sion process. Indeed, noble and near-noble metals have been reported to diffuse by an interstitial mechanism in Pb and Sn (*49*).

Conclusion

We have attempted to illustrate the nature of thin film reactions with some cogent examples which have come principally from the field of integrated circuits. Our objective has been to show that the occurrence of low temperature solid-phase reactions can be analyzed by a variety of existing techniques. We hope that, as we come to an understanding of the physical nature of these films and their interfaces, correlations can be made with other physical parameters such as Schottky-barrier heights or superconducting transition temperatures.

In conclusion, therefore, we would like to emphasize that the study of solid-phase reactions in thin film systems and interface properties was prompted by the increased use of thin films in modern technology. The study of these systems was greatly facilitated by the development of three analytical concepts—depth profiling by nuclear techniques, the combination of surface analytical techniques combined with layer removal by sputtering, and glancing angle x-ray diffraction. These three, along with the more established tools of scanning electron microscopy, electron microprobe, and transmission electron microscopy, have provided pronounced new insights into the behavior of thin film systems.

The development of analytical tools with capability for depth microscopy on a submicron scale offers a unique opportunity for the study of low temperature reactions. Although we have stressed the study of thin film systems, these same tools have been used in studies of oxidation and corrosion, ion implantation in metals and semiconductors, and front wall degradation in fusion reactors.

References and Notes

1. J. W. M. Dumond and J. P. Youtz, *Phys. Rev.* **48**, 703 (1935).
2. ———, *J. Appl. Phys.* **11**, 357 (1940).
3. H. E. Cook and J. E. Hilliard, *ibid.* **40**, 2191 (1969); E. M. Philofsky and J. E. Hillard, *ibid.*, p. 2198.
4. K. N. Tu and B. S. Berry, *ibid.* **43**, 3283 (1972).
5. C. Weaver and L. C. Brown, *Philos. Mag.* **7**, 1 (1962); C. Weaver and D. T. Parkinson, *ibid.* **22**, 377 (1970).
6. K. N. Tu, *Acta Metall.* **21**, 347 (1973).
7. K. Nakamura, J. W. Mayer, M-A. Nicolet, C. A. Evans, R. J. Blattner, *J. Appl. Phys.*, in preparation.
8. K. Nakamura and S. S. Lau, unpublished result.
9. S. Herd, P. Chaudhari, M. H. Brodsky, *J. Non-Cryst. Solids* **7**, 309 (1972); G. Ottaviani, D. Sigurd, V. Marrello, J. W. Mayer, J. O. McCaldin, *J. Appl. Phys.* **45**, 1730 (1974).
10. M. P. Lepselter and J. M. Andrews, in *Ohmic Contacts to Semiconductors*, B. Schwartz, Ed. (Electrochemical Society, New York, 1969), p. 169; J. A. Borders and S. T. Picraux, *Proc. IEEE* **62**, 1224 (1974).
11. S. U. Campisano, G. Foti, E. Rimini, S. S. Lau, J. W. Mayer, *Philos. Mag.* **31**, 903 (1975).
12. K. N. Tu and D. A. Chance, *J. Appl. Phys.* **46**, 3229 (1975).
13. W. J. DeBonte and J. M. Poate, *Thin Solid Films* **25**, 441 (1975).
14. D. Gupta, *J. Appl. Phys.* **44**, 4455 (1973).
15. R. W. Bower and J. W. Mayer, *Appl. Phys. Lett.* **20**, 359 (1972); L. D. Locker and C. D. Capio, *J. Appl. Phys.* **44**, 4366 (1973).
16. J. M. Poate, P. A. Turner, W. J. DeBonte, J. Yahalom, *J. Appl. Phys.*, in press.
17. H. Sankur, J. O. McCaldin, J. Devaney, *Appl. Phys. Lett.* **22**, 641 (1973).
18. M-A. Nicolet, J. W. Mayer, I. V. Mitchell, *Science* **177**, 841 (1972).
19. J. M. Morabito, *Thin Solid Films* **19**, 21 (1973).
20. W. K. Chu, J. W. Mayer, M-A. Nicolet, T. M. Buck, G. Amsel, F. Eisen, *ibid.* **17**, 1 (1973).
21. J. A. Cairns, A. D. Marwick, I. V. Mitchell, *ibid.* **19**, 91 (1973).
22. G. Amsel, T. P. Nadai, E. D'Artemare, D. David, E. Girard, J. Moulin, *Nucl. Instrum. Methods* **92**, 481 (1971).
23. C. C. Chang, *Surf. Sci.* **25**, 53 (1971).
24. C. A. Evans and J. P. Pensler, *Anal. Chem.* **42**, 1060 (1970).
25. D. P. Smith, *Surf. Sci.* **25**, 171 (1971); D. J. Ball, T. M. Buck, D. MacNair, G. H. Wheatley, *ibid.* **30**, 69 (1972).
26. K. N. Tu, W. K. Chu, J. W. Mayer, *Thin Solid Films* **25**, 403 (1975).
27. J. Ziegler and W. K. Chu, *At. Data Nucl. Data Tables* **13**, 463 (1974).
28. W. K. Chu, M-A. Nicolet, J. W. Mayer, C. A. Evans, *Anal. Chem.* **46**, 2136 (1974).
29. R. Feder and B. S. Berry, *J. Appl. Phys.* **43**, 3283 (1972).
30. M. H. Read and D. H. Hansler, *Thin Solid Films* **10**, 123 (1972).
31. L. R. Testardi, R. L. Meek, J. M. Poate, W. A. Royer, A. R. Storm, J. H. Wernick, *Phys. Rev. B* **11**, 4304 (1975).
32. S. S. Lau, W. K. Chu, J. W. Mayer, K. N. Tu, *Thin Solid Films* **23**, 205 (1974).
33. J. W. Mayer and K. N. Tu, *J. Vac. Sci. Technol.* **11**, 86 (1974).
34. K. N. Tu, *Appl. Phys. Lett.* **27**, 221 (1975).
35. E. O. Kirkendall, *Trans. Am. Inst. Min. Metall. Pet. Engl.* **147**, 104 (1942).
36. W. K. Chu, H. Krautle, J. W. Mayer, H. Muller, M-A. Nicolet, K. N. Tu, *Appl. Phys. Lett.* **25**, 454 (1974).
37. A. K. Sinha and J. M. Poate, *ibid.* **23**, 666 (1973).
38. K. N. Tu, J. F. Ziegler, C. J. Kircher, *ibid.*, p. 493.
39. J. O. McCaldin, *J. Vac. Sci. Technol.* **11**, 990 (1974).
40. R. W. Bower, *Appl. Phys. Lett.* **23**, 99 (1973).
41. J. M. Caywood, *Metall. Trans.* **4**, 735 (1973).
42. J. O. McCaldin and A. Fern, *Proc. IEEE* **60**, 1018 (1972).
43. V. Marrello, T. A. McMath, J. W. Mayer, I. L. Fowler, *Nucl. Instrum. Methods* **108**, 93 (1973).
44. P. M. Hall, J. M. Morabito, J. M. Poate, in preparation.
45. K. N. Tu and R. Rosenberg, *Thin Solid Films* **13**, 163 (1972).
46. E. Philofsky, *Solid State Electron.* **13**, 1391 (1970).
47. S. T. Picraux, *Jpn. J. Appl. Phys.* (Suppl. 2, Pt. 1) (1974), p. 657.
48. K. N. Tu and R. Rosenberg, *ibid.*, p. 633.
49. W. Warburton and D. Turnbull, *Thin Solid Films* **25**, 71 (1975).

50. We acknowledge the contributions of our colleagues at Bell Laboratories, IBM–Watson Research Center, and Caltech to many of the concepts presented here. The Caltech work was supported by grants from the Ford Motor Foundation and the Office of Naval Research (L. Cooper). The IBM work was supported in part by ARPA contract F19628-74-C-0077.

Membrane Electrode Probes for Biological Systems

New sensors expand measurement horizons.

G. A. Rechnitz

Scientific progress is limited not only by the rate of generation of new ideas but also by the state of development of appropriate experimental tools and techniques. As a result, there is a constant interplay between theoretical advances and the development of new instrumentation, with one or the other leading at any particular time. Since most scientific hypotheses cannot be tested without experimentation, measurement capabilities and associated instrumentation provide a common ground for theoretical and experimental scientists and serve as a powerful integrating force between scientific specialties.

At the present time, it seems that conceptual advances in the biological and biochemical sciences have temporarily outrun our ability to perform unambiguous measurements in real systems with regard to the nature, concentration, and time-dependent fate of chemical species on the cellular and molecular level. Certainly, much of the experimental work being carried out now employs simplified "model" systems or relies upon highly indirect observations.

This situation could be changed through the development of new instrumental techniques designed to permit selective and nondestructive measurements to be made in complex systems on a direct and, preferably, continuous basis. There is reason to believe that several membrane electrode

probes have now reached a developmental stage at which they could make a useful contribution toward such measurements in biological systems ranging from bulk body fluids to material in single cells.

Ion-Selective Membrane Electrodes

While extremely valuable contributions have been made by using polarographic and other faradic techniques in conjunction with membrane systems (*1*), much of the current research involves the use of ion-selective membrane electrodes as direct probes or as the major component of multimembrane sensors (*2*). Ion-selective sensors can be conveniently grouped into three major categories on the basis of the nature of the active membrane material employed, for instance, glass electrodes, liquid membrane electrodes, and crystal membrane electrodes.

Glass electrodes employ a thin but rugged membrane of silicate glass, such as Na_2O-Al_2O_3-SiO_2 mixture, as the active sensor material (*3*). Such electrodes respond to univalent cations such as H^+, Na^+, K^+, Li^+, NH_4^+, Rb^+, Cs^+, Ag^+, TL^+, and Cu^+ but are not appreciably responsive to anions or multivalent cations (*4, 5*). Selectivity among univalent cations is achieved by altering the glass composition in order to produce anionic sites of appropriate charge and geometry on the outer layers of the glass surface (*4, 5*). The overall mechanism of glass electrode response and selectivity is complex (*5, 6*), but involves a combination of surface ion-exchange and ion-diffusion steps (*7*). There is no net transport of ions across the glass membrane during normal use.

By appropriate choice of an ion-exchanging or -carrying material, liquid membrane electrodes can be designed with response to practically any desired ion. Typically, the active liquid phase is held in an inert support membrane, made of fritted glass or porous plastic (*8*). Liquid membrane electrodes have been prepared successfully for such ions as Ca^{2+}, NO_3^-, CO_3^{2-}, ClO_4^-, Cu^{2+}, BF_4^-, Mg^{2+}, Mn^{2+}, and Cl^- as well as several charged organic species. Selectivity for a given ion depends on the nature of the active liquid phase. Both organic ion exchangers dissolved in a compatible solvent and natural or synthetic neutral ion carriers, such as antibiotics (*9*), have been employed widely for this purpose. In practice, liquid membrane electrodes often are designed to have a gradual outward flow of the liquid material from the electrode so as to avoid contamination of the electrode interior by the sample to be measured. Alternatively, the active liquid phase may be immobilized in polyvinyl chloride plastic (*10*) or coated on wires with the aid of a binding material (*11*).

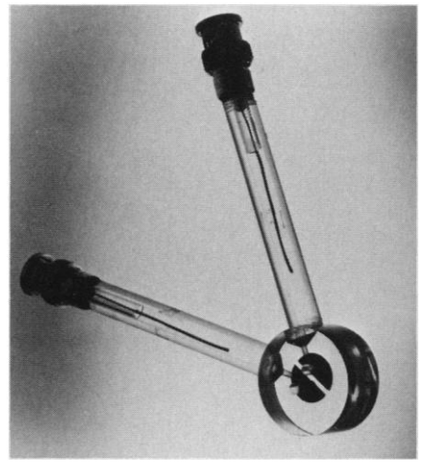

Fig. 1. Dual flow through Pb^{2+} electrode constructed by using a "split-crystal" membrane.

A particularly rugged type of sensor can be made by using solid-state or crystalline materials as the membrane phase. Such materials should be ionic conductors with low water solubility. An outstanding example of this category is the F^--selective electrode (*12*), which uses a single crystal of sparingly soluble LaF_3 as the active membrane material. This compound is an ionic conductor for F^-; high selectivity is achieved because other ions cannot enter the crystal lattice. Other successful crystal membrane electrodes are available (Fig. 1) for such ions as S^{2-}, Cu^{2+}, Pb^{2+}, Cd^{2+}, I^-, Br^-, Cl^-, CN^-, and Ag^+, all based upon Ag_2S (an ionic conductor for Ag^+) as the crystal matrix. A powerful advantage of such solid-state electrodes is that they can be prepared easily without liquid internals by directly connecting a metallic lead to the crystalline membrane material.

All three types of membrane electrodes described measure the activity of ions. When operating properly, the electrodes obey the Nernst equation,

$$E = \text{constant} + (RT/nF)\ln a_M$$

where E is the measured electromotive force, R is the gas constant, T is the absolute temperature, F is the Faraday, n is the ionic charge, and a_M is the activity of the ion to be measured. For a mixture of ions, M_1^+ and M_2^+, the equation is expanded to

$$E = \text{constant} + (RT/nF)\ln(a_{M_1^+} + Ka_{M_2^+})$$

where the new quantity, K, is the potentiometric selectivity factor, a quantitative but activity-dependent measure of an electrode's ability to discriminate among ions. In practice, selectivity factors in excess of 10^6 have been achieved for several electrodes.

The fact that potentiometric membrane electrodes measure the activity rather than the concentration of ions seems to have produced a psychological barrier to their adoption by biological scientists. Perhaps this is so because much of the scientific literature deals with total analytical concentrations; certainly, biomedical scientists have become accustomed to "normal" and "abnormal" clinical values expressed entirely in concentration terms.

This is unfortunate, because it is becoming increasingly evident that ionic activities are the variables of interest in many biochemical situations and in the diagnosis of certain disease states. According to Moore (*13*), for example, Ca^{2+} is the physiologically active variable in such processes as bone formation and resorption, nerve conduction, muscle contraction, cardiac conduction and contraction, cerebral function, renal tubular function, intestinal secretion and absorption, blood coagulation, membrane and capillary permeability, enzyme function, and hormonal release from various endocrine glands.

Moore's extensive studies with the liquid membrane type of Ca^{2+} electrode have shown that Ca^{2+} activity measurements in conjunction with total calcium determinations provide more meaningful information for studies on hyperparathyroidism, cirrhosis, and hypercalcemia of cancer than do total concentration measurements alone. One reason for this is that Ca^{2+} activity measurements can be used to estimate ion binding to body fluid proteins such as albumin. Because of the electrode's ability to measure the activity of the free metal ion in equilibrium with the ligand or ligands, it is possible to obtain not only thermodynamic formation constants but also information on the number of binding sites and the stoichiometry of the various complexes formed.

Such ion-binding studies with biological molecules are of potential importance not only to clinical measurements but to the elucidation of fundamental biochemical reaction systems of crucial significance to bioenergetics, enzyme activation, and membrane transport. Consider, for example, one of the processes of primary importance to cellular energy systems, the adenosine triphosphate (ATP)–adenosine diphosphate (ADP) cycle. Energy is released by the hydrolysis of ATP according to the reaction

$$ATP + H_2O \rightleftharpoons ADP + P_i$$

where P_i represents inorganic phosphate. In the cell, this reaction is much more complicated because the hydrolysis of

The author is professor of chemistry at the State University of New York, Buffalo 14214.

Table 1. Dissociation constants of ATP and ADP complexes measured with ion-selective membrane electrodes. All measurements were made at 25°C and zero ionic strength. [Source: (*27*)]

Reaction	K_D	Electrode
$NaATP^{3-} \rightleftarrows Na^+ + ATP^{4-}$	4.37×10^{-3}	Glass
$KADP^{2-} \rightleftarrows K^+ + ADP^{3-}$	6.66×10^{-3}	Valinomycin-based liquid membrane
$CaADP^- \rightleftarrows Ca^{2+} + ADP^{3-}$	3.48×10^{-5}	Liquid membrane Ca^{2+}
$Ca_2ADP^+ \rightleftarrows Ca^{2+} + CaADP^-$	3.70×10^{-3}	Liquid membrane Ca^{2+}
$MgADP^- \rightleftarrows Mg^{2+} + ADP^{3-}$	2.26×10^{-5}	Divalent liquid membrane
$CaATP^{2-} \rightleftarrows Ca^{2+} + ATP^{4-}$	4.27×10^{-7}	Liquid membrane Ca^{2+}
$Ca_2ATP^0 \rightleftarrows Ca^{2+} + CaATP^{2-}$	9.09×10^{-4}	Liquid membrane Ca^{2+}
$MgATP^{2-} \rightleftarrows Mg^{2+} + ATP^{4-}$	8.69×10^{-7}	Divalent liquid membrane
$Mg_2ATP^0 \rightleftarrows Mg^{2+} + MgATP^{2-}$	2.47×10^{-3}	Divalent liquid membrane
$KATP^{3-} \rightleftarrows K^+ + ATP^{4-}$	4.37×10^{-3}	Valinomycin-based liquid membrane

ATP is dependent on *p*H, metal ion activities, ligand concentrations, and other variables, so that the free energy of hydrolysis could vary by several kilocalories with changing conditions. If one could obtain precise thermodynamic information about the binding of ATP and ADP to the principal metal ions present, that is, Na^+, K^+, Ca^{2+}, and Mg^{2+}, it would be possible to construct a free energy "map" relating $\Delta G^{0\prime}$ for the hydrolysis reaction to actual metal ion levels.

Ion-selective membrane electrodes provide the means to measure the thermodynamics of interaction between ATP and ADP and the relevant metal ions. Table 1 shows dissociation constants for various metal ion–nucleotide complexes, all determined by using membrane electrodes sensitive to the respective ion of interest. The electrode methodology is much less ambiguous than previous indirect techniques, exactly because the activity of the free ion in equilibrium with the ligand is measured and, moreover, because this measurement can be carried out selectively in the presence of other reagents that may be necessary to adjust conditions of *p*H, ionic strength, and so forth.

A surprising finding of this study is the discovery of significant 2 : 1 complexes for alkaline earth ions with some of the nucleotides. The presence of these 2 : 1 complexes in equilibrium with 1 : 1 complexes has a striking effect upon the free energy of the ATP hydrolysis at higher levels of metal ion activity. As illustrated in Fig. 2, which is a free energy map calculated for the hydrolysis by using data from Table 1 for the various alkali and alkaline earth metal ion complexes of ATP and ADP, the free energy reaches a fairly constant maximum value at Mg^{2+} levels higher than $10^{-3}M$ when 2 : 1 complexes are taken into account. The same map, calculated without taking the 2 : 1 complexes into account, shows a continuing change of free energy in the direction of increasing spontaneity. If the latter case were correct, organisms that derive energy via ATP hydrolysis would benefit from internal environments having very high metal ion levels. This is not so, and the ability of ATP and ADP to form both 1 : 1 and 2 : 1 complexes provides for a fairly constant free energy of ATP hydrolysis over a range of metal ion levels. A logical future step for this research would be to measure the interaction of metal ions with enzymes such as adenosine triphosphatase, by using ion-selective membrane electrodes as probes, in the hope of finding correlations between patterns of enzyme activation and metal ion binding.

Few instruments are more commonly found in scientific laboratories than *p*H meters and *p*H-type glass electrodes. Why, then, has the entirely parallel array of ion electrodes and associated apparatus been so slow to gain similar acceptance? Reasons surely include problems with commercial availability and reliability, a lack of impartial electrode evaluation and comparison with independent methods, as well as an insufficient effort on the part of practitioners in the field to define and explain the underlying principles in a clear, jargon-free manner. Many laboratories are now adopting membrane electrodes, however, and biological scientists are likely to feel more comfortable with the techniques involved as rational activity scales (*14*) for alkali metal and alkaline earth ions become more widely accepted and as reliable standards based on these scales become readily available.

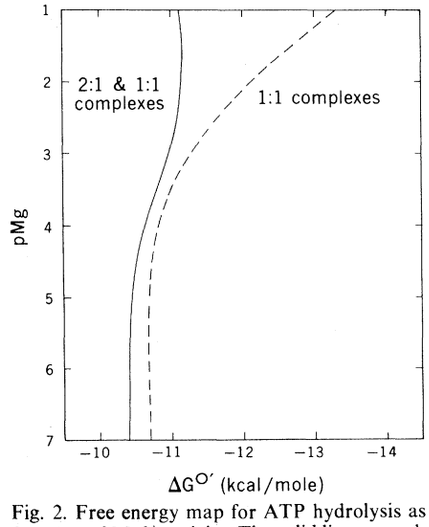

Fig. 2. Free energy map for ATP hydrolysis as function of Mg^{2+} activity. The solid line was calculated for both 1 : 1 and 2 : 1 complexes; the broken line, for 1 : 1 complexes only (25°C, *p*Ca 4.0, *p*H 9.0).

Gas-Sensing Membrane Electrodes

Some 15 years after the development of the Severinghaus (*15*) potentiometric CO_2 electrode, there has been a renaissance for this type of membrane electrode, with a resultant development of several new gas sensors, notably for NH_3 and SO_2, but with wide possibilities for other gaseous species. These electrodes employ a double membrane system in which a thin layer of an appropriate filling solution separates an external gas-permeable membrane from an internal sensing electrode of the ion-selective membrane type. The solution layer and internal sensing membrane electrode are so chosen that the gaseous species to be measured, after passing through the outer membrane, shifts a chemical equilibrium in the filling solution to produce an ion activity change that can be sensed by the internal membrane electrode (*16*).

In the Severinghaus electrode this is accomplished by interposing a dilute sodium bicarbonate solution between a gas-permeable membrane and a *p*H-type glass membrane inner electrode. The same basic principle can be utilized for other gas-sensing electrodes by taking advantage of new developments in ion-selective membrane electrodes and the availability of improved microporous hydrophobic membranes (*16*). Such gas-sensing electrodes are now commercially available not only for the measurement of CO_2 but also for NH_3, SO_2, and NO_2. As noted by Ross *et al.* (*16*), the development of additional gas electrodes should be feasible by judicious selection of inner ion-sensing electrodes, for instance, an H_2S sensor with the S^{2-} electrode, an HF sensor with the F^- electrode, an HCN sensor with an Ag^+ electrode, and a Cl_2 sensor with a Cl^- electrode.

Such electrodes are certain to have a major impact on measurement in biological systems. Recently, Ladenson *et al.* (*17*) used both gas- and ion-sensing electrodes for continuous real-time monitoring of metabolic parameters in growing bacterial cultures. Using commercially available components, they monitored changes in *p*H, CO_2, and NH_3 in situ during growth of *Proteus* cultures and demonstrated the advantages of such continuous, nonde-

structive measurements over earlier sampling techniques. If the size of the sensing electrodes could be sufficiently scaled down, similar measurements within individual cells might become possible.

For the routine analysis of patient samples in hospitals or service laboratories, there is still some question whether gas-sensing or ion-sensing electrodes should be chosen in those cases where a choice is possible. Consider the determination of CO_2 content in blood serum, for example. Current practice is to collect patient samples, remove the red cell material, and determine the CO_2 content of the serum by measurement of *p*H and pCO_2 with glass and gas-sensing electrodes, respectively. Difficulties arise, however, owing to volatilization of CO_2 during sample handling and treatment. Moreover, at physiological *p*H much of the CO_2 is present as HCO_3^- and it is necessary to have precise knowledge of both the *p*H and pCO_2 values in order to obtain meaningful results. Ideally, one would like to have an HCO_3^--selective membrane electrode for these measurements, but no satisfactory HCO_3^- electrode is available. However, a CO_3^{2-}-selective electrode was recently reported (*18*). Since CO_2, HCO_3^-, and CO_3^{2-} are all dynamically related via *p*H-dependent equilibria ($K_1 = 4.5 \times 10^{-7}$, $K_2 = 4.8 \times 10^{-11}$), the CO_2 content of a solution could also be determined by measuring CO_3^{2-} activity provided the *p*H of the sample is raised to above ~ 8. This step would also prevent volatilization of CO_2 from the sample; if the *p*H adjustment is carried out by the addition of a buffer, a separate *p*H measurement would no longer be required. Thus, the CO_3^{2-} electrode measurement provides, in principle, an alternative to the pCO_2 electrode technique. Figure 3 shows a comparison of CO_2 values determined on 28 serum samples from hospital patients by both the pCO_2 electrode and the CO_3^{2-} electrode (*19*). The correlation coefficient of 0.931 is reasonable for such samples and suggests that further development of the ion electrode approach might be attractive in this instance, especially if an automated analysis is desired.

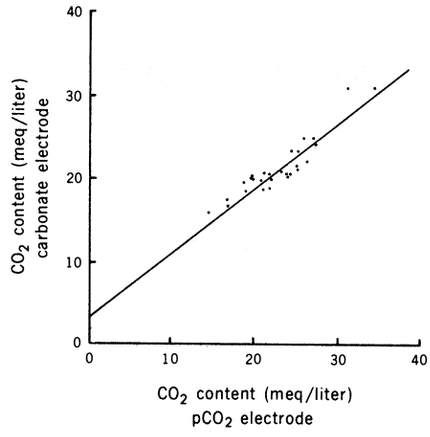

Fig. 3. Comparison of CO_2 determination in serum samples of hospital patients by pCO_2 gas electrode and CO_3^{2-}-selective liquid membrane electrode. Correlation coefficient = 0.931.

Enzyme Electrodes

Both ion-selective and gas-sensing membrane electrodes can be used in conjunction with enzymes to make selective biosensors for numerous important substrates. Typically, the appropriate enzyme (or enzymes) is held or immobilized at the surface of the potentiometric sensing electrode, which is chosen to respond to changes in the surface steady-state concentration of one of the reactants or products of the enzyme-catalyzed reaction.

Enzyme electrodes based on conventional ion-selective membrane electrodes have been reviewed in *Science* (*20*), but relatively little attention has been given to the benefits and potentialities of enzyme probes using the newer gas-sensing electrodes as building blocks. A principal advantage of the latter type of enzyme electrode is that it can be constructed to be free of both ionic and protein interferences. This has been demonstrated (*2, 21*) in the case of urea sensors in which urease enzyme layers are used in conjunction with the NH_3 gas-sensing membrane electrode for the determination of urea in whole blood, without prior removal of the red cell material.

The use of the NH_3 gas-sensing electrode in making enzyme electrodes is illustrated in Fig. 4, a schematic diagram of an AMP-responsive sensor currently under development (*22*). For this electrode, a thin layer of the enzyme AMP deaminase (E.C. 3.5.4.6) in glycerol suspension is held on the sensing surface of the NH_3 electrode by means of a thin dialysis membrane, which also serves to screen out proteins and other materials of high molecular weight. The overall electrode operates by the selective enzymatic deamination of AMP to produce stoichiometric amounts of NH_3, which diffuse through the gas-permeable hydrophobic membrane of the gas electrode to produce a change in the electrode potential. This change in potential can be related to the original AMP concentration via a calibration curve of the type shown in Fig. 5. Although the AMP sensor is still in a stage of early development, it shows promise as a useful tool for monitoring protein synthesis and for numerous other biochemical studies and may be a forerunner of other nucleotide-selective sensors.

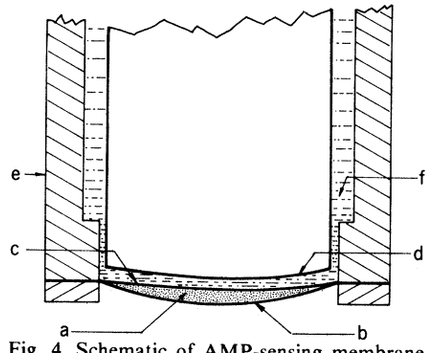

Fig. 4. Schematic of AMP-sensing membrane electrode; *a*, AMP deaminase enzyme layer; *b*, cellophane dialysis membrane; *c*, NH_3 gas-permeable membrane; *d*, internal sensing element; *e*, plastic electrode body; and *f*, internal filling solution.

The design and refinement of enzyme electrodes require considerable attention to detail if a sensor of practical usefulness is to be attained. In particular, careful consideration must be given to the factors that determine the selectivity, sensitivity, response time, and lifetime of enzyme electrodes. Selectivity will be primarily determined by the biological selectivity of the enzyme itself, but also by the potentiometric selectivity of the sensing electrode used inside the enzyme layer. In the case of the AMP electrode, one would expect limitations in this regard arising from *p*H extremes as well as from the presence of any volatile amines in the sample. The analytical sensitivity of the enzyme probe is determined by both the levels of enzyme activity in the reaction layer and the inherent sensitivity of the inner potentiometric electrode. Sensitivities as low as 10^{-5} or $10^{-6}M$ should be relatively easy to attain, but such limits may not be low enough for some measurements in biological systems. In that event, possible use of enzyme activators or amplification reactions could be investigated.

The response time of these electrodes also depends on the level of enzyme activity, since the enzyme acts as a catalyst, but even more critically on the design geometry of the probe assembly because reaction products must reach the sensing element by diffusion. Generally, the response times of such triple membrane electrodes are of the order of 1 to 5 minutes for the electrode geometry shown in Fig. 4. The lifetime of an enzyme electrode is mainly a function of the stability of the enzyme itself, which, in turn, depends upon storage conditions and operating temperature; data are not yet available for the AMP electrode, but the earlier urea sensor had no appreciable loss of activity during 3 weeks of nearly constant use.

Possible enzyme electrodes are by no

means restricted to systems using the NH_3 gas electrode. Since many enzymes produce CO_2 as a reaction product, the construction of enzyme probes based on CO_2-sensing electrodes for such substrates as uric acid and lysine should be feasible. Indeed, there is no basic reason to limit the concept of an enzyme electrode to a single enzyme immobilized or trapped at the sensor surface. If a single enzyme reaction does not produce a reaction product conveniently measurable with a membrane electrode, a second enzyme could be added to break down the primary products to suitable species.

As more and improved enzyme electrodes become available (some are now being produced commercially), imaginative applications for measurements in biological systems are certain to follow. Such applications are likely to include clinical monitoring and analysis, production monitoring in the pharmaceutical industry, as well as environmental measurements. With miniaturization of sensors, direct implantation in living organisms and measurements on the intracellular level become attractive. Significant progress has already been reported on applications to neurophysiology (*1*) and on implantation of a glucose sensor for development of an artificial pancreas (*23*). At the same time, with more critical data and accumulated experience on actual electrode assemblies (*24*) and model systems (*25*), the biological scientist will be able to make an intelligent judgment on whether or not the necessary investment in time and effort needed to utilize electrodes as biological probes is justified by the potential benefits.

Implications for the Future

There is reason to believe that membrane electrodes could also become useful for measurements of such biological materials as proteins and hormones, and for monitoring of immunochemical processes

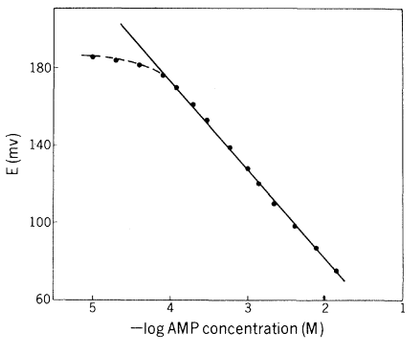

Fig. 5. Potentiometric response of AMP-sensing membrane electrode. The calibration curve was constructed with aqueous AMP solutions in 0.05*M* tris(hydroxymethyl)aminomethane buffer (*p*H 7.48, 27°C). Slope = −46 mv per concentration decade.

(*2*). Increasing knowledge (*26*) about the specificity of antibodies in terms of their binding functions suggests that antibodies or antigens might be used as selective layers in conjunction with membrane electrodes.

Beyond the technical implications of the membrane electrode field and its future possibilities, there is the basic dilemma facing scientists working in instrumentation-oriented areas, especially those that are "interdisciplinary." Should the researcher's work be directed toward the development and eventual perfection of a specific but limited measurement system, or should a more fundamental approach to the field in terms of broad principles be emphasized? This choice is crucial to young scientists entering the field in an era of "relevance" which, at the same time, limits scientific support and opportunities. The issue, moreover, challenges the academic view of measurement and instrumentation as an applied field. Its long history of practical contributions has overshadowed the fundamental nature of measurement science, that is, that our measurement capabilities serve to define the scope of science and, in themselves, create new areas of science.

References and Notes

1. L. C. Clark, Jr., *Am. J. Ment. Defic.* **77**, 633 (1973).
2. G. A. Rechnitz, *Chem. Eng. News* **53** (No. 4), 29 (1975).
3. G. Eisenman, D. O. Rudin, J. U. Casby, *Science* **126**, 831 (1957).
4. G. Eisenman, *Adv. Anal. Chem. Instrum.* **4**, 213 (1965).
5. ———, *Biophys. J.* **2**, 259 (1962).
6. J. Koryta, *Ion-Selective Electrodes* (Cambridge Univ. Press, Cambridge, England, 1975), chap. 2.
7. G. Eisenman, in *Glass Electrodes for Hydrogen and Other Cations*, G. Eisenman, Ed. (Dekker, New York, 1967), chap. 5; R. H. Doremus, in *ibid.*, chap. 4; B. P. Nicolsky, M. M. Shultz, A. A. Belijustin, A. A. Lev, in *ibid.*, chap. 6.
8. G. M. Shean and K. Sollner, *Ann. N.Y. Acad. Sci.* **137**, 759 (1966); O. D. Bonner and J. Lunney, *J. Phys. Chem.* **70**, 1140 (1966); M. Kahlweit, *Pfluegers Arch. Gesamte Physiol. Menschen Tiere* **271**, 139 (1960); G. Eisenman, *Anal. Chem.* **40**, 310 (1968).
9. Z. Stefanac and W. Simon, *Microchem. J.* **12**, 125 (1967); W. E. Morf, D. Ammann, E. Pretsch, W. Simon, *Pure Appl. Chem.* **36**, 421 (1973).
10. G. J. Moody and J. D. R. Thomas, *Selective Ion Sensitive Electrodes* (Merrow, Watford, England, 1971), chap. 7.
11. R. W. Cattrall and H. Freiser, *Anal. Chem.* **43**, 1906 (1971); M. D. Smith, M. A. Genshaw, J. Greyson, *ibid.* **45**, 1782 (1973).
12. M. S. Frant and J. W. Ross, *Science* **154**, 1553 (1966).
13. E. W. Moore, in *Ion Selective Electrodes*, R. A. Durst, Ed. (National Bureau of Standards Special Publication 314, Government Printing Office, Washington, D.C., 1969), chap. 7.
14. R. G. Bates, *Pure Appl. Chem.* **36**, 407 (1973).
15. W. Severinghaus and A. F. Bradley. *J. Appl. Physiol.* **13**, 515 (1958).
16. J. W. Ross, J. H. Riseman, J. A. Krueger, *Pure Appl. Chem.* **36**, 473 (1973).
17. J. H. Ladenson, M. Huebner, J. J. Marr, *Anal. Biochem.* **63**, 56 (1975).
18. H. B. Herman and G. A. Rechnitz, *Science* **184**, 1074 (1974).
19. G. J. Nogle, thesis, State University of New York, Buffalo (1975).
20. D. A. Gough and J. E. Andrade, *Science* **180**, 380 (1973).
21. G. G. Guilbault and M. Tarp, *Anal. Chim. Acta* **73**, 355 (1974).
22. D. S. Papastathopoulos and G. A. Rechnitz, unpublished results.
23. K. W. Chang, S. Aisenberg, J. S. Soeldner, J. M. Hiebert, *Trans. Am. Soc. Artif. Int. Organs* **19**, 352 (1973); S. P. Bessman and R. D. Schultz, in *Ion Selective Microelectrodes*, H. J. Berman and N. C. Hebert, Eds. (Plenum, New York, 1974), pp. 189–197.
24. M. Nanjo and G. G. Guilbault, *Anal. Chim. Acta* **73**, 367 (1974); *ibid.* **75**, 169 (1975); M. D. Smith and C. L. Olson, *Anal. Chem.* **46**, 1544 (1974).
25. P. Davies and K. Mosbach, *Biochim. Biophys. Acta* **370**, 329 (1974); D. F. Ollis and R. Carter, in *Enzyme Engineering*, E. K. Pye and L. B. Wingard, Eds. (Plenum, New York, 1974), vol. 2, pp. 271–278.
26. F. F. Richards, W. H. Konigsberg, R. W. Rosenstein, J. M. Varga, *Science* **187**, 130 (1975).
27. E. J. Fogt and G. A. Rechnitz, *Arch. Biochem. Biophys.* **165**, 604 (1974).

28. I thank the National Institutes of Health and National Science Foundation for grants in support of this work.