Reports

New Property of the Crystalline Style of Crassostrea virginica

During experiments in which oysters were fed cultures of the red-colored alga *Cryptomonas* sp. (*1*), oyster feces were consistently green in color. Oysters sacrificed during feeding experiments revealed (i) that red pigment was distributed in the stomach juice and surrounding tissues and (ii) that green masses of disintegrated *Cryptomonas* cells were wound around the head of the crystalline style and packed in the intestine. Normal-appearing *Cryptomonas* cells were found at the esophageal end of the stomach. Samples of stomach juice taken nearer the style showed few motile cells, most being rounded and less red in color. Neither motile nor red cells were found in the intestine.

To explain the rapid action on the cells it seemed advisable to study (i) the direct effect of low acidity comparable to that found in the stomach and (ii) the effect of the style and its strong amylase (*2, 3*).

Oysters actively feeding on *Cryptomonas* were dissected quickly, and Beckman electrodes were inserted directly into the juice of the exposed stomachs. The *p*H of the stomach juice ranged between 6.0 and 6.3. The *p*H of styles, obtained by placing freshly removed styles across a one-drop electrode, ranged from 5.8 to 6.0. Yonge (*3*) reported lower *p*H ranges in *Ostrea edulis*—namely, 5.4 to 5.9 for stomach juice and 5.2 to 5.4 for the style. Cultures of *Cryptomonas* as well as those of *Monochrysis* sp. and *Isochrysis* sp. (*4*) were centrifuged and resuspended in sea water adjusted to different *p*H levels with Sørensen phosphate buffer. The lowest *p*H's at which these different cells retained their motility and normal appearance for 12 hours or longer were 5.5, 5.7, and 6.0, respectively. Hence, oyster style or stomach acidity could not alone be responsible for disintegration of *Cryptomonas*.

The effect of styles upon algal cells in vitro was studied by means of the hanging-drop technique. Styles removed from actively pumping oysters were washed twice in sea water buffered at *p*H 6.0. Extracts were made by dissolving three to seven washed styles in 1 to 10 ml of sea water buffered at *p*H 6.0. Algal cells were centrifuged and resuspended in sea water buffered at *p*H 6.0. Approximately 0.75-ml quantities of algal suspensions and style extract, or algal suspensions and one-third to one style, were used in hanging drops. The effect of styles upon algal cells was determined by continuous microscopic observation in over 75 experiments at 19 ± 1.5°C.

Disintegration of *Cryptomonas* cells, in both hanging drops and stomach juice, follows a uniform sequence. The first step involves two to three rapid backward jerking movements followed by loss of motility as a globule is everted from the gullet. Within 1 to 2 minutes the cell becomes round and the red pigment slowly fades until only a green mass of indefinite shape remains. Within 30 minutes after the first step, the green material fades until it is barely perceptible. The limiting membrane of the cell is usually indistinguishable after about 10 to 15 minutes.

In drops containing undissolved styles, *Cryptomonas* swimming near the style disintegrated immediately. As the styles dissolved, the area in which cells disintegrated gradually spread from the style toward the periphery of the drop. Disintegration of all cells occurred in 20 ± 5 minutes. In a few preparations containing small pieces of style, the wave of disintegration stopped short of the periphery of the drop. Shortly (within about 5 minutes) after final dissolution of the style, the motile cells were able to swim in any section of the drop, including the area of the dissolved style, without ill effect. In drops containing style extracts, disintegration occurred in three preparations only. In these instances the extracts were used prior to the complete dissolution of styles, the extract containing small pieces of style.

A similar wave was observed to immobilize *Monochrysis* in 20 ± 5 minutes. *Monochrysis* were also observed to swim back through the region of immobilization in the few instances in which the wave stopped short of the periphery of the drop. No change in pigment or other cell contents was observed in *Monochrysis cells*. In contrast to the other species, *Isochrysis* were able to swim near, and even touch, the style without observable effect for more than 72 hours. All algal species appeared normal in boiled style and buffer controls.

These studies demonstrate a property of the oyster style not heretofore reported—namely, the ability to attack certain algal cells only during, or for a very short period after, the dissolution of the style. The substance responsible for this phenomenon is heat-labile and is thought to be an enzyme. This "enzyme" may be a protease, a lipase, or a more powerful amylase than has been previously reported. Although proteases are reportedly absent and lipases have been infrequently found, it is noted that, of the investigations for lamellibranch style enzymes, only in those of George (*5*) have undissolved styles been used. George studied only fat digestion and found lipase present in styles of several lamellibranchs. Further studies on this enzyme may help explain the cause of the rapidly disintegrating animal forms in lamellibranch stomachs reported by Nelson (*6*) and Mansour (*7*).

The differences in algal "response" to style preparations are noteworthy in view of increased experimentation in pure-culture feeding of various Metazoa. It is known that certain algae are good foods and others poor foods for various developmental stages of animals. The tendency seems to be to interpret this in terms of possible differences in nutritive quality of the foods. Observations reported here indicate the differences may be due to "resistance" to digestion by some of the algae.

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References and Notes

1. D. Dean, doctoral thesis, Rutgers University (1957). I am grateful to Dr. Harold H. Haskin for his counsel in these studies.
2. T. C. Nelson, *J. Morphol.* 31, 53 (1918).
3. C. M. Yonge, *J. Marine Biol. Assoc. United Kingdom* 14, 295 (1926).
4. Dr. L. Provasoli of Haskins Laboratories, New York, kindly furnished cultures of *Cryptomonas*, *Monochrysis*, and *Isochrysis* for this study.
5. W. C. George, *Biol. Bull.* 102, 118 (1952).
6. T. C. Nelson, *Proc. Soc. Exptl. Biol. Med.* 30, 1287 (1933).
7. K. Mansour, *Nature* 158, 378 (1946).

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].