fects in many instances does not appear to be specific for the *agent* used but may be more clearly related to the intensity of the treatment and the period of pregnancy at which it is given, as well as to the interplay of these factors with genetic determinants in the developing organism (*7*).

LESTER M. GELLER
Division of Neuropathology, Department of Pathology, College of Physicians and Surgeons, Columbia University, New York

References and Notes

1. H. Nishimura and K. Nakai, *Science* 127, 877 (1958).
2. This work was supported in part by a grant from the Association for the Aid of Crippled Children and by research grant B-1145, from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.
3. J. M. Essenberg, J. V. Schwind, A. R. Patros, *J. Lab. Clin. Med.* 25, 708 (1940).
4. C. H. Thienes *et al.*, *J. Pharmacol. Exptl. Therap.* 87, 1 (1946).
5. T. H. Ingalls, F. J. Curley, R. A. Prindle, *New Engl. J. Med.* 247, 758 (1952); F. J. Curley and T. H. Ingalls, *Proc. Soc. Exptl. Biol. Med.* 94, 87 (1957).
6. J. Warkany in *Advances in Pediatrics* (Interscience, New York, 1947), p. 1; S. P. Hicks, *J. Am. Med. Assoc.* 154, 115 (1954); U. Murakami, *Acta Pathol. Japon.* 5, 495 (1955).
7. T. H. Ingalls, F. R. Avis, *et al.*, *J. Heredity* 44, 185 (1953); M. N. Runner, *J. Natl. Cancer Inst.* 151, 637 (1954).

24 July 1958

Alginase in the Sea Urchin Strongylocentrotus purpuratus

Abstract. Viscosimetric evidence of alginase activity is given for the intestine and the intestinal contents of a sea urchin. The alginase activity of the gut wall and that of the contents of the gut differ in *p*H optima; this suggests that there may be two sources of alginase. The enzyme (or enzymes) depolymerizes algin.

Alginase may be a common digestive enzyme in invertebrates which feed upon brown algae. The presence of the enzyme has been noted in intestinal extracts of an abalone, *Haliotus giganteus*, and a sea urchin, *Sphaerechinus pulcherissima* (*1*), and in a sea hare, *Aplysia punctata* (*2*).

The alginase from Haliotus (*2*), like that of certain bacteria (*3*), appears to hydrolyze algin to free mannuronic acid, after a period of incubation. As with pectic-acid hydrolysis by pectin-polygalacturonase (*4*), the viscosity of the algin solution is much reduced before measurable reducing sugar appears (*2*). By analogy with the nomenclature of pectic enzymes (*4*), this alginase may be termed algin-polymannuronase.

In *Cryptochiton stelleri* (*5*) and in the sea urchin *Strongylocentrotus purpuratus*, alginase activity has not yet been demonstrated by the appearance of reducing sugar, in tests in which the Somogyi-Nelson reagents are used (*6*). However, we have found that intestinal extracts of the sea urchin very quickly reduce the viscosity of algin solutions.

The presence of an alginase in marine animals and production of this enzyme by marine decomposing microorganisms may have considerable ecological significance in the economy of the intertidal and subtidal zones. Waksman *et al.* claim that bacteria are most important in algin decomposition and assign a negligible role to marine fungi (*7*).

A further importance of alginase may lie in its use in clarifying the structure of algin, which is known to be a linear polymer of mannuronic acid, although details of the structure are still not fully known (*8*). Miwa (*2*) used the enzyme from *Haliotus* in a study of the anatomy of brown algae.

Because algin is a major constituent of brown algae, which, at times, make up a large portion of the diet of *S. purpuratus*, our purpose was to examine this animal for the existence of an alginase and to determine whether such an enzyme is active at the reported *p*H of the gut.

Crude enzyme preparations were obtained from (i) whole gut plus gut contents; (ii) the gut wall, washed five times with sterile sea water; (iii) the liquid gut contents; and (iv) the solid contents of the gut (in this case mostly coralline red algae). The filtered gut liquid contents were used directly. The other preparations were ground in a mortar with cold 0.5*M* tris buffer (tris-hydroxymethylaminomethane) at *p*H 7.5, then filtered through Whatman No. 1 and No. 42 filter papers before use.

These extracts were mixed with approximately 0.1 percent of sodium alginate (*9*), and subsequent changes in viscosity were followed by means of a rolling ball (Hoeppler type) viscosimeter. Decrease in viscosity was taken as evidence for digestion of the algin (*2*).

For the determination of activity with varying *p*H, tissues were ground in distilled water and filtered as before, then mixed with alginate made up in the appropriate buffer. Variation in the initial algin viscosity, especially marked in McIlvaine's buffers, was corrected for in the activity determinations. The digestion experiments never exceeded 4 hours' duration, and no preservative was used.

Potassium oxalate (final concentration 0.03 percent) was added to the gut liquid and gut solids extracts to eliminate the effects of calcium salts (from the coralline red algae) on the viscosity of the algin solutions. The same amount of oxalate, added to extracts low in calcium, was without effect on the enzyme activity.

Tests for reducing-sugar production during the reaction period were made by the Somogyi-Nelson method (*6*). Tests for mannuronic acid were made on aliquots, after precipitation of polyuronides with 10 percent calcium chloride, in a naphthoresorcinol test (*10*).

All of the extracts appeared to contain an enzyme or enzymes capable of digesting algin. At the same time, no increase in reducing sugar could be demonstrated, nor did any calcium-soluble, naphthoresorcinol-positive products appear. Only the marked decreases in viscosity observed indicated that digestion had occurred. Again, by analogy with pectic enzyme nomenclature, this alginase may be called an algin depolymerase.

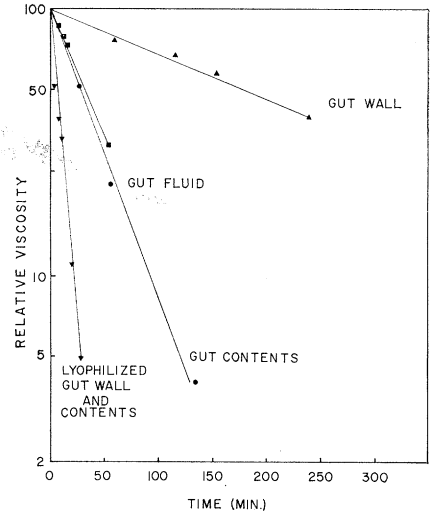

Fig. 1. Reduction in viscosity of sodium alginate solution in the presence of alginase extracts from the sea urchin *Strongylocentrotus purpuratus*. Relative viscosity = (100 minus percentage of viscosity change). Reaction mixtures contained: (i) gut fluid, gut solids, and gut wall; (ii) 3 ml of extract, 14 ml of 0.1-percent sodium alginate, and lyophilized gut wall and contents; (iii) 10 ml of extract, 20 ml of sodium alginate.

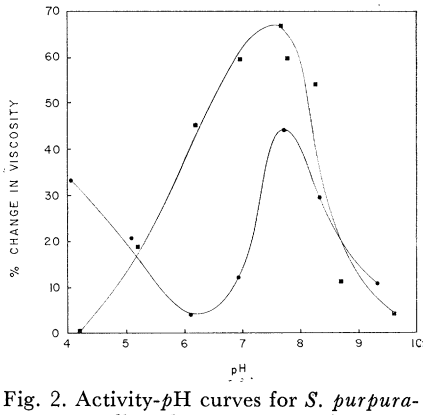

Fig. 2. Activity-*p*H curves for *S. purpuratus* gut wall and gut contents (squares) and gut wall (circles). Tris buffer was used for *p*H 7.5 and above; McIlvaine's buffer, for *p*H 7.5 and below. Reaction mixtures contained: (i) gut wall and gut contents; (ii) 4 ml of enzyme preparation, 5 ml of buffer, 10 ml of 0.1-percent sodium alginate, gut wall; (iii) 1.5 ml of enzyme preparation, 5 ml of buffer, 11 ml of sodium alginate. Reaction period, 30 min.

The gut wall was less active than were the liquid contents, the gut solid extracts, or the combined gut contents and gut wall, containing approximately the same concentration of homogenate. The most rapid digestion of algin occurred with a lyophilized gut-wall-plus-gut-contents preparation. This extract decreased the viscosity of the polysaccharide by more than one-half in 7½ min, and the reduction in viscosity was complete in 50 min (Fig. 1).

Figure 2 shows the *p*H activity curve for the alginase of the gut wall compared with that of the pooled gut wall and gut contents. Although both have optima in the range of the intestinal *p*H (7.2 to 7.3) (*11*), the alginase from the gut wall has an additional optimum toward *p*H 4 (*p*H values below 4 were not used because of the increase in algin viscosity at low *p*H).

Different *p*H activity curves, obtained for the alginase of the gut wall and of the gut contents, respectively, suggest that there may be two alginases involved. Because the gut contents of this animal are known to contain microorganisms capable of degrading whole brown algal blades (*5*), it is possible that these may be a source of alginase in the gut. On the other hand, precursors of alginase in the gut wall could contribute to the alginase found in the gut contents. Clarification of the role of intestinal bacteria in algin digestion by *Strongylocentrotus purpuratus* is needed. It would also be of interest to characterize the products of algin digestion by these preparations and to determine their usefulness to the sea urchin.

RICHARD W. EPPLEY
Department of Biology,
University of Southern California,
Los Angeles

REUBEN LASKER*
Life Sciences Division,
Compton College, Compton, California

References and Notes

1. K. Oshima, *Bull. Agr. Chem. Soc. Japan* 7, 17 (1931); *J. Agr. Chem. Soc. Japan* 7, 329 (1931) [*Chem. Abstr.* 25, 5681 (1931)].
2. T. Miwa, *Japan. J. Botany* 11, 41 (1940). This report includes a brief review of earlier Japanese work on alginase.
3. P. Kooiman, *Biochim. et Biophys. Acta* 13, 338 (1954).
4. Z. I. Kertesz, *The Pectic Substances* (Interscience, New York, 1951).
5. H. Huang and A. C. Giese, *Science* 127, 475 (1958).
6. M. Somogyi, *J. Biol. Chem.* 195, 19 (1952).
7. S. A. Waksman, C. L. Carey, M. C. Allen, *J. Bacteriol.* 28, 213 (1934).
8. S. K. Chanda, E. L. Hirst, E. G. V. Percival, A. G. Ross, *J. Chem. Soc.* 338, 1833 (1952).
9. The sodium alginate (Kelcosol) used in this study was a gift from the Kelco Company, San Diego, Calif.
10. H. D. Ratish and J. G. M. Bullowa, *Arch. Biochem.* 2, 381 (1943).
11. R. Lasker and A. C. Giese, *Biol. Bull.* 106, 328 (1954).

* Present address: U.S. Fish and Wildlife Service, Scripps Institution of Oceanography, University of California, La Jolla.

15 September 1958

Functional Hermaphroditism and Self-fertilization in a Serranid Fish

Abstract. Each mature individual of *Serranellus subligarius,* regardless of size, has at the same time both motile sperm and eggs. Embryos and larvae were produced from artificial fertilizations and from isolated fish kept in aquaria. Mating behavior of pairs and groups of hermaphrodites shows two types of behavioral patterns involving different color changes.

Although teratological hermaphroditism has been reported in a wide variety of teleosts, only two groups, the serranids and the sparids, have been seriously considered to have members which are regularly and functionally hermaphroditic. Evidence for this has been based on purely anatomical and similar evidence. A recent review of this situation is given by Bertin (*1*). The present report presents data on the reproductive behavior, including self-fertilization, of the serranid *Serranellus subligarius* (Cope) (*2*). Such observations were possible because this species is small enough to be kept conveniently in the usual laboratory aquaria.

During July, August, and early September of 1958, all of the mature individuals of this species appeared to have slightly to greatly distended abdomens. They are common in depths of from 8 to 65 ft on rocky bottoms in areas of the Gulf of Mexico near the Cape Haze Marine Laboratory, off Sarasota and Madeira Beach, Florida, where I have observed thousands of these fish while skin diving. Over two hundred specimens have been collected by steering them into glass jars by hand (*3*). Individuals over 28 mm in standard length which were examined had sizable ovaries with a thin winding patch of white tissue, on the ventral surface, from which motile sperm smears can be made. Within 1 or 2 days after they have been collected, greatly distended individuals can be made to release quantities of mature sperm and hundreds of ovulated eggs simultaneously by means of very slight pressure on the abdomen. If this pressure is applied while the genital area of the fish is being viewed under a dissecting scope it can be seen clearly that the eggs are released from a separate exit of the oviduct into a small vestibule under a flap with a bilobed edge, just posterior to the anus. Posterior to the exit for the ova is a pigmented genital papilla which erects slightly when sperm is released from a small opening at the tip of the papilla. A clear fluid is sometimes released with the cloud of sperm. Eggs have been artificially fertilized by sperm from the same individual by washing both eggs and sperm from the genital area into a fingerbowl with sea water. Ovulated eggs in good condition taken from live or recently dead fish are readily fertilized by sperm from the same or another fish.

In cases of both self- and cross- artificial fertilizations, embryonic development has been followed through to the hatching of the larvae (18 to 22 hours at 82 to 88°F). The egg is buoyant and nonadhesive and has a single oil drop. The developing embryo and newly hatched larva have a distinctive set of round dark pigmented areas (two on the head, two just anterior to the anus, and four forming a ring around the tail, halfway along its length).

I have observed spawning activity in nature while diving with an Aqualung, and also in laboratory aquaria. Studies so far indicate that spawning activity usually takes place between two individuals in the late afternoon between 4 and 7 P.M. It seems to be initiated by a fish with a distended abdomen, who puts its body into an "S" curve, spreading its fins and sometimes quivering in this position directly before or near the head end of another fish. During S-curving the white area of the abdominal region stands out conspicuously. The other fish may be obviously carrying ripe ova as well as sperm, or it may be comparatively slim, carrying only immature ova but with sperm which can be squeezed out easily. A fish with only mature sperm will often ignore an S-curving fish or nip it and show aggressive behavior toward it. In other cases it will start to follow an S-curving fish until both fish are swimming with slow jerky movements, often upwards to the surface of the aquarium, and the pairing fish may separate momentarily or for long periods after a splash at the surface. In nature, however, the fish stayed within a few inches of the bottom on the occasions when they showed spawning behavior. The fish that is following often touches with its mouth the dorsal region of the S-curving fish, or it may follow from below and gently mouth the abdominal region. Sometimes the S-curving fish will lie down on its side with the other fish curved over it.

As S-curving activity becomes more marked, being repeated at more frequent intervals, and while the fish are close together, there is a noticeable color-pattern change in the S-curving fish. The fish blanches, the usual dark vertical bands on the sides of the body completely disappear, and the large black spot at the base of the dorsal fin suddenly turns pale gray. In addition, the evenly rounded profile of the abdomen changes, and the front half is pulled up flatter while the posterior part of the abdomen is lowered conspicuously and sharply just anterior to the genital area, forming approximately a right angle with the genital area. The other fish of the pair stays in the normal darker-banded color phase, and only in a few instances is there