the gulf. The earthquake epicenters show a remarkable parallelism with the fracture zones that form the prominent bathymetric and structural lineations.

Profiler records, crossing the two southernmost offsets (Fig. 3, *V* and *W*), emphasize the faulted nature of the bathymetric lineations. To the east of the fault bordering the southernmost offset, the Mexican mainland margin is made up of a thick section of sediments within which there is a prominent unconformity, with probably Tertiary sediments forming much of the section. West of the fault, beneath the actively spreading section, only very thin sediments cover a basement reflector (Fig. 3, *V*).

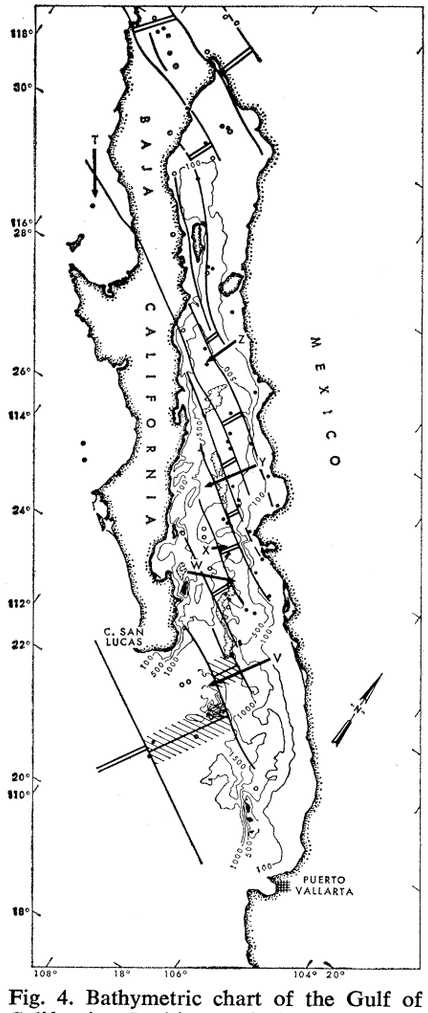

Fig. 4. Bathymetric chart of the Gulf of California. Positions of fracture zones (solid black lines) are based on reflection profiles. The crest of the East Pacific Rise, based on magnetic and seismic-reflection profiles at the mouth of the gulf, is hatched. Proposed positions of spreading centers are shown by double black lines; epicenters (*14*), by open (Gutenberg and Richter) and by solid circles (Sykes). Positions and directions of reflection profiles of Fig. 3 are shown by solid arrows.

Farther up the gulf, seismic-reflection profiles again give positive verification of the faulted nature of the basin flanks. The section of profile *X* (Figs. 3 and 4) shows faulting on the south side of a spreading cell, and profile *Y* (Figs. 3 and 4) shows a rifted trough defined by the bordering fracture zones. The latter profile also indicates that the next fracture zone, well up the slope, projects into the section as a distorted zone. Profile *Z* (Figs. 3 and 4) crosses the mainland continental terrace to its abrupt termination at a major eastern boundary lineation; again the structure indicates that the next-northeastern fracture zone may be projected into the section.

Two major faults that can be seen crossing the peninsula are the previously documented Agua Blanca fault (*15*) and, to the south, a fault newly verified by Fyfe (*16*); between them lies most of the granite pluton of the peninsula. The Agua Blanca, in particular, is known for its recent dextral movements and, by our placement of spreading axes, would be a transform fault.

Additional verification of the transpeninsular nature of the recently defined fault to the south is shown by the reflection profile *T* (Fig. 4) on the west side of the peninsula. Fyfe found that the onshore fault turned more to the west before heading out to sea. Projected on this course it coincides neatly (Fig. 3) with the major structural discontinuity seen in the reflection record.

On the basis of an axial growth rate of 3 cm/year and the distance of 120 km from the present crest of the ridge to the 1000-fathom (1830-m) isobath off the tip of the peninsula, the present cycle of spreading began about 4 million years ago. Because the spreading cell is believed to have migrated to the west, translation of the peninsula, from near the west edge of the thick pelagic sediments to its present location, occurred at a doubled rate of about 6 cm/year. Therefore, from about 4 to 10 million years ago, during Late Miocene and Pliocene time, a proto-Gulf of California existed. The earlier period of spreading of the sea floor, which is now buried by the thick pelagic sediments, also would have required about 4 million years if the peninsula was originally situated next to the mainland, south of Puerto Vallarta, and moved to its Late Miocene or Early Pliocene position at a rate comparable to that of the present cycle. The presence of relatively thick, probably Tertiary sediments on the mainland continental margin of Mexico (Figs. 3 and 4: *V*, *Y*, and *Z*) complements the idea of existence of a Late Miocene–Pliocene gulf, and is additional evidence of the existence of a regional lapse in spreading of the sea floor during much of Pliocene time.

DAVID G. MOORE
EDWIN C. BUFFINGTON
Marine Environment Division, Naval Undersea Warfare Center, San Diego, California 92132

References and Notes

1. Larson, Menard, and Smith's more recent independent magnetic survey [*Science* **161**, 781 (1968)] of the East Pacific Rise, in the region of its approaches to the gulf, resulted in a comprehensive chart of magnetic anomalies on and flanking the critical part of the rise as it approaches and enters the gulf. Their studies have reached several common conclusions based on significantly different sets of data and are complementary.
2. H. W. Menard, *Science* **132**, 1737 (1960); B. C. Heezen, *Sci. Amer.* **203**, 98 (1960).
3. J. C. Crowell, *Geol. Soc. Amer. Spec. Paper 71* (1966).
4. G. A. Rusnak and R. L. Fisher, *Amer. Assoc. Petrol. Geologists Mem. 3* (1964), p. 144; D. C. Krause, *Bull. Geol. Soc. Amer.* **76**, 617 (1965).
5. W. Hamilton, *Bull. Geol. Soc. Amer.* **72**, 1307 (1961).
6. F. J. Vine and D. H. Matthews, *Nature* **199**, 947 (1963).
7. J. T. Wilson, *ibid.* **207**, 343 (1965).
8. H. W. Menard, *J. Geophys. Res.* **71**, 682 (1966).
9. F. J. Vine, *Science* **154**, 1405 (1966).
10. L. R. Sykes, in *History of the Earth's Crust* (NASA, in press).
11. W. R. Normark and J. R. Curray, *Bull. Geol. Soc. Amer.*, in press.
12. A. Cox, R. R. Doell, G. B. Dalrymple, *Science* **144**, 1537 (1964).
13. D. A. Ross and G. G. Shor, *J. Geophys. Res.* **70**, 5551 (1965).
14. B. Gutenberg and C. F. Richter, *Seismicity of the Earth* (Princeton Univ. Press, 1954); L. R. Sykes, *J. Geophys. Res.* **72**, 2131 (1967).
15. C. R. Allen, L. T. Silver, F. G. Stehli, *Bull. Geol. Soc. Amer.* **71**, 467 (1960).
16. D. L. Fyfe, thesis, San Diego State College.

17. Sponsored by NAVSHIPS under SR 104 03 01 (Task 0539).

28 June 1968

Taste-Modifying Protein from Miracle Fruit

Abstract. *The active principle of miracle fruit* (Synsepalum dulcificum) *is a basic glycoprotein with a probable molecular weight of 44,000. Application of the protein to the tongue modifies the taste so that one tastes sour substances as sweet.*

A native shrub (*Synsepalum dulcificum*) in tropical West Africa yields a small, red berry that, once its pulp is chewed, causes sour substances to taste sweet. Local people often use it to make their stale and acidulated maize bread more palatable and to give sweetness

Table 1. Amino acid composition of taste-modifying protein. The data are given as residues per 100 total residues. Three milligrams of the lyophilized taste-modifying protein was hydrolyzed in 6*N* HCl at 110°C for 22 hours. The amino acid analysis was performed on the Beckman model 120 amino acid analyzer. Sugar components comprised 6.7 percent of the protein as determined by the tryptophan-sulfuric acid reaction (*9*). Paper chromatography was carried out by the method of Masamune and Yosizawa (*10*) to identify the sugar component. The sugars were detected by the aniline hydrogen phthalate-spray method (*11*). Two red spots were identified as L-arabinose and D-xylose, respectively, by comparison with known spots of sugars.

Amino acid	residue per 100 total residues	Amino acid	residue per 100 total residues
Lysine	7.9	Alanine	6.3
Histidine	1.8	Half cystine	2.3
(Ammonia)	17.4	Valine	8.0
Arginine	4.7	Methionine	1.0
Aspartic acid	11.3	Isoleucine	4.7
Threonine	6.1	Leucine	6.5
Serine	6.1	Tryptophan	—
Glutamic acid	9.2	Tyrosine	3.6
Proline	6.0	Phenylalanine	5.0
Glycine	9.8		

to sour palm wine and beer. Daniell (*1*) first described the unusual properties of the berry and called it miraculous berry. Others call it miracle fruit.

Inglett *et al.* (*2*) stated that "Miracle Fruit has the unique property of causing sour materials to taste sweet after the mouth has been exposed to the fruit's mucilaginous material. Sour foods, such as lemons, limes, grapefruit, rhubarb, and strawberries taste pleasantly sweet; dilute organic and mineral acids also taste sweet." They attempted to isolate the active principle of the fruit, but the active principle could not be solubilized by any treatment including extraction with water, salt solution, or organic solvents or treatment with detergents or enzymes (*2*). We now report our studies on the isolation and characterization of the active principle from miracle fruit.

The berries were grown in a greenhouse at the Florida State University. Since the active principle is labile, the berries were stored in a deep freezer (−70°C) until needed. The sweetening activity was assayed on four subjects. Five milliliters of a solution containing the active principle was kept in the mouth for 2 minutes and was spit out. The mouth was rinsed with distilled water, and 0.02*M* citric acid solution was tasted. For a quantitative measurement of the activity, the subject was asked to choose one out of a series of ten sucrose solutions (0.1 to 1.0*M*) which best approximated the intensity of sweetness of the given citric acid.

The skin and seed of 300 berries were removed by hand, the pulp was homogenized with distilled water, and the homogenate was centrifuged (*3*). The active principle did not appear in the supernatant. The insoluble slurry was extracted with carbonate buffer (*p*H 10.5). The extracts had a strong activity, whereas the residue of the slurry had no activity after the extraction procedure was repeated three times. We applied the combined extracts to a column of diethylaminoethyl-Sephadex A-25 to remove colored materials (*4*). The effluent was colorless and had a strong activity. The effluent, concentrated with Aquacide (*5*), was used to obtain preliminary information on properties of the active principle.

Activity was destroyed when the solution was boiled or exposed to a high concentration of organic solvents at room temperature. Activity was greatly decreased by exposure to *p*H above 12.0 or below 2.5 at room temperature, whereas activity was stable at *p*H 3.7 and 4°C for at least 1 month. When the solution was dialyzed against distilled water for 48 hours, no activity was observed in the dialyzate. Addition of trypsin or pronase destroyed the activity. These characteristics suggest that the active principle is protein.

The molecular weight of the active principle was estimated with a Sephadex G-75 column (*6*). The elution pattern of the active principle was typical of that of a single homogeneous polymer. Elution volumes of proteins with known molecular weights were determined on the same column, and the observed elution volumes were plotted against the logarithm of the molecular weights according to the method of Andrews (*7*). The molecular weight of the active principle was estimated as 44,000.

Further purification of the active principle was carried out on a cation-exchange column. The concentrated effluent from the diethylaminoethyl-Sephadex column was dialyzed against 0.03*M* acetate buffer (*p*H 5.5) and applied to a column of carboxymethyl-Sephadex C-25. The active principle was absorbed on the column and eluted with a gradient of phosphate buffer. The active fraction from the column had characteristics typical of protein; its reaction to biuret reagent was positive, and its absorption maximum was at 278 nm. The elution pattern of the

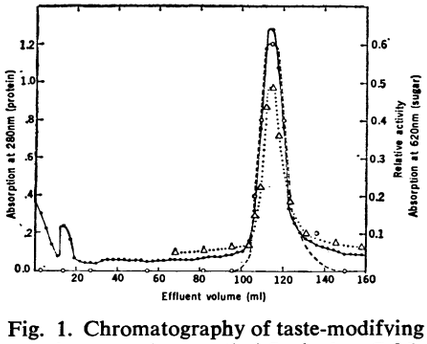

Fig. 1. Chromatography of taste-modifying protein on carboxymethyl-Sephadex C-25. A sample solution (100 ml) was applied to a column (1.1 by 42 cm) of carboxymethyl-Sephadex C-25 (100 to 270 mesh) buffered with 0.03*M* acetate buffer, *p*H 5.5. The column was eluted with a linear gradient between 0.03*M* phosphate, *p*H 6.0 (80 ml), and 0.2*M* phosphate, *p*H 8.8 (80 ml); flow rate 20 ml/hour. The volume of each fraction was 2.7 ml. Protein was detected by absorption at 280 nm. Sugar content was determined at 620 nm after 0.25 ml of each fraction was colored by reaction with the tryptophan-sulfuric acid. Activity of the protein was assayed with 0.1 ml of each fraction after dilution with water to 5 ml, and expressed as the concentration of sucrose whose sweetness is equal to that of the 0.02*M* citric acid solution. The yield of the protein from 300 berries was 10 mg. Closed circles, protein; open circles, activity; and triangles, sugar.

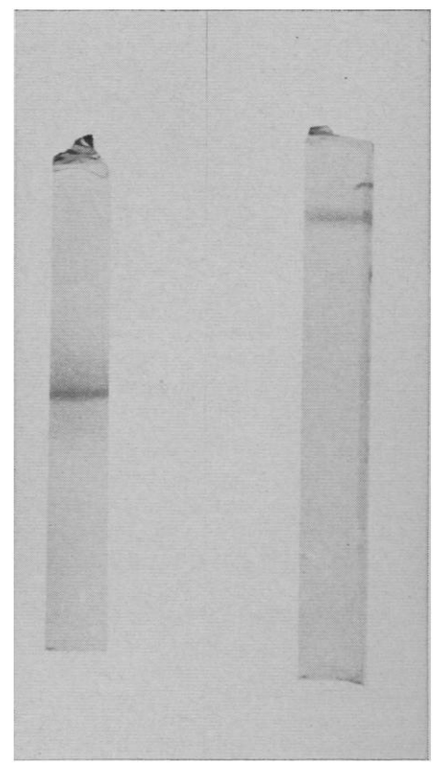

Fig. 2. Polyacrylamide disc electrophoresis of taste-modifying protein. Left, without urea; right, in 8*M* urea. A concentrated active fraction from carboxymethyl column was applied to gels. Electrophoresis was conducted at 4°C and 3 ma for 2 hours at *p*H 4.5. The gels were stained with 1 percent amido black in 7.5 percent acetic acid solution.

active protein, measured by ultraviolet absorption, correlates well with the activity curve (Fig. 1). This purified protein seems to be an aggregate of the protein monomer, because application of the protein to the Sephadex G-75 column indicated that the molecular weight of the purified protein, measured by activity and ultraviolet absorption, was more than 80,000 (*8*). Aggregation proceeded during the dialyzing process against acetate buffer of low ionic strength, although this process did not change the activity of the protein.

Purity of the isolated protein was checked with disc electrophoresis on polyacrylamide gel. The protein migrated to the cathode at *p*H 4.5 (Fig. 2) but did not migrate at *p*H 8.3. This electrophoretic behavior was in agreement with that expected from the result obtained with the cation exchange column, and indicated that the protein is basic. A single sharp band accompanied a light diffused band. These bands changed into a single sharp band (Fig. 2) when electrophoresis was carried out in 8*M* urea. This fact indicated that the isolated protein does not contain any protein other than the active component.

An amino-acid analysis of the protein was carried out after the lyophilized protein was hydrolyzed in 6*N* HCl (Table 1). Sugar components comprise 6.7 percent of the protein. The sugar content of each fraction from a carboxymethyl-Sephadex column was determined by the tryptophan-sulfuric acid reaction (Fig. 1, triangles) (*9*). The possibility that the sugars are an impurity was eliminated by the fact that the curve of sugar content correlated well with the chromatographic pattern of the active protein (Fig. 1) and that the sugar content was not changed by dialysis or rechromatography of the fractions on a carboxymethyl-Sephadex column. Paper chromatography of the protein hydrolyzate gave two sugar spots, which were identified as L-arabinose and D-xylose. These results indicated that the active principle of miracle fruit is a glycoprotein.

The sweetening of the subsequent taste of acids was observed at a $5 \times 10^{-8} M$ concentration of the protein solution and reached a maximum at $4 \times 10^{-7} M$. The sweetening effect at high concentration of the protein solution slowly declines over a period up to 2 hours. The purified protein itself has no inherent taste. A mixture of the protein with sour substances initially tastes sour, and slowly changes to sweet if the mixture is held in the mouth for about 1 minute. This fact ruled out the possibility that a complex of the protein with the sour substance itself has a sweet taste. It is believed that the protein binds to receptors of the taste buds and modifies their function. We call the protein "taste-modifying protein."

KENZO KURIHARA
LLOYD M. BEIDLER
Department of Biological Science, Florida State University, Tallahassee

References and Notes

1. W. F. Daniell, *Pharm. J.* **11**, 445 (1852).
2. G. E. Inglett, B. Dowling, J. J. Albrecht, F. A. Hoglan, *J. Agr. Food Chem.* **13**, 284 (1965).
3. Pulp (100 g) from 300 berries was homogenized with 200 ml of distilled water in a Waring blender for 1 minute. The homogenate was centrifuged at 10,000 rev/min for 10 minutes, and the sediment was washed with water once more. The washed sediment was homogenized with 100 ml of 0.1*M* $NaHCO_3$-Na_2CO_3 buffer, *p*H 10.5, for 1 minute. After the homogenate was stirred for 5 minutes, it was centrifuged at 10,000 rev/min. The extraction was repeated three times with the carbonate buffer. All of the preparative experiments in this paper were carried at 0 to 4°C.
4. Combined extracts with carbonate buffer were applied to a column (3.6 by 45 cm) of DEAE-Sephadex A-25 (50 to 140 mesh) buffered with a 0.025*M* phosphate buffer, *p*H 6.0. The column was eluted with 0.1*M* carbonate buffer, *p*H 10.5, and 600 ml of active fraction was collected.
5. The *p*H of the effluent from DEAE-Sephadex column was adjusted to 3.7, and the effluent was placed in dialyzing tube. The powder of Aquacide II (Calbiochem) was placed outside the tube and kept overnight. The volume (600 ml) of the solution in the tube was concentrated to 100 ml.
6. One milliliter of 0.5 percent protein solution (hemoglobin, chymotrypsin, ribonuclease, and cytochrome c) and a solution of taste-modifying protein was applied to a column (1.5 by 61.5 cm) of Sephadex G-75 (0.1*M* phosphate buffer, *p*H 7.0; flow rate 20 ml/hour). Activity was measured with 1 ml of each fraction after dilution with water to 5 ml.
7. P. Andrews, *Biochem. J.* **91**, 222 (1964).
8. An ultracentrifugal pattern of the purified protein showed a single peak, but the pattern diffused quickly.
9. J. Badin, C. Jackson, M. Shubert, *Proc. Soc. Exp. Biol. Med.* **84**, 288 (1953).
10. H. Masamune and Z. Yosizawa, *Tohoku J. Exp. Med.* **59**, 1 (1953).
11. S. M. Partridge, *Nature* **154**, 443 (1949).
12. Supported by NSF grant GB-4068X and AEC grant AT-(40-1)-2690.

24 May 1968; revised 9 August 1968 ■

Sea Urchin Response to Foreign Substances

Abstract. *An assessment of the response of* Strongylocentrotus purpuratus *to substances injected into the coelom shows that (i) foreign molecules are cleared from the coelomic fluid more rapidly than native molecules, (ii) coelomocytes can respond selectively to albumins of human and bovine origin, and (iii) immunization attempts fail to elicit accelerated coelomocyte uptake or accelerated clearance of foreign protein.*

The vertebrate immune response is characterized by (i) increased responsiveness to a foreign substance as a result of prior exposure to it, and (ii) specificity, permitting this response to proceed only against the immunizing substance and closely related substances. No invertebrate has yet been found which possesses both of these two characteristics (*1*). Conceivably, during evolutionary history, one of these characteristics might have developed before the other, and if so, one might expect to find animal species which have in their response to foreign substances only one of these characteristics. Recent evidence suggests that some invertebrates possess a degree of receptor specificity toward antigens (*2*). We report here that the sea urchin (phylum Echinodermata), while not developing a state of increased responsiveness to foreign protein, nonetheless without prior exposure can respond specifically, discriminating between two structurally related proteins. The finding of such discriminative capacity suggests an evolutionary connection between this sea urchin response and the vertebrate immune response, a possibility which seems more plausible because of the known phylogenetic affinities of echinoderms and chordates (*3*).

Sea urchins *Strongylocentrotus pur-*

Table 1. Clearance of radioactive molecules from the coelomic fluid of *S. purpuratus*.

Molecule	No. of animals per group	Protein present (μg/ml, $\pm$ 1 S.D.) at various times after injection			Protein remaining after 22 hours (mean percent)
		1 hour	13 hours	22 hours	
BSA-C^{14}	11	2.30 $\pm$ .24	0.70 $\pm$ .29	0.28 $\pm$ .15	12.2
HSA-C^{14}	6	2.97 $\pm$ .44	0.96 $\pm$ .19	0.31 $\pm$ .10	10.4
S. purpuratus molecules*	4	2.55 $\pm$ .20	1.44 $\pm$ .26	1.36 $\pm$ .19	53.2

* See text for preparation procedure.