

present in human tissue, as has been suggested by Sutherland (26), concomitant PR during near-UV irradiation, interacting with efficient dark DNA repair systems, should reduce but not eliminate DNA damage from solar UV radiation. However, if an effective PR enzyme is not present in human skin or the human eye, stringent precautions are appropriate when the possibility exists of exposure to moderate to high fluences of near-UV radiation.

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Calcium-Bearing Objects Elicit Shell Selection Behavior in a Hermit Crab

Abstract. *Hermit crabs explore empty gastropod shells by touching, rolling, and probing them before choosing one for a home. This component of shell selection behavior was examined in *Pagurus hirsutiusculus hirsutiusculus* (Dana) with binary choice tests between natural shells and accurate replicas of the shells with different chemical compositions. The results show that calcium emanating from the surface of shells is responsible for the behavior. Sensitivity to calcium may be a factor that enables the hermit crab to locate partially buried shells and discriminate empty shells from ones housing living gastropods or from small pebbles.*

Many hermit crab species carefully select the gastropod shells they inhabit (1-4) and exhibit a complex behavioral sequence before accepting a shell (2, 5). Briefly, the selection process begins with the hermit crab grasping a shell with its walking legs and running its opened chelae over the surface. It then rolls the shell over between its appendages until it finds the aperture and probes the opening with the chelae. If the hermit crab lacks a shell, aperture probing is always followed by rapid insertion of the crab's abdomen into the shell (Fig. 1).

The specific features of shells that the crab examines and how each independently affects the choice of a shell was not known (6, 7). To study this, replicas of natural shells, including their fine textural features, were constructed. Original shells served as a standard for arti-

cial replicas of different compositions. Independent variation of shell parameters allowed assessment of the contribution of each to shell selection (8).

Using this technique, I found that cal-

cium promotes the exploration of shells and further examination of other features by *Pagurus hirsutiusculus hirsutiusculus* (Dana). Calcium-bearing objects such as pieces of minerals also elicited shell-like exploration behavior. Although, for other species visual characteristics promote investigation as well (2), shells lacking calcium were rarely investigated by *P. hirsutiusculus* (9), suggesting that initial recognition and examination of a shell is primarily dependent on a chemical cue (10).

The intertidal hermit crab *P. hirsutiusculus* prefers gastropod mollusc shells of the genus *Nucella* (11). Both male and female crabs with cephalothorax lengths in the range 0.7 to 1.6 cm were used. Hermit crabs were collected off Heceta Head, Oregon, from March through September and maintained in aquariums 1 m in diameter in a 12:12 hour, light:dark cycle. Natural seawater was used with a pH range of 7.8 to 8.0, at a constant temperature of 15°C.

Replicas of natural shells were made from inside and outside molds between which reagent grade, plaster of Paris ($\text{CaSO}_4 \cdot 1/2\text{H}_2\text{O}$, J. T. Baker Company) and water were poured (12). Natural shells were boiled, and both replica and natural shells were handled with disposable gloves (13) to control for additional chemical cues. Shell selection was examined by binary choice tests between a natural and replica shell or a pair of replicas of the gastropod shell *Nucella emarginata* or *N. canaliculata*. Crabs were removed from the shells in which they were found and placed in an opaque, round test chamber with an exit to a larger container. Two shells (choices), side by side with their apertures down, were placed directly outside the exit. Animals were tested only once. Shell positions were alternated between trials to control for side preferences. Except for animals tested in complete darkness, crabs were scored only if they contacted both shells.

To test the effects on behavior of particular minerals, a sample was placed in the test chamber at a fixed position just outside the crab's refuge (a few rocks). Animals were given a maximum of five contacts with only one sample and scored if full exploratory behavior was observed (14).

In tests of selection between plaster replica shells and natural shells, 26 of 26 hermit crabs preferred replica shells. Hermit crabs that were in contact with both shells explored the surface of the plaster replica shells first; further exploration was followed by acceptance of the plaster shells.

Table 1. Results obtained from binary choice tests with the hermit crab *P. hirsutiusculus*.

Condition	Number of animals choosing		Number of animals
	Natural shell	Replica shell	
Seawater	0	26	26
Seawater saturated with CaSO_4	13*	9*	22
No visual information, seawater	0	8	8

*Not significantly different by the chi-square test.

A possible feature of plaster replicas that might have influenced the crabs was a greater amount of free calcium; plaster shells are made exclusively of $\text{CaSO}_4 \cdot \text{H}_2\text{O}$, whereas natural gastropod shells are composed primarily of slightly soluble CaCO_3 . Plaster replicas dissolve in seawater within a few days, and the high solubility of $\text{CaSO}_4 \cdot \text{H}_2\text{O}$ suggested that it might be solubilized calcium at the shell surface that triggers shell exploration. When the amount of calcium dissociating from the plaster replicas was lowered by nearly saturating the seawater with $\text{CaSO}_4 \cdot \text{H}_2\text{O}$, the preference for plaster shells was abolished (Table 1) (15).

To test for the possibility that calcium stimulates exploratory behavior, crabs were presented with small and smooth randomly shaped pieces of calcium- and noncalcium-containing minerals. The samples lacked both the texture of shell surfaces and an aperture. In the presence of noncalcium-containing minerals, crabs ($N = 40$) did not exhibit any exploratory behavior. However, crabs ($N = 30$) examined samples containing calcium and rolled them several times. The mineral celestite (SrSO_4) was used to test for possible effects of SO_4 contained in plaster replicas (16). In addition, SrSO_4 was used to examine the specificity for calcium since strontium mimics the effects of calcium in other biological systems (17). No crabs explored the celestite samples or other noncalcium-containing minerals

Table 2. Responses of *P. hirsutiusculus* to pieces of minerals. Samples were in the same weight range as shells which were explored, between 0.8 and 2 g. Each animal was allowed a maximum of five contacts with each type of mineral. All pieces of minerals which contained calcium elicited exploratory behavior within two contacts.

Mineral	Number of animals showing exploratory behavior	Number of animals
CaCO_3 (calcite)	10	10
CaCO_3 (aragonite)	10	10
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (natural gypsum)	10	10
SrSO_4 (celestite)	0	10
MnCO_3 (rhodochrosite)*	0	10
FeCO_3 (siderite)	0	10
SiO_2 (quartz)	0	10

*Rhodochrosite was found to elicit some surface examination, but the sample was not rolled over; the purity of the mineral was not analyzed with respect to calcium.

tested (Table 2). To control for the possible influence of size and shape of samples, each mineral sample was replicated in plaster. The 40 hermit crabs that previously ignored the minerals lacking calcium, fully explored the replicas.

To examine the effect of calcium emanating from replica or natural shells on exploratory behavior, a thin layer of sealant was applied over the shell surfaces (9). When coated shells were presented as alternatives to uncoated ones,

90 hermit crabs (100 percent) selected uncoated natural and replica shells over coated ones. Although hermit crabs came in contact with coated shells and did not actively avoid them, the behaviors associated with shell selection were not elicited (9). A final possibility was that a difference in color between natural and replica shells influenced selection. To control for this, crabs were tested in complete darkness or with paint covering the crabs' eyes. Under these conditions animals selected replica shells.

To further demonstrate that exploratory behavior is dependent on the presence of calcium, crabs were presented with a choice between a small piece of calcite and a natural shell of equal weight which had been coated with a thin Au-Pd film of approximately 100 Å. This ensured that most of the microsurface structure of the shell was intact but that little or no calcium was present at the shell surface. The 12 animals which had contact with both objects explored the calcite mineral but ignored the coated shell.

Calcium may play a role in the shell selection behavior of other species of hermit crabs. Hertz (18) observed that *Clibanarius misanthropus* did not abandon long, narrow plaster containers even when they could exchange them for natural shells. Containers were explored for longer times than were natural shells of three species, and it was concluded that the stimulus was the shape of the plaster models. Sensitivity to calcium would be an alternative explanation. Reese (2), using a procedure that did not involve a choice, concluded that the hermit crab *P. samuelis* (Stimpson) does not rely on a chemical cue in selecting a shell. I observed that when given a choice 16 of 16 crabs of this species preferred shells which had calcium at the surface (19).

I conclude from these results that for *P. hirsutiusculus* a single inorganic substance, calcium, is the active factor at the surface of a shell which triggers shell exploration leading to the selection of a shell. It has been shown that hermit crabs are capable of finely tuned behavior in response to chemical signals other than feeding attractants (20, 21), and small molecules (possibly peptides), released by partially digested gastropods, attract hermit crabs (22). In such situations the crabs do not generally feed but exhibit behaviors associated with acquisition of a newly emptied shell (20, 21).

For a hermit crab survival is enhanced by having a shell (23), especially an adequate one (24, 25). Fecundity (26, 27), population densities (28, 29), and growth rates (27, 30) increase as the

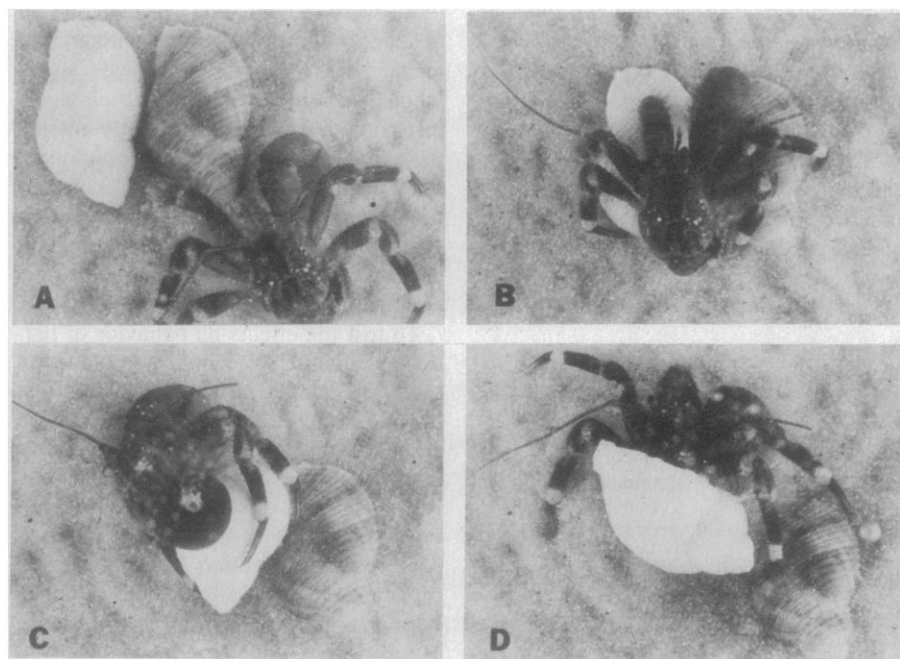


Fig. 1. The hermit crab *P. hirsutiusculus* given a choice between a natural shell (*Nucella canaliculata*) on the right and its plaster replica on the left. (A) Hermit crab approaching shells and (B) in contact with both shells, a requirement for scoring. (C) The plaster shell is explored and rolled until the aperture is probed. (D) The replica plaster shell is selected.

number of adequate shells increases. *Pagurus hirsutiusculus* lives in an environment where adequate shells are scarce (11, 28). Sensitivity to calcium may enable this species to locate partially buried shells, thus increasing the number of shells available for habitation. Living in an area with a siliceous-based substrate and little coral, *P. hirsutiusculus* may be able to discriminate empty shells from those housing living gastropods (31) as well as from pebbles or unsuitable homes.

Calcium is one of the most important control and regulatory substances in physiological systems (32), but reports of the elicitation of behavior by metal ions such as calcium are rare (33). Studies of the effects of calcium on whole animal systems may reveal a role for calcium sensitivity in other species.

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9. Coated shells (shells without a calcium cue) were only explored if the crab's chelae accidentally entered the shells' aperture and chelae probing was released or the crab was presented with one in a no-choice situation after being without a shell for hours. Shells were coated with Au-Pd film, paraplax wax, varathane, silicone rubber, or cyanoacrylate glue. At least ten crabs were tested with each type of coating.
10. This contrasts with the findings of E. S. Reese (2) who reported that the hermit crab species *P. samuelis* will quickly explore shells that are coated and hence lack a calcium cue.
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12. Outside molds of natural shells were made from silicone rubber. Inside molds were made from Blend Impression Material (Westone Labs., Inc.). The two molds were well aligned with one another to ensure accurate wall thickness. Core molds were carefully twisted out from both the original and plaster shells and could be used over again many times.
13. Hermit crabs were not found to prefer boiled shells over those that were well scrubbed and air or oven dried. Gloves were polyvinyl chloride (VWR Scientific).
14. Full exploratory behavior consisted of grasping and extensive scratching of the object with the chelae and repeatedly turning it over between the appendages. Because the objects did not have an aperture, chelae probing could not be fully expressed.
15. The same hermit crabs were also observed to select (100 percent) natural and replica shells over ones that had been coated and lacked a calcium cue in the same CaSO₄ saturated seawater. Shell selection behavior also appeared normal under these conditions. It was concluded that the crab's detection of calcium or sulfate was not impaired by raising the levels of CaSO₄ in the seawater and that animals could still detect a greater amount of calcium at the shell surface.
16. Possible effects of SO₄ are not completely ruled out because the solubility of celestite is less than that of gypsum. However, the calcium carbonates are even less soluble than SrSO₄ and they were effective in promoting exploratory behavior.
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19. *Pagurus samuelis* was tested under the same conditions as *P. hirsutiusculus*. Crabs were given a choice between natural shells (*Tegula funebralis*) or their plaster replicas and natural or replica shells coated with paraplax wax or silicone rubber. Because *P. samuelis* is visually attracted to particular shells (2) animals were tested in complete darkness or with black paint covering their eyes [K. A. Mesce, *Am. Zool.* **20**, 727 (1980)].
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33. A significant influence of calcium was demonstrated in the homing of sock-eye salmon [D. Bodznick, *J. Comp. Physiol.* **127**, 157 (1978)], which are sensitive to calcium ion concentrations in the range of 10⁻⁶ to 10⁻³M.
34. I thank G. Hoyle, S. Zill, R. Franklin, J. Mittenhal, K. Thompson, L. Muske, C. Cypher, and the reviewers for valuable discussions and comments on the manuscript. I also thank H. Howard for photographic assistance and B. Orr for providing the minerals. Supported in part by NSF grant BNS-79 04513 to D. Hoyle.

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Monoclonal Antibody to Acetylcholine Receptor: Cell Line Established from Thymus of Patient with Myasthenia Gravis

Abstract. A human B cell line producing a monoclonal antibody to an antigenic determinant of acetylcholine receptors was established by cloning B cells that had been transformed in vitro by Epstein-Barr virus. The B cells were obtained from the thymus of a patient with myasthenia gravis. The antibody produced by the cell line precipitated acetylcholine receptors from denervated and innervated rat muscle and from human muscle, but did not show detectable response to the acetylcholine receptors from the electric organs of *Narke japonica*. The monoclonal antibody showed identical binding patterns in innervated and denervated rat muscles. Passive transfer of the monoclonal antibody into rats induced moderate muscle weakness and electromyographic changes characteristic of myasthenia gravis.

Myasthenia gravis (MG) is an autoimmune disease in which neuromuscular transmission is impaired by autoantibodies to acetylcholine receptors (AChR). Monoclonal antibodies against *Torpedo* AChR produced by hybridoma cells have been used to identify individual determinants of AChR (1, 2). Only some of the antigenic determinants of *Torpedo* AChR proved to be shared with mammalian AChR and to induce MG (2).

Using B cells from the thymus of a patient with MG, we have now established a human B cell line producing a monoclonal antibody against AChR by cloning the cells after their transformation in vitro by Epstein-Barr virus. We chose the thymus as a source of B cells sensitized with AChR because antibodies to AChR have been found in 70 percent of the tissue extracts and culture fluids of the thymus and in germinal centers in the thymus of patients with MG (3-5).

Thymus lymphocytes from a patient with MG were suspended in RPMI-1640 medium with 20 percent fetal calf serum (6). Eight parts of thymus lymphocyte suspension (2 × 10⁷ cells per milliliter) were mixed with two parts of the culture fluid of B95-8 cells (used as a source of Epstein-Barr virus) (7). After incubation

at 37°C for 3 hours, enough culture medium was added to adjust the cell concentration to 2 × 10⁶ per milliliter. The lymphocytes were cultured further for 1 month in Linbro plates (76-033-05) in a humidified atmosphere of 5 percent CO₂ in air at 37°C. Half the volume of medium was changed once or twice a week. One month after the culture was started, the amount of antibody to AChR in the supernatant of each well of the Linbro plates was assessed, and the cell clusters producing relatively high titers of the antibody were dispersed to form new clusters. This procedure was repeated two times, until a cluster producing a reasonably high titer of the antibody was obtained. This cluster was used as a source for cloning the antibody-producing cells. Cells producing antibody to AChR were cloned twice by limiting dilution (0.4 cell in 0.2 ml per well) in Microtest II plates (Falcon 3040) in the presence of feeder cells (10⁴ cells per well) prepared by x-ray irradiation (2000 rads) of autologous lymphocytes that did not secrete antibody to AChR or by treating the cells with mitomycin C at a concentration of 50 µg/ml for 30 minutes at 37°C. Feeder cells were not proliferative under these conditions. Four weeks after the cells were cloned, supernatants