Wood-boring animals belonging to at least two different metazoan phyla have been shown to possess cellulolytic enzyme systems (1, 2). Marine borers of the genus Teredo have been shown (3) to contain up to 50 per cent glycogen on a dry weight basis. This observation, combined with the fact that the animal spends its entire adult life span embedded in wood, prompted an investigation of cellulase activity in this form. It is the dual purpose of this communication briefly to report the existence of such an enzyme system and to describe some of the observed conditions of its activity in vitro.

Methods

Preliminary studies established that entire worms could be removed from their burrows into chilled sea water without serious immediate physiological consequences. If handled with reasonable care to avoid tissue damage, they will survive for many hours (4). When a sufficient number of animals had been assembled, tissues were removed from all for further study. To localize any activity which might appear, two tissue samples were initially collected; the first ("prececal") contained all the internal organs anterior to the capacious cecum, only the shell musculature and the appended shells being excluded; the second sample ("postcecal") contained the cecum, some postcecal gut, gonad, and some shreds of mantle tissue. No attempt was made to assay the activity of the individual derivatives of the gut. In subsequent experiments only the tubular gut was employed since these preliminary observations suggested that maximal cellulase activity takes place there.

The dissected tissues were homogenized in \( \frac{1}{15} \) phosphate buffer, made isotonic with sea water by the addition of NaCl. Isotonicity was confirmed by freezing point determinations. \( \Delta T_f \) of the final suspending medium was 2.06°; the medium was 0.620 m NaCl. A known quantity of tissue was used for each incubation, a similar quantity being set aside to
determine weight, moisture, and residual carbohydrate content. Tests were run at pH 5.0, 5.6, 6.7, and 7.6. Regenerated cellulose prepared by the method of Parkin (5) was the substrate. Bacterial growth was inhibited by toluene. All the incubations were conducted at 25.0°.

The rate of cellulose breakdown was determined by periodic analyses of the reducing substance present in the medium, corrected, of course, for that introduced in the homogenate. An aliquot of the incubation mixture was withdrawn and centrifuged to remove particulate material. The supernatant fluid was treated with 5 per cent (weight per volume) trichloroacetic acid and recentrifuged. This supernatant fluid was freed of glycogen and salts by the addition of 1 volume of ethanol, and by centrifugation. The final supernatant fluid was analyzed for reducing sugar by the method of Park and Johnson (6), with either the Beckman model DU spectrophotometer or the Coleman model 6A spectrophotometer. The analysis of known glucose solutions by this procedure showed that the maximal error introduced by the manipulation did not exceed 2 per cent.

Control incubations consisted of (1) suspending medium alone, (2) suspending medium plus regenerated cellulose, (3) heat-inactivated homogenate alone, and (4) heat-inactivated homogenate plus regenerated cellulose. To assess the contribution of symbiotic microorganisms to the total cellulose-digesting ability of Teredo, sea water nutrient agar containing 1 per cent regenerated cellulose was inoculated with fresh tissue homogenate and incubated at 25.0°.

RESULTS AND DISCUSSION

There was no increase in reducing substance when gut homogenates which had been heated to 100° for 5 minutes were incubated with 1 per cent regenerated cellulose. No cellulose-digesting bacteria or Protozoa were found in the digestive system of Teredo. Some colonies appeared on plates of sea water nutrient agar inoculated with homogenates of shipworm gut, but these colonies showed no cellulolytic activity.

In Fig. 1 is shown the relationship between pH and the conversion of cellulose to reducing substance by gut homogenates during a 24 hour incubation period. The two fractions differ qualitatively in their response to identical physical conditions. The conversion of cellulose to reducing substance by prececal material appears independent of pH within the range studied. The postcecal fraction, on the other hand, shows maximal activity within the pH range 5.6 to 6.7. This regional difference in response suggests that more than a single enzyme may be involved in the total cellulose-digesting ability of this marine borer. It may be recalled that cellulolytic enzymes of many fungi (7) also exhibit optimal activity under weakly acid conditions.
If these two segments of the digestive system are compared on the basis of their ability to convert cellulose to reducing substance at pH 5.6, then the postcecal portion of the gut appears to contain approximately twice the activity of the prececal segment. This may signify either that the enzyme is produced chiefly in the postcecal portion of the gut or that it is

**Fig. 1.** Relationship between pH and formation of reducing substance (RS) by homogenates of the ship-worm gut. Substrate was 1 per cent regenerated cellulose in isotonic M/15 phosphate buffer. Concentration of dry, carbohydrate-free tissue was 0.48 mg. per ml. Analyses were made after 21 hours incubation.

**Fig. 2.** Effect of concentration of tissue on formation of reducing substance (RS) by homogenates of the postcecal gut of the ship-worm. (a) The concentration was 0.48 mg. of dry, carbohydrate-free tissue per ml. of suspending medium; (b) the concentration was 4.2 mg. of dry, carbohydrate-free tissue per ml. Substrate was 1 per cent regenerated cellulose in isotonic M/15 phosphate buffer, pH 6.0.
secreted into the gut in the more anterior portions and is concentrated by
differential absorption as the intestinal contents move posteriorly.

It should be emphasized that no attempt was made to assay the wall of
the gut separately from its contents, nor was the enzymic activity of gut
diverticula determined separately. Therefore it is not possible to localize
the site of enzyme elaboration with strict morphological precision.

Fig. 2 illustrates the effect of tissue concentrations on the course of the
incubation. The lower curve represents the activity of a mixture in which
the concentration of tissue was approximately 10 times that represented
by the upper curve. At higher concentrations of tissue there is evidently
a greater concentration of inhibitory substances, or an enhanced rate of
glycogenesis. Either or both of these factors would effectively conceal the
ture rate of cellulose breakdown. These factors are proportionately attenu-
ated in the experiments with lower concentration, which reveals the true
rate of cellulysis.

SUMMARY

Ship-worm gut, homogenized in m/15 phosphate buffer made isotonic
with sea water, has been shown to cause the liberation of reducing sugar
from regenerated cellulose. This activity is destroyed by heat. The op-
timal pH range for this reaction extends from pH 5.6 to 6.7. The activity
of the postcecal portion of the gut is approximately double that of the
prececal segment. Some evidence suggests that there may be more than
a single enzyme involved in the total ability of the ship-worm to digest
cellulose.

BIBLIOGRAPHY

ibbon, 2, 385 (1952).
(1951).