hydrolysis would be small, so it is still possible to estimate preindustrial CCl_4 levels accurately from measurements in slowly renewed deep waters (*1*).

In contrast, loss of CH_3CCl_3 would be considerable even in cold waters over a period of only 40 years (that is, since CH_3CCl_3 was introduced into the environment), and it has an input function that is not dramatically different in nature from those of F-11 and F-12 (*1*). We thus reiterate that no great advantage will be gained from its use as an additional tracer.

We recommend that CCl_4 be measured together with F-11, F-12, and F-113 during future oceanographic sampling expeditions. We also encourage our colleagues to perform hydrolysis rate measurements for these compounds and the chlorofluorocarbons in seawater: data concerning the latter class of compounds, and measurements in seawater in general, are notably lacking in the literature.

D. W. R. WALLACE
Oceanographic Sciences Division, Department of Applied Science, Brookhaven National Laboratory, Upton, NY 11973
M. KRYSELL
Department of Analytical and Marine Chemistry, Chalmers University of Technology, and *University of Göteborg, S412 96, Göteborg, Sweden*

REFERENCES

1. M. Krysell and D. W. R. Wallace, *Science* **242**, 746 (1988).
2. M. Stuiver, P. D. Quay, H. G. Ostlund, *ibid.* **219**, 849 (1983).

13 March 1989; accepted 20 September 1989

Caterpillars and Polymorphisms

Greene reports (*1*) that two seasonal morphs of the herbivore *Nemoria arizonaria* are adapted for feeding and hiding on their respective substrates, oak catkins and leaves. While we do not dispute the occurrence of this polymorphism, the striking resemblance of each to its substrate, or its purported adaptiveness, we contend that the proposed mechanism for its induction, dietary tannin, is unlikely.

Greene's conclusion that the "developmental polymorphism is triggered by the dietary concentration of defensive compounds [tannins]" is based on two lines of evidence. First, he observed that caterpillars eating spring catkins remain as catkin morphs while those feeding on summer foliage of four Arizona oak species develop into twig-like morphs. He assumes that catkins are low in tannins relative to leaves on the basis of citations (*1*, note 16) that do not deal explicitly with catkin phytochemistry. We examined this assumption by analyzing tannin content of catkins of *Quercus emoryi*, one of the host plants of *N. arizonaria* (*2*). Condensed tannin content of catkins is relatively low [three trees, 0.59% (± 0.05), 0.39% (± 0.01), 0.29% (± 0.10)] but comparable to that of mature leaves (*2*). Hydrolyzable tannin content, however, is relatively high [three trees, 15.08% (± 2.28), 18.50% (± 0.82), 17.65% (± 2.66) (*2*)]. Clearly, low tannin content is not a common feature of catkins, weakening Greene's contention that dietary tannin level is the mechanism for the polymorphism.

Second, Greene found that diets of catkins plus the tannin quebracho, of catkins plus leaves, or of only leaves induced the twig morph. The latter two artificial diets included new, spring leaves (*3*), but in nature the twig morphs feed on mature, summer leaves. Something common to mature leaves and artificial diet plus new leaves or quebracho must induce the twig morph; Greene proposes it is either specific tannin compounds or tannin concentrations, but neither are likely to be shared among the three diets. *Quercus emoryi* and *Q. arizonica*, two hosts of *N. arizonaria*, vary seasonally in tannin types and concentrations (*4*). New and mature leaves differ in condensed tannin content (new, <1% dry mass; mature, ≈5% dry mass) and hydrolyzable tannin content (new, 25% dry mass; mature, 10 to 12% dry mass), as well as in other phenolic compounds (*4*). Quebracho is mostly condensed tannins, yet it triggers the same response as new leaves with little condensed tannin. Further, condensed tannins are unlikely to be the inducing factor since they are typically bound indissolubly to plant tissues (*5*) and are too large to pass through the peritrophic membrane of insects (*6*). Finally, Greene presents no evidence, and we are not aware of any, for the existence of tannin "receptors" in insects.

A more parsimonious explanation for Greene's results is that the twig morph is simply induced by a low protein diet. Low protein is a shared feature of the twig morph's natural and artificial diets. New and mature oak leaves are low in protein (for example, *Q. emoryi*, 2 to 5% dry mass; *Q. arizonica*, 2 to 6% dry mass). Adding quebracho or ground leaves that contain phenolic compounds to an artificial diet would lower relative protein, since tannins bind to plant proteins in agar media (*7*). Higher protein levels in catkins could inhibit development of twig morphs.

Low dietary protein triggers polymorphisms in other insects (*8*) and in vertebrates (*9*). Low protein is also consistent with slow development and decreased mass and fecundity of the twig morph (*10*). Toughness (fiber content) of leaves could also induce the twig morph. Consumption of hard tissues can induce morphological changes in insects (*11*) and in vertebrates (*9*). Greene's dietary experiments do not distinguish among increased tannins, reduced protein, or increased toughness as the inducing mechanism because tannins interfere with protein availability and fiber content was not controlled. Dietary experiments with varying levels of protein without tannins or fiber could resolve this issue.

STANLEY H. FAETH
KYLE E. HAMMON
Department of Zoology, Arizona State University, Tempe, AZ 85287-1501

REFERENCES AND NOTES

1. E. Greene, *Science* **243**, 643 (1988).
2. Catkins were collected from three trees of *Q. emoryi* at Oak Flat, Pinal County, AZ, on 2 April 1989 and immediately frozen on dry ice. Catkins were lyophilized, ground into a fine powder, extracted, and analyzed for condensed and hydrolyzable tannin content according to methods reported elsewhere [S. H. Faeth, *Ecology* **67**, 479 (1986)]. Values reported are means (% dry mass) and standard deviations of subsamples analyzed in triplicate from each tree.
3. Leaves used in diet experiments were collected within 2 weeks of budbreak (E. Greene, personal communication).
4. S. H. Faeth, *Oecologia* **68**, 34 (1985); *Ecology* **67**, 479 (1986).
5. A. Scalbert and E. Haslam, *Phytochemistry* **26**, 3191 (1987).
6. E. Bernays, *Ecol. Entomol.* **6**, 353 (1981).
7. A. E. Hagerman, *J. Chem. Ecol.* **13**, 437 (1987).
8. R. F. Denno, in *Variable Plants Herbivores in Natural and Managed Systems*, R. F. Denno and M. S. McClure, Eds. (Academic Press, New York, 1983), pp. 291–341.
9. A. Meyer, *Evolution* **41**, 1357 (1987).
10. J. M. Scriber and F. Slansky, Jr., *Ann. Rev. Entomol.* **26**, 183 (1981); P. W. Price, *Insect Ecology* (Wiley, New York, ed. 2, 1984); W. J. Mattson and J. M. Scriber, in *Nutritional Ecology of Insects*, F. Slansky, Jr., and J. G. Rodriguez, Eds. (Wiley, New York, 1987), pp. 105–146.
11. E. Bernays, *Science* **231**, 495 (1986).
12. We thank J. Howard and R. Rooney for comments. Supported by NSF grant BSR 8715743.

20 March 1989; accepted 19 September 1989

Response: Faeth and Hammon are correct in pointing out that the feeding experiments with *Nemoria arizonaria* (*1*) do not establish what differences between catkin and leaf diets are responsible for inducing the development of the two morphs. Polyphenolic compounds may trigger the development of

the twig morph directly, or as Faeth and Hammon suggest, through their interaction and binding with proteins, thus lowering the amount of digestible protein. The feeding experiments (*1*, table 2) do not distinguish between these two alternatives.

The main purpose of the paper (*1*) was to report the existence of this diet-induced developmental polymorphism. Although I suggested that polyphenolic compounds may be important, I had hoped to make it clear that we still do not know what it is that induces the development of the different morphs by stating, "This developmental polymorphism *may* be triggered by the concentration of defensive secondary compounds in the larval diet" (emphasis added) and "a possible mechanism is that receptors respond to tannin levels. . . ." I thank Faeth and Hammon for clarifying these points and for stating more explicitly another hypothesis concerning the mechanism involved.

ERICK GREENE
Department of Avian Sciences, University of California, Davis, CA 95616

REFERENCES

1. E. Greene, *Science* **243**, 643 (1989).

17 May 1989; accepted 19 September 1989

Does Voltage Affect Excitation-Contraction Coupling in the Heart?

In their report of experiments investigating excitation-contraction coupling in cardiac muscle, Näbauer *et al.* (*1*) say they have demonstrated that "it is *exclusively* Ca^{2+} influx through calcium channels . . . that regulates Ca^{2+} release in cardiac myocytes (emphasis added)." The experiments they present demonstrate that internal Ca^{2+} release can occur when the membrane is depolarized under conditions that permit calcium influx and that if Ca^{2+} influx is prevented, Ca^{2+} release does not occur. Their experiments therefore confirm previous work demonstrating that membrane depolarization alone cannot trigger release of Ca^{2+} from internal stores (*2–4*). However, they did not examine the effects of varying membrane potential under conditions that allow Ca^{2+} influx. Thus their data do not contradict the records of Cannell *et al.* (*3*), which show that repolarization can switch off calcium release from the sarcoplasmic reticulum (*3, 4*), nor do they refute our suggestion (*3*) that "Ca^{2+}release may be mediated by a mechanism that requires a Ca^{2+} influx but which may be modulated by changes in voltage directly." (We did not suggest that voltage alone can trigger Ca^{2+} release, as might be inferred from the context of the citation to our work.) Since modulation of calcium release by voltage can explain all experimental data to date, more critical tests of this hypothesis are needed.

M. B. CANNELL
Department of Pharmacology, University of Miami School of Medicine, Miami, FL 33143
J. R. BERLIN
Graduate Hospital, Bockus Research Institute, Philadelphia, PA 19146
W. J. LEDERER
Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201

REFERENCES

1. M. Näbauer, G. Callewaert, L. Cleemann, M. Morad, *Science* **244**, 800 (1989).
2. H. A. Fozzard and D. C. Hellam, *Nature* **218**, 688 (1968); W. R. Gibbons and H. A. Fozzard, *J. Gen. Physiol.* **65**, 345 (1975); R. Ochi and W. Trautwein, *Pflügers Arch. Eur. J. Physiol.* **323**, 187 (1971); W. Trautwein, T. F. McDonald, O. Tripathi, *ibid.* **354**, 55 (1975); G. W. Beeler and H. Reuter, *J. Physiol. (London)* **207**, 211 (1970); C. Leoty, *ibid.* **239**, 237 (1974; B. London and J. W. Krueger, *J. Gen. Physiol.* **88**, 475 (1986).
3. M. B. Cannell, J. R. Berlin, W. J. Lederer, *Science* **238**, 1419 (1987)
4. D. J. Beukelmann and W. G. Wier, *J. Physiol. (London)* **405**, 233 (1988).

15 June 1989; acccepted 15 September 1989

Response: The main issue addressed in our report (*1*) is the regulation of Ca^{2+} release from the sarcoplasmic reticulum in cardiac myocytes. Our experiments showed that there is no Ca^{2+} release when there is no Ca^{2+} current through the Ca^{2+} channel. Nor was there any release if Na^+ or Ba^{2+} carried the charge through the Ca^{2+} channel [this finding was independent of holding potentials in the range from −60 mV to −90 mV, which rules out possible inactivation of gating charge (*2*)]. We feel justified, therefore, in maintaining that it is exclusively the influx of Ca^{2+} through the Ca^{2+} channel which regulates Ca^{2+} release in cardiac cells.

Our findings also suggest that membrane potential plays no direct role in the release process. Previously we showed that the voltage dependence of the intracellular Ca^{2+} transients closely follows that of the inward Ca^{2+} current (*3*). It is not correct, therefore, to say that we "did not examine the effects of varying membrane potentials." In addition, others have also found that early estimates of the Ca^{2+} transients are well correlated with the inward Ca^{2+} current, even when a more slowly developing Ca^{2+} transient is present (as it may be in Na^+–loaded cardiac cells) (*4*). The confusion surrounding the voltage dependence of the Ca^{2+} transient may result from comparison of the Ca^{2+} current with the much later occurring maximum value of the Ca^{2+} transient (*5*). Such a comparison may be flawed, not only because of the presence of other Ca^{2+} transport mechanisms, but also because of saturation of the Ca^{2+} indicator dye or exhaustion of the releasable Ca^{2+} pools.

As for the question of whether membrane potential plays a limited modulatory role, Cannell *et al.* point to their observation that the rising phase of the Ca^{2+} transient at 0 mV is interrupted by early repolarization to the holding potential (−40 to −80 mV). We repeated these experiments and found in addition that the rising phase of the Ca^{2+} transient could also be interrupted by further depolarization to +80 mV (ECa), where Ca^{2+} transients are generally completely suppressed. We interpret this as indicating that the Ca^{2+} release is stopped when the Ca^{2+} current is interrupted either by repolarization-induced deactivation or by depolarization to the reversal potential. Repolarizing pulses to −80 mV, in addition, would activate the Na^+-Ca^+ exchange to extrude Ca^{2+} and help suppress the Ca^{2+} transients. Therefore, even these experiments support our basic idea that it is not the membrane potential as such which is important for Ca^{2+} release; rather it is the degree to which the membrane potential activates the Ca^{2+} current.

M. MORAD
L. CLEEMANN
G. CALLAWAERT
Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104

REFERENCES

1. M. Näbauer, G. Callewaert, L. Cleemann, M. Morad, *Science* **244**, 800 (1989).
2. G. Brum, E. Rios, E. Stefani, *J. Physiol.* **398**, 441 (1988).
3. G. Callewaert, L. Cleemann, M. Morad, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2009 (1988).
4. G. Isenberg *et al.*, in *Biology of Isolated Adult Cardiac Myocytes*, W. A. Clark, Ed. (Elsevier, New York, 1988), p. 354.
5. M. B. Cannell, J. R. Berlin, W. J. Lederer, *Science* **238**, 1419 (1987).

3 July 1989; accepted 18 September 1989