served external larval shell sculpture, which appears to be of taxonomic significance in certain Recent species (*8, 9*), may also be helpful in the study of fossil forms (Fig. 1C). Detailed examination of sequential growth series from single localities or horizons will permit generic or even specific identification of numerous larval bivalves (*14*) and should be of assistance in phylogenetic studies. The Late Mesozoic was marked by a spectacular bivalve radiation (*15*), and Cretaceous growth series will give insight into the ancestral larval morphologies and hence the relationships between some of the major families of heterodont eulamellibranchs. In addition, it will be possible to test Kauffman's suggestion (*16*) that the vulnerability of certain benthic groups to massive extinction at the end of the Late Cretaceous may be traced to their planktotrophic larval stages. Nonplanktotrophic groups (*17*) might be expected to be relatively unaffected by the Cretaceous-Tertiary boundary event.

Kauffman (*18*) and Scheltema (*19*) have stressed the significant role that pelagic dispersal must have played in shaping bivalve paleobiogeography, particularly in the light of shifting paleocontinental configurations. It appears that an ontogenetic history is available for at least some fossil species. The interpretation of these fossil larvae, linked with the distribution of the adult stages, will be an important step in our understanding of molluskan paleodistributions.

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References and Notes

1. S. Loven, *K. Sven. Vetenskapsacad. Handl.* **4**, 299 (1848); N. H. Odhner, *Zool. Anz.* **44**, 156 (1914); M. V. Lebour, *J. Mar. Biol. Assoc. U.K.* **23**, 119 (1938); B. Werner, *Zool. Jahrb. Abt. Anat. Ontog. Tiere* **66**, 1 (1939); C. B. Jørgensen, *Medd. Dan. Fisk. Havunders.* **4**, 277 (1946); C. M. Sullivan, *Bull. Fish. Res. Board Can.* **77**, 1 (1948); K. A. Zakhvatkina, *Trudy Sevastop. Biol. Stn. Akad. Nauk Ukr. SSR* **11**, 108 (1959); I. Miyazaki, *Jpn. Soc. Sci. Fish. Bull.* **28**, 955 (1962); G. E. Newell and R. C. Newell, *Marine Plankton: A Practical Guide* (Hutchinson Educational Ltd., London, 1963); V. L. Loosanoff and H. C. Davis, in *Advances in Marine Biology*, F. S. Russell, Ed. (Academic Press, London, 1963), vol. 1, p. 1; ——, P. E. Chanley, *Malacologia* **4**, 351 (1966).
2. M. LaBarbera, *J. Paleontol.* **48**, 265 (1974).
3. P. Huddleston [personal communication, cited in (*2*)] has suggested that LaBarbera's material came from sediments of Middle Pliocene, rather than Miocene, age.
4. The clayey silt-fine sands of the Ripley Formation of Mississippi and Georgia, the Owl Creek Formation of Mississippi, the Coon Creek Formation of Tennessee, and the Monmouth Formation of Maryland appear to protect original aragonitic and calcitic shells from groundwater dissolution or recrystallization (or both). All of these horizons thus produce an unusually complete record of the carbonate-secreting fauna. S. Weiner, H. A. Lowenstam, and L. Hood [*Proc. Natl. Acad. Sci. U.S.A.* **73**, 2541 (1976)] have also examined mollusk shell proteins from the Coon Creek Formation.
5. W. B. Clark [in *Maryland Geological Survey, Upper Cretaceous* (Johns Hopkins Univ. Press, Baltimore, 1916), p. 23], J. Gardner (in *ibid.*, p. 371), and J. P. Owens *et al.* [*A Field Guide to Cretaceous and Lower Tertiary Beds of the Raritan and Salisbury Embayments, New Jersey, Delaware and Maryland* (American Association of Petroleum Geologists/Society of Economic Paleontologists and Mineralogists convention, Washington, D.C., 1977), p. 52] recently proposed that this massive clayey silty sand be renamed the Severn Foundation.
6. Larval shells were found at a density of roughly one specimen per cubic centimeter of sediment fraction.
7. P. Chanley and J. D. Andrews, *Malacologia* **11**, 45 (1971).
8. Various workers have also made extensive use of soft-part anatomical features beyond the reach of the paleontologist [R. D. Turner and P. J. Boyle, *Am. Malacol. Union Inc. Bull.* **1974**, 59 (1974)].
9. P. J. Boyle and R. D. Turner, *J. Exp. Mar. Biol. Ecol.* **22**, 55 (1976).
10. C. B. Rees, *Hull Bull. Mar. Ecol.* **3**, No. 19, 73 (1950).
11. E. Pascual, *Invest. Pesq.* **35**, 549 (1971); *ibid.* **36**, 297 (1972); M. Le Pennec, *Cah. Biol. Mar.* **15**, 475 (1974); M. LaBarbera, *Malacologia* **15**, 64 (1975); P. Dinamani, *J. Moll. Stud.* **42**, 95 (1976).
12. R. D. Turner and A. C. Johnson, *Annu. Rep. Am. Malac. Union Inc.* **1969**, 9 (1969); R. S. Scheltema, *Mar. Biol.* **11**, 5 (1971); J. L. Culliney and R. D. Turner, *Ophelia* **15**, 149 (1976); P. E. Chanley, *Chesapeake Sci.* **6**, 162 (1965).
13. For Recent larval mytilid hinge structure descriptions, see M. Le Pennec and M. Masson, *Cah. Biol. Mar.* **16**, 113 (1976); S. E. Siddall, *Proc. Natl. Shellfish. Assoc.*, in press; R. A. Lutz, *ibid.*, in press.
14. D. Jablonski and R. A. Lutz, in preparation.
15. S. M. Stanley, *J. Paleontol.* **42**, 214 (1968).
16. E. G. Kauffman, *ibid.* **51**, No. 2 (Suppl.), 16 (1977).
17. Criteria for distinguishing planktotrophic from nonplanktotrophic larval shells are discussed by LaBarbera (*2*) and references therein.
18. E. G. Kauffman, *Geol. Assoc. Can. Spec. Pap.* **13**, 163 (1975).
19. R. S. Scheltema, in *Concepts and Methods of Biostratigraphy*, E. G. Kauffman and J. E. Hazel, Eds. (Dowden, Hutchinson and Ross, Stroudsburg, Pa., 1977), p. 73.
20. We thank R. D. Turner for comments and aid in identification of larval shells; A. S. Pooley for technical assistance with the scanning electron microscopy; M. LaBarbera, R. D. Turner, D. C. Rhoads, E. G. Kauffman, T. R. Waller, and M. R. Carriker for reviews of the manuscript; and W. K. Sacco for his assistance with photographic reproductions. Supported in part by NOAA Sea Grant 04-7-158-44034 and a grant-in-aid of research from the Society of Sigma Xi. Contribution 109 from the Ira C. Darling Center, University of Maine, Walpole.

20 September 1977

Glutamine-Selective Membrane Electrode That Uses Living Bacterial Cells

Abstract. *A novel bioselective membrane electrode for* L-*glutamine has been constructed by coupling living bacteria of the strain* Sarcina flava *to a potentiometric ammonia gas sensor. Tests in aqueous standards and human serum show that the electrode combines excellent sensitivity and selectivity with rapid response and a useful lifetime of at least 2 weeks.*

The development of membrane electrode probes (*1*) for biological substances need not be limited to the use of immobilized enzymes as mediators between the material to be measured and the internal potentiometric sensor; indeed, an entire hierarchy of possible mediators, ranging from enzymes through immunoagents and intact vesicles, has recently been proposed (*2*). We describe here a novel membrane electrode probe which uses intact living bacterial cells in situ to produce a highly selective and sensitive potentiometric response to the amino acid L-glutamine in aqueous standards and in human serum. The bacterial electrode also shows a greatly improved lifetime over earlier potentiometric sensors (*3*) based on the unstable enzyme glutaminase (E.C. 3.5.1.2).

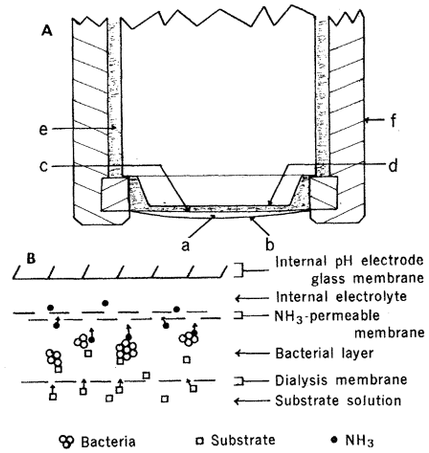

Fig. 1. (A) Schematic diagram of the bacterial electrode: *a*, bacterial layer; *b*, dialysis membrane; *c*, gas-permeable membrane; *d*, internal sensing element; *e*, internal filling solution; and *f*, plastic electrode body. (B) Detail of the membrane phases.

One prepares the glutamine electrode by holding a layer of whole cells of the bacterium *Sarcina flava* (American Type Culture Collection 147) at the surface of an ammonia-sensing membrane electrode (Orion 95-10) with a dialysis membrane, as shown in Fig. 1A. The bacteria are freshly grown on agar slants of nutrient broth at 30°C for 3 days, then harvested and washed by centrifugation in tris-HCl buffer (*p*H 7.5) containing 0.01 mole per liter of $MnCl_2$ as activator. No special sensitization or treatment of the bacteria is required.

Figure 1B illustrates in detail the vari-

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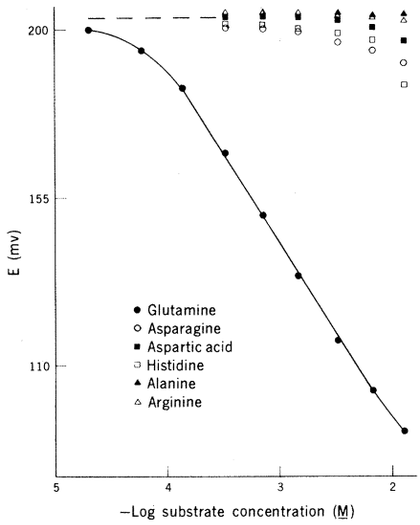

Fig. 2. Response and nominal selectivity of the bacterial sensor in tris-HCl buffer (*p*H 7.5) with 0.01*M* $MnCl_2$; *E* is the potential.

ous phases and processes at the sensing tip of the bacterial electrode. Essentially, the bacteria function as a selective biocatalyst to convert glutamine to NH_3, which produces a change in the measured potential.

The resulting potentiometric response to varying concentrations of glutamine is shown in Fig. 2. We took these data with a 10-day-old bacterial electrode by adding increments of freshly prepared glutamine stock solution to 25 ml of tris-HCl buffer (*p*H 7.5) (0.01*M* $MnCl_2$) thermostated at 30°C. Steady-state potentials were reached in 5 minutes or less. Over the linear range of $10^{-4}M$ to $10^{-2}M$ glutamine, the response slope of the electrode is −48.5 mv per concentration decade (correlation coefficient = 0.9997), but a useful response is obtained to concentrations as low as $2 \times 10^{-5}M$. Since the normal serum glutamine concentration falls (*4*) in the range from 4.2 to $7.6 \times 10^{-4}M$, the electrode sensitivity is more than adequate.

We evaluated electrode selectivity by measuring the potentiometric response to a variety of other amino acids over the same concentration range. The data in Fig. 2, taken with the same 10-day-old bacterial electrode, show that the response to these amino acids is negligible except at the very highest concentrations. To further demonstrate the favorable electrode selectivity, we compared the response to glutamine in buffer with that to glutamine in buffer also containing the maximum normal physiological concentrations of the other amino acids. As can be seen in Fig. 3, the electrode response curves are identical for glutamine alone and for the mixtures. The electrode showed no response to urea, lysine, valine, glycine, glutamic acid, serine, or threonine. In view of the low values of K_m (Michaelis constant) (approximately $10^{-4}M$) for bacterial glutaminases (*5*, *6*), the favorable electrode response to glutamine is entirely reasonable.

Finally, we tested the response of the bacterial electrode in human serum by making known additions of glutamine to reconstituted pooled serum (General Diagnostics, Calibrate, lot 1956035, 1 : 5 dilution) and obtained the behavior shown in Fig. 4. The response slope is the same as that found in aqueous standards, but the curve is shifted slightly along the potential axis owing to viscosity differences. Termination of the plot of the lowest concentrations reflects an unavoidable glutamine background in the control serum. For practical measurements, calibration standards for the bacterial electrode, indeed for all membrane electrodes, should be prepared in the same medium as the final test solution. It is clear, however, that the proposed glutamine electrode functions effectively in serum and in synthetic sample solutions.

We found it desirable to precondition the bacterial electrode by soaking it in the buffer medium prior to initial use and also obtained best results by storing the electrode in the buffer between measurements. When used in this fashion, the electrode was found to be an effective glutamine sensor for at least 2 weeks; this may be compared to an effective lifetime of only 1 day for the earlier enzymatic electrode (*3*).

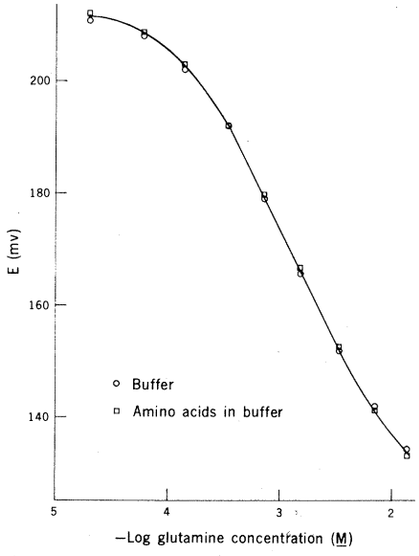

Fig. 3. Reponse of the bacterial sensor to glutamine alone and to glutamine in the presence of amino acids ($8.6 \times 10^{-5}M$ asparagine, $1.2 \times 10^{-4}M$ histidine, $5.0 \times 10^{-4}M$ alanine, $1.5 \times 10^{-4}M$ arginine, and $5.4 \times 10^{-5}M$ aspartic acid).

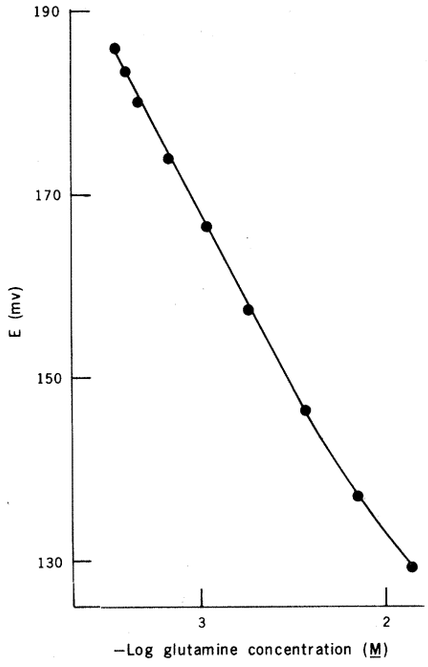

Fig. 4. Response of the bacterial sensor to glutamine in reconstituted control serum (1:5 dilution).

The novel concept described here for the specific case of the bacterial glutamine-selective electrode lays the groundwork for the development of other sensors through the appropriate combination of various bacterial strains with internal ion or gas-sensing elements. In view of the very large number of known microorganisms and of continuing advances in the development of potentiometric membrane electrodes (*1*), it is not unreasonable to expect that other bacterial electrodes of fundamental and practical significance would be feasible.

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References and Notes

1. G. A. Rechnitz, *Science* **190**, 234 (1975).
2. ———, paper presented at the 30th Annual Summer Symposium on Analytical Chemistry, Amherst, Mass., 13 June 1977.
3. G. G. Guilbault and F. R. Shu, *Anal. Chim. Acta* **56**, 333 (1971).
4. F. A. Ibbott, in *Clinical Chemistry, Principles and Techniques*, R. J. Henry, D. C. Cannon, J. W. Winkelman, Eds. (Harper & Row, New York, ed. 2, 1974), p. 584.
5. S. C. Hartman, in *The Enzymes*, P. D. Boyer, Ed. (Academic Press, New York, ed. 3, 1971), vol. 4, chap. 4.
6. E. Ehrenfeld, S. J. Marble, A. Meister, *J. Biol. Chem.* **238**, 3711 (1963).

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