References and Notes

1. D. M. Fambrough, D. B. Drachman, S. Satyamurti, *Science* **182**, 293 (1973).
2. D. B. Drachman, I, Kao, A. Pestronk, K. V. Toyka, *Ann. N.Y. Acad. Sci.* **274**, 226 (1976).
3. D. P. L. Green, R. Miledi, A. Vincent, *Proc. R. Soc. London* **189**, 57 (1975).
4. E. X. Albuquerque, J. E. Rash, R. F. Mayer, J. R. Satterfield, *Exp. Neurol.* **51**, 536 (1976).
5. A. G. Engel, J. M. Lindstrom, E. H. Lambert, V. A. Lennon, *Neurology* **27**, 307 (1977).
6. S. Satyamurti, D. B. Drachman, F. Slone, *Science* **187**, 955 (1975).
7. D. B. Drachman, *N. Engl. J. Med.* **298**, 136 (1978).
8. R. R. Almon, C. G. Andrew, S. H. Appel, *Science* **186**, 55 (1974).
9. A. M. Bender, W. K. Engel, S. P. Ringel, M. P. Daniels, Z. Vogel, *Lancet* **1975-I**, 607 (1975).
10. J. M. Lindstrom, M. E. Seybold, V. A. Lennon, G. Whittingham, D. D. Duane, *Neurology* **26**, 1054 (1976).
11. T. Mittag, P. Kornfeld, A. Tormay, C. Woo, *N. Engl. J. Med.* **294**, 691 (1976).
12. K. V. Toyka, D. B. Drachman, A. Pestronk, I. Kao, *Science* **190**, 397 (1975).
13. K. V. Toyka, D. B. Drachman, D. E. Griffin, A. Pestronk, J. A. Winkelstein, K. H. Fischbeck, Jr., I. Kao, *N. Engl. J. Med.* **296**, 125 (1977).
14. I. Kao and D. B. Drachman, *Neurology* **27**, 364 (1977); *Science* **196**, 527 (1977).
15. S. H. Appel, R. Anwyl, M. W. McAdams, S. Elias, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2130 (1977).
16. S. Heinemann, S. Bevan, R. Kullberg, J. Lindstrom, J. Rice, *ibid.*, p. 3090.
17. J. P. Brockes, D. K. Berg, Z. W. Hall, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 253 (1975).
18. A. Vincent, *Nature (London)* **254**, 182 (1975).
19. A. G. Engel, E. H. Lambert, F. M. Howard, *Mayo Clin. Proc.* **52**, 267 (1977).
20. K. Heide and H. G. Schwick, *Handbook of Experimental Immunology* (Blackwell, Oxford, ed. 2, 1973).
21. C. Y. Lee, *Annu. Rev. Pharmacol.* **12**, 265 (1972).
22. D. K. Berg and Z. W. Hall, *J. Physiol. (London)* **252**, 771 (1975).
23. C. C. Chang and M. D. Huang, *Nature (London)* **253**, 643 (1975).
24. The mean rates of degradation were calculated from the regression lines fitted by the least-squares method to the log of the fraction remaining bound to the ACh receptors versus time. To avoid assumptions about the kinetics of this process that are implicit in the determination of degradation *rates*, we have also compared the amount of radioactivity remaining bound to control and myasthenic Ig-treated diaphragms at the end of 4 days of treatment and found a highly significant difference ($P < .001$). The control Ig-treated diaphragms had 63.0 ± 6.8 percent remaining radioactivity; the diaphragms of mice treated with myasthenic Ig had 21.5 ± 1.5 percent to 42.6 ± 2.0 percent remaining.
25. P. N. Devreotes and D. M. Fambrough, *J. Cell Biol.* **65**, 335 (1975).
26. J. P. Merlie, J. P. Changeux, F. Gros, *Nature (London)* **264**, 74 (1976).
27. J. Patrick, M. McMillan, H. Wolfson, J. C. O'Brian, *Proc. Natl. Acad. Sci. U.S.A.* **252**, 2143 (1977).
28. P. N. Devreotes and D. M. Fambrough, *Cold Spring Harbor Symp. Quant. Biol.* **40**, 237 (1975).
29. To determine whether BuTx is broken down by myasthenic Ig, we incubated free ^{125}I-α-BuTx with culture medium containing myasthenic Ig at 37°C for 20 hours. Column chromatography showed that 99 percent of the radioactivity remained in the BuTx peak. This indicated that myasthenic Ig itself does not have the ability to degrade BuTx.
30. J. E. Rash, E. X. Albuquerque, C. S. Hudson, R. F. Mayer, J. R. Satterfield, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4584 (1976).
31. R. B. Taylor, W. P. H. Duffus, M. C. Raff, S. de Petris, *Nature (London) New Biol.* **233**, 225 (1971); E. Boyse and L. J. Old, *Annu. Rev. Genet.* **3**, 269 (1969).
32. P. DeMeyts, J. Roth, D. M. Neville, Jr., J. R. Gavin, III, M. A. Lesnick, *Biochem. Biophys. Res. Commun.* **55**, 154 (1973); J. R. Gavin, III, J. Roth, D. M. Neville, P. DeMeyts, D. N. Buell, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 84 (1974).

33. We thank R. N. Adams and J. Michelson for technical assistance and C. Barlow for help in the preparation of the manuscript. Supported by NIH research grants 5PO1-NS109 and 5RO1-HD04817 and by a fellowship to E.S. from the Muscular Dystrophy Association.

17 January 1978

Is Pump Stimulation Associated with Positive Inotropy of the Heart?

Abstract. *A purified sodium and potassium dependent adenosinetriphosphatase isolated from cat heart was not stimulated by any concentration of ouabain that produced positive inotropy of cat papillary muscle. Only inhibition of enzyme activity was observed. Concentrations of ouabain used ranged from 3.3 × 10^{-10} molar to 5 × 10^{-7} molar and produced an increased force of contraction without any evidence of toxicity. The results are inconsistent with a concept that stimulation of sodium pump activity is associated with positive inotropy.*

Cardiac glycosides, in concentrations that are presumed to be nontoxic, produce a positive inotropic effect on the heart. The mechanisms that produce this improvement in cardiac muscle function are investigated at many levels including intact heart preparations, subcellular organelle function, ionic fluxes across membranes, and electrophysiological studies (*1*). In 1956, Solomon *et al.* (*1*) provided the first quantitative data on K^+ transport and cardiac glycoside action, thus implicating the Na^+- and K^+-dependent adenosinetriphosphatase (Na^+, K^+-ATPase; E.C. 3.6.1.3) pump system in the mechanism of digitalis action. Recent work has focused on the effect of ouabain on membrane currents altered by extracellular potassium ($[K]_o$) and on the coincident inotropic effect as a consequence of a supposed electrogenic pump stimulation or inhibition. For example, Cohen *et al.* (*2*) studied membrane currents in sheep Purkinje fibers. At high values of $[K]_o$, that is, 5.4 to 8 m*M*, ouabain (5×10^{-7}*M*) shifted the reversal potential (E_{rev}) for the pacemaker current (I_{k2}) in a negative direction; at low values of $[K]_o$, that is, 2.7 or 4 m*M*, this dose of ouabain shifted E_{rev} in a positive direction. In another experiment, 1×10^{-7}*M* ouabain at $[K]_o$ of 5.4 m*M* caused a transient negative shift in E_{rev} during the first 5 minutes of drug perfusion followed by a positive E_{rev} shift in the succeeding 30 minutes. A positive shift in E_{rev} was interpreted by these authors as an inhibition of the Na^+,K^+-pump and a buildup of potassium, $[K]_e$, in a restricted extracellular space, whereas a negative shift in E_{rev} was taken to imply Na^+,K^+-pump stimulation with depletion of $[K]_e$. Changes in $[K]_o$ might indicate corresponding changes in outward current, i_{k1}, and pacemaker current, i_{k2}, or the currents could be the same but, more or less, K^+ would be pumped back. The assumption by Cohen *et al.* (*2*) that E_{rev} may simply represent E_K may be incorrect. Voltage clamp techniques may have sources of error that obviate definitive conclusions about Na^+,K^+-pump activity. Very recently, Kass *et al.* (*3*) reported that 10^{-6}*M* strophanthidin increased a slow inward current (i_{Si}) in short Purkinje fibers from the calf, concomitant with an augmented twitch tension. This occurred at an early stage of drug action.

Blood (*4*) reported that doses of ouabain in the range of 10^{-10} to 10^{-7}*M* produced positive inotropic effects in sheep Purkinje fibers bathed with 5.4 m*M* $[K]_o$ Tyrodes solution. He indicated that the effects of low concentrations of ouabain could be mimicked by reducing $[K]_o$; a reduction from 5.4 m*M* to 4.05 m*M* produced a positive inotropic response similar to that resulting from 5×10^{-8}*M* ouabain, but no values for the degree of inotropy were given. Anderson *et al.* (*5*) reported that $[K]_o$ raised from 2.7 to 5.4 m*M* caused a decrease in the maximum diastolic potential from 96 to 89 mV, respectively. In the presence of 2.1×10^{-7}*M* ouabain, an increase in $[K]_o$ from 2.7 to 5.4 m*M* caused an increase in maximum diastolic potential from −52 to −65 mV. Using amphibian atrial trabeculae, Loh (*6*) observed a positive inotropic effect in response to the cardiac glycoside desacetyl-lanatoside C, 7.4×10^{-7}*M*; simultaneously, there was an increase in $[K]_i$ at 10 m*M* $[K]_o$, no increase or decrease at 4 m*M* $[K]_o$, and a decrease at 1.25 to 3.5 m*M* $[K]_o$.

Some years ago it was suggested (*7*) that stimulation of a Na^+,K^+-ATPase exchange pump may produce a positive inotropic state, although this concept is not now generally held (*1*). Since recent electrophysiological data have again raised the possibility, we reexamined this hypothesis. Cats were anesthetized with sodium pentobarbital (25 mg/kg) and the hearts were quickly excised. Isolated right ventricular papillary muscles were placed in an isometric myograph and stimulated to contract at a rate of 0.25 Hz with pulses 20 percent above

0036-8075/78/0616-1287$00.50/0

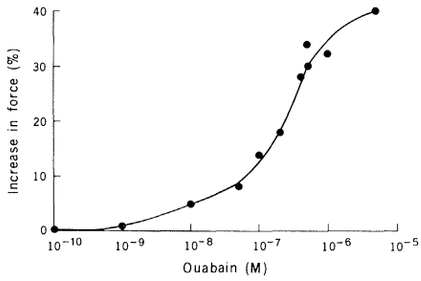

Fig. 1. Effect of ouabain on force development of cat right ventricular papillary muscles. Increase in force is expressed as percentage of control (without ouabain). Force was allowed to reach steady-state level for each ouabain concentration. Average cross-sectional area of the 11 papillary muscles was 0.90 ± 0.23 mm², and average developed force at L_{max} was 4.8 ± .40 g.

threshold voltage and 5 msec in duration. The muscle bath contained, in millimolar concentrations, NaCl, 117.4; $NaHCO_3$, 25; Na_2HPO_4, 1.2; $MgSO_4$, 1.2; KCl, 3.6; $CaCl_2$, 2.5; and glucose, 11.1. The temperature was maintained at 29°C. After 3 hours of equilibration the muscle length at which maximum force developed, L_{max}, was set. Various concentrations of ouabain, starting from the lowest and increasing in increments, were added to the bath. Force was altered from the control state in response to various concentrations of ouabain (Fig. 1). Concentrations of ouabain higher than $10^{-6}M$ invariably produced arrhythmia and ultimately contracture if allowed to remain in contact with the tissue for sufficient time (that is, 45 minutes to 2 hours). No negative inotropic effect was ever recorded for doses less than $5 \times 10^{-7}M$.

The dose response of papillary muscles exposed to ouabain was also determined with 5.4 mM K^+. This concentration of K^+, which supposedly promotes pump stimulation, slightly decreased the force increase at each concentration of ouabain but caused no decrease in force below 5×10^{-6}. The Na^+,K^+-ATPase (NaI-treated fraction) was isolated from cat heart (*8*), and the effect of ouabain was measured by means of the spectrophotometric pyruvate kinase–lactic acid dehydrogenase-coupled enzyme system (Fig. 2). There was no measurable stimulation or inhibition of the enzyme when it was observed continuously for a period of 2 hours after the addition of 3.3×10^{-10} or $10^{-9}M$ ouabain to the enzyme reaction medium (Fig. 3); however, $10^{-8}M$ ouabain inhibited the enzyme by approximately 20 percent, but no stimulation was observed at anytime during the 2-hour incubation period. Note that at $10^{-7}M$ ouabain, the increase in force was about 12 percent, but inhibition of Na^+,K^+-ATPase was about 65 percent. This substantiates the complexity of the events and difficulty of quantitating inhibition of enzyme activity in vivo with effects on contractile force (*1*).

No significant inhibition or stimulation was observed when the concentration of ouabain was $10^{-9}M$ or less. Stimulation of a Na^+,K^+-ATPase enzyme system has been reported sporadically in the past (*9*), but recently there have been new reports of Na^+,K^+-pump stimulation (*10*). Godfraind and Ghysel-Burton (*10*), reporting a decrease in Na^+ content coincident with an increase in K^+ content of guinea pig atria at ouabain concentrations of 10^{-8} and $10^{-9}M$, concluded that a pump stimulation occurred since the inulin space was not altered. In a previous communication by Ghysel-Burton and Godfraind (*11*), a positive inotropic effect of ouabain occurred at concentrations which (they presumed) stimulated the Na^+ pump; however, it should be noted that Na^+,K^+-ATPase was not actually measured. Using the highly digitalis-sensitive species, the cat, we have not seen a stimulation of the Na^+,K^+-ATPase at any concentration of ouabain; furthermore, in past work (*1*) with rabbit, dog, and guinea pig heart Na^+,K^+-ATPase, the dose-response curves have never revealed a stimulatory effect. We believe that the Na^+,K^+-ATPase is the receptor for digitalis and that prior to the inotropic action, digitalis must bind to this receptor (*1*). The sequential events that follow must involve other cell membrane and possibly subcellular events which ultimately produce an improved muscle performance (*1*).

Very recently, Blood and Noble (*12*) presented evidence which suggested the

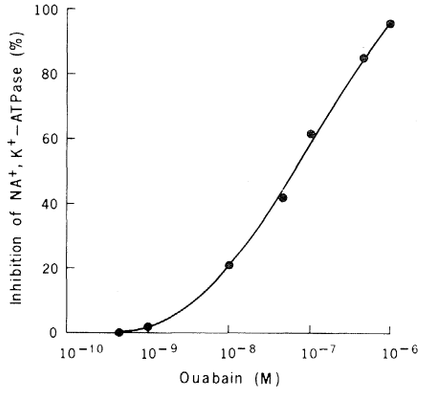

Fig. 2. Effect of ouabain on Na^+,K^+-ATPase hydrolytic activity. Inhibition of activity is expressed as percentage of control (without ouabain) measured after 60 minutes of incubation at 37°C. Non-ouabain-inhibitable ATPase activity that could not be inhibited by ouabain was less than 10 percent in this NaI-treated enzyme.

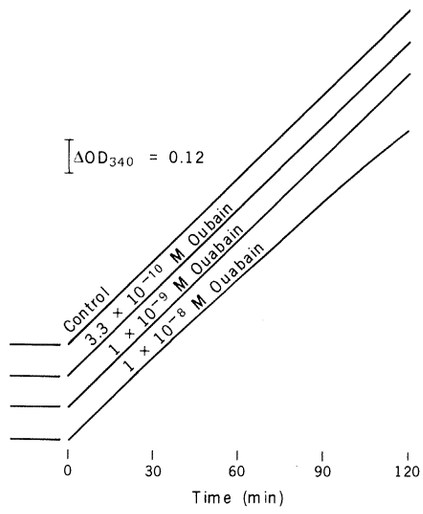

Fig. 3. Time course of Na^+,K^+-ATPase activity and the effect of ouabain concentrations: $3.3 \times 10^{-10}M$, $1 \times 10^{-9}M$, and $1 \times 10^{-8}M$, respectively. At time zero, 20 μg of Na^+,K^+-ATPase was added to each cuvette.

possibility of a "low and high dose inotropism." Using sheep Purkinje fibers stimulated at 2½ times threshold, they found that a "low" dose of ouabain ($10^{-8}M$) produced a fast (5 minutes to peak) inotropism and a "high" dose ($10^{-6}M$) produced a slow (25 to 30 minutes to peak) inotropism. The authors speculated that there may be two mechanisms: a "low dose mechanism" not related to speed of action on Na^+ and K^+ gradients and a "high dose mechanism" depending on reduction of Na^+ gradient by pump inhibition. However, no actual pump exchange data were presented. The presumed transient stimulation of the Na^+,K^+-exchange pump by low concentrations of ouabain, that is, $1 \times 10^{-7}M$ ouabain and $[K]_o$ 5.4 mM (*12, 13*), has not been reflected in our experiments using cat heart Na^+,K^+-ATPase by an increase in adenosine triphosphate hydrolysis. The data are not inconsistent with a transient alteration of E_{rev} in cat heart. However, there is very little evidence to support the concept that the final state of positive inotropy or inhibition of the Na^+,K^+-ATPase enzyme does reflect such a membrane voltage fluctuation. If stimulation of the Na^+,K^+-exchange pump occurs in a living cell at low concentrations of digitalis glycosides, the significance of this is unknown. A positive inotropic state induced by a cardiac glycoside is always demonstrated at the level of supposed pump stimulation ($10^{-8}M$) as well as when the pump is definitely inhibited ($10^{-7}M$). It is a difficult at present to fit both stimulation and inhibition of the pump into a mechanism requiring delivery of an increased quantity of calcium to troponin C. The effect of

K^+ on inhibiting the rate of ouabain binding to Na^+,K^+-ATPase is well known (*1*). Similar effects of $[K]_o$ on the binding of cardiac glycoside to intact cells has also been discussed by several authors (*1*) who used reconstituted red blood cell ghosts and by Anderson *et al.* (*5*) who used canine Purkinje fibers. Although it has been demonstrated that E_{rev} of Purkinje fibers is altered by ouabain or a change in $[K]_o$, or both, it remains to be shown that this same phenomenon occurs in the atrial or ventricular myocytes which are the major components generating the positive inotropic state in heart. It is possible that Purkinje tissue is qualitatively different from contracting myocytes with respect to Na^+,K^+-ATPase. For instance, Kübler *et al.* (*14*) reported that cardiac conducting tissue and ventricular muscles differ both in amount of Na^+,K^+-ATPase and in sensitivity to ouabain; however, Palfi *et al.* (*15*) found no such ouabain sensitivity distinction. Uptake of [^{3}H]labeled digoxin by sheep ventricle was 27.5 percent greater than by bundle of His and Purkinje fibers as reported by Hammerman *et al.* (*16*).

Our purified Na^+,K^+-ATPase hydrolytic activity was not stimulated by any concentration of ouabain, and positive inotropy of cat papillary muscles prevailed at concentrations below $5 \times 10^{-7} M$. Thus, it would be difficult to attribute the negative change in E_{rev}, reported by other authors who used low concentrations of ouabain ($10^{-8} M$), to a stimulation of Na^+,K^+-ATPase. Neither E_{rev} nor isolated Na^+,K^+-ATPase are entirely reflective of the exchange pump in the whole cell. The enzymatic mechanism, however, is generally accepted as the Na^+,K^+-exchange pump from the cell membrane (*1*). We recognize, however, that the isolated enzyme may behave differently from the enzyme in situ. It is clear that the important issue of "pump stimulation" deserves further work.

LLOYD MICHAEL*
BARRY J. R. PITTS*
ARNOLD SCHWARTZ†
Department of Cell Biophysics,
Baylor College of Medicine,
Houston, Texas 77030

References and Notes

1. A. Schwartz, G. Lindenmayer, J. C. Allen, *Pharmacol. Rev.* **27**, 3 (1975); A. K. Solomon, T. J. Gill, G. L. Gold, *J. Gen. Physiol.* **40**, 327 (1956); W. B. Van Winkle and A. Schwartz, *Annu. Rev. Physiol.* **38**, 247 (1976); M. Rosen, A. Wit, B. F. Hoffman, *Am. Heart J.* **89**, 391 (1975); G. W. Beeler, Jr., *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 2209 (1977).
2. I. Cohen, J. Daut, D. Noble, *J. Physiol. (London)* **249**, 42 (1975).
3. R. S. Kass, R. W. Tsien, R. Weingart, *Biophys. J.* **17**, 4a (1977).
4. B. E. Blood, *J. Physiol. (London)* **251**, 69 (1975).
5. G. J. Anderson, J. C. Bailey, J. Reiser, A. Freeman, *Circ. Res.* **39**, 717 (1976).
6. C. Loh, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, (Abstr. 1208) (1975).
7. W. Wilbrandt, *Proceedings of the 1st International Congress for Pharmacology, Stockholm* (Pergamon, New York, 1963), vol. 3, p. 196.
8. B. J. R. Pitts and A. Schwartz, *Biochim. Biophys. Acta.* **401**, 184 (1975).
9. I. Bihler, *Myocard. Biol.* **4**, 209 (1974); R. F. Palmer, K. C. Lasseter, S. Melvin, *Arch. Biochem. Biophys.* **113**, 629 (1966); P. Dal Pra, L. Rossini, G. Serge, *Pharmacol. Res. Commun.* **2**, 177 (1970); H. Bader, A. Wilkes, D. Jean, *Biochem. Biophys. Acta* **198**, 583 (1970); T. Peters, R. Raben, O. Wasserman, *Eur. J. Pharmacol.* **26**, 166 (1974).
10. T. Godfraind and J. Ghysel-Burton, *Nature (London)* **265**, 165 (1977).
11. J. Ghysel-Burton and T. Godfraind, *Br. J. Pharmacol.* **55**, 249 (1975).
12. B. E. Blood and D. Noble, *J. Physiol. (London)* **226**, 76 (1977).
13. I. Cohen, J. Daut, D. Noble, *ibid.* **260**, 75 (1976).
14. V. Kübler, G. Endres, P. Smekal, *Verh. Dtsch. Ges. Kreislaufforsch.* **36**, 305 (1970).
15. F. Palfi, H. R. Besch, A. M. Watanabe, *Circulation* **52** (Suppl. 2), (Abstr. 329) (1975).
16. H. Hammerman, A. Hernandez, D. Goldring, *J. Lab. Clin. Med.* **78**, 799 (1971).
17. This work was supported in part by PHS grant HL 7906-13 (now HL 22039-01) and by the Section of Myocardial Biology of the National Heart and Blood Vessel Research and Demonstration Center; Baylor College of Medicine; a grant-supported research project of the National Heart, Lung, and Blood Institute, HL 17269-03; myocardial biology training grant HL 7219-01 (A.S.) (L.M. was a myocardial biology trainee); and contract NO 1 HV 52988 (A.S.) with the National Institutes of Health. We thank Lonnie Franks for his technical assistance and Linda Miner for editorial assistance.

* Present address: Section of Cardiovascular Sciences, Department of Medicine, Baylor College of Medicine, Houston, Texas 77030.
† Present address: Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

26 September 1977; revised 24 February 1978

Deep-Sea Foraging Behavior: Its Bathymetric Potential in the Fossil Record

Abstract. *Spiral and meander foraging traces in the deep sea are not distributed in proportion to assumed food availability. Data collected by means of deep-sea photography failed to reveal a bathymetric gradient in behavioral complexity or sensitivity. The foraging paradigm developed by numerous trace fossil studies does not adequately predict the modern environment.*

In this report, a rigorous analysis is presented of modern spiral and meander foraging patterns on the ocean floor; the presence of such patterns in ancient strata has been interpreted to indicate bathyal to abyssal depths. Because of the difficulties of study in the deep sea, a paucity of traces have been identified in modern environments. Spiral and meander foraging patterns produced in modern sediments represent trace fossil counterparts that could possibly be used as direct standards for depth. If trace fossil theory predicts modern deep-sea trace morphology, diversity, or density (or all), its bathymetric potential will be substantiated.

Seilacher (*1–3*) suggested that trace fossils occur in depth-controlled communities. He postulated that in nutrient-enriched shallow marine systems, sediment ingesters need not conserve energy and therefore may forage inefficiently. Recurrent crossings of older traces and encounters between individuals are common. Conversely, in nutrient-limited deep-sea systems, sediment grazers must maximize areal coverage while minimizing energy output. Complex spiral and meander patterns that avoid recrossings and encounters between individuals are common. Subsequent studies (*4*) indicated the bathyal to abyssal *Nereites* community is closely depth-controlled. Its component grazing traces reflect highly organized foraging strategy.

Seilacher (*2*) argued against direct comparison with data from the modern seafloor because organisms responsible for many trace fossils remain unknown in terms of taxonomic affinity and body structure. However, optimal foraging strategy is a behavioral principle (*5*) that has been elevated to the status of paradigm in a recent paper by Seilacher (*6*). Its bathymetric potential should represent an adaptive response to an increasingly limited resource, independent of organism identity.

A digital simulation model for two-dimensional feeding patterns preserved on bedding planes of ancient flysch deposits was developed by Raup and Seilacher (*7*). Their work demonstrated that what paleontologists viewed as highly variable and complex feeding traces could be precisely simulated by 1 to 4 behavioral commands. The simplest command produced a scribble; four commands produced a meander. Spirals were of intermediate complexity. More recently, computer evolutionary experiments by Papentin (*8*) demonstrated that selection for avoidance of crossing will result in both spiral and meander foraging strategies.

We hypothesized that corroboration of trace fossil theory required these data from a modern analog: (i) a bathymetric gradient in increased sensitivity to behavioral commands; hence, an increased organization with depth; (ii) a bathymetric gradient in behavioral complexity from scribbles to spirals to meanders;

0036-8075/78/0616-1289$00.50/0