Pierce Chemical Co., Rockford, Ill.), introduced a column (200 μm to 2 mm long) of the liquid ion-exchanger solution into their tips, and filled them with 0.5 or 0.1*M* NaCl. Calibration and intracellular recording were performed as described elsewhere (*6*).
11. P. Läuger and B. Neumke, in *Membranes*, G. Eisenman, Ed. (Dekker, New York, 1973), vol. 2, p. 1.
12. Aside from the Na^+-selective ligand (*9*), this solution is similar in composition to Corning 477317 K^+-exchanger. We found that a solution of the ligand in Corning 477317 can be used to make Na^+-selective microelectrodes similar to those described here.
13. Supported by USPHS grant AM 12715. J. F. G.-D. was partially supported by the Ministerio de Educacion y Ciencia, Madrid.

23 October 1978; revised 19 December 1978

Membrane Surface Potential Changes May Alter Drug Interactions: An Example, Acetylcholine and Curare

Abstract. *Curare is known to be less effective as an acetylcholine antagonist when the divalent cation concentration of the extracellular solution is increased. This observation can be accounted for by the negative surface potential on the end plate; an increase in divalent cation concentration decreases the negativity of the surface potential and thereby lowers the concentrations of cations at the membrane-solution interface. The concentration of divalent cations, such as curare, will be reduced more than the concentration of univalent cations, such as acetylcholine. The observations can be accounted for by a surface potential of about −50 millivolts. The same principle can explain the reported actions of divalent cations on the affinity of receptors for acetylcholine. The effects of surface potential on concentrations at active sites may play an important role in drug interactions.*

The potency of drugs that act on the acetylcholine (ACh) receptor at the neuromuscular junction can be modified appreciably by changes in the concentrations of ions in the extracellular solution. Increasing the concentration of either Ca^+ or Mg^{2+} decreases the effectiveness of tubocurarine as an antagonist of ACh or of carbachol-induced depolarizations (*1*). However, the effectiveness of curare is enhanced when the Na^+ concentration in the extracellular solution is reduced (*2*). These observations can be accounted for by the negative surface potential found at the end plate.

Most biological membranes have an excess of fixed, anionic groups at the membrane-solution interface. The membrane potential as measured with an intracellular microelectrode is the total voltage drop across the system. In reality this is the sum of at least three subcomponents: the surface potentials at the outer and inner interfaces and the voltage drop through the membrane itself. Increasing the divalent ion concentration in the extracellular solution reduces the negativity of the external surface potential, by screening or binding to the fixed anionic groups. If the membrane potential is held constant (with a voltage clamp, for example) the decrease in the external surface potential is accompanied by a corresponding increase in the voltage drop within the membrane. Consequently, an increase in the extracellular divalent ion concentration has the same effect on the voltage gradient through the end-plate membrane as a hyperpolarization produced by a voltage clamp (*3*). Both treatments increase the duration of miniature end-plate currents (MEPC's). Mallart and Molgo (*4*) showed that a similar lengthening is produced by lowering the extracellular *p*H. This can be accounted for if the hydro-

Table 1. Estimates of the surface potential of the muscle end plate based on curare-ACh interactions in a variety of experimental solutions. See Jenkinson (*1*) for details of the solutions. The mean ψ_s value is 49.7 ± 14.2 mV (± standard error of mean).

Experimental solution	Method	K_1'/K_1	$\Delta\psi$ (mV)	ψ Control (mV)
1. 5.4 m*M* Ca^{2+}	Microelectrode	0.52	+8.15	−87
2. 8.6 m*M* Mg^{2+}	Microelectrode	0.63	+5.87	−42
3. 5.4 m*M* Ca^{2+}	External recording	0.72	+4.07	−45
4. 2/3 Na^+, 1/3 sucrose	External recording	1.11	−1.28	−7
5. 1/3 Na^+, 2/3 sucrose	External recording	2.41	−11.01	−74
6. 8.6 m*M* Mg^{2+}	External recording	0.62	+6.08	−43

0036-8075/79/0330-1351$00.50/0