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## Blood Glucose of the Crab *Hemigrapsus nudus*

Studies in crustaceans of blood reducing substances, commonly referred to as "blood sugar," have been numerous. Until recently, however, there has been no means of establishing that the values obtained actually represent glucose, or indeed any other single substance. Attempts to increase specificity by use of fermentation methods (1, 2) still leave some question but indicate clearly that a substantial fraction of the reducing substances is not glucose. Recently Hu (3), using chromatographic methods, has shown that acid extracts of the shore crab *Hemigrapsus nudus* contain a variety of carbohydrates, some of which, at least, will probably also be present in the blood. It therefore appeared essential, as a basis for studies of carbohydrate metabolism in crustaceans, to learn whether glucose is actually present in normal blood and, if so, at what levels of concentration (4).

Crabs (*Hemigrapsus nudus*) were collected near Charleston, Oregon, and brought to the laboratory in Eugene, where they were maintained in sea-water aquaria until blood was drawn, but no more than 5 to 10 days after capture. The stage of the animals in the intermolt cycle was determined by the method of Drach (5), as modified for this species by Kincaid and Scheer (6). Blood was drawn with a syringe through the coxal membrane of the fifth walking leg and was deproteinized by being heated

for 15 to 30 seconds in a boiling water bath. Control experiments with other methods of deproteinization showed that this procedure gave reliable glucose values, and in view of the very low glucose concentrations found, it was considered desirable to avoid the dilution involved in other methods. Moreover, most other methods render the blood unsuitable for enzymatic procedures. Glucose was determined by the highly specific hexokinase-glucose-6-phosphate dehydrogenase (*Zwischenferment*) method (7), which depends on a spectrophotometric measurement of triphosphopyridine nucleotide reduction in the presence of adenosine triphosphate.

The mean blood glucose values, for the various intermolt cycle stages, for normal animals and for animals from which eyestalks had been removed 1 to 4 days before blood collection, are summarized in Table 1. From the values presented, it appears that the blood glucose of these crabs is very much lower than previous determination of blood reducing substances would indicate. No parallel measurements of reducing substances were made in these crabs, but determinations by means of the Folin-Wu method give values of about 15 mg/100 ml, and determinations of "total carbohydrate" with the anthrone method (8) give values of about 10 mg/100 ml. Evidently, then, less than 20 percent of the "blood sugar" is in fact glucose.

The variation with the intermolt cycle is also of interest. Renaud (9) found a gradual increase in blood reducing substances as the molt approaches in *Cancer pagurus*, as had other workers earlier. We had very few animals available in the premolt stages (D), but there is no sign of a premolt increase in blood glucose. Rather, the maximum values appear in stage C<sub>1</sub>, in the early intermolt period; the mean for this stage is significantly higher than the means for stages B<sub>2</sub> or C<sub>3</sub>, at a probability level of 0.5 percent on the basis of the *t* test.

The values for eyestalkless animals in Table 1 are, in general, lower than the values for normal animals in the same stage of the cycle; however, the differences are not statistically significant. Scheer and Scheer (2) found a decrease in total and fermentable reducing substances in spiny lobsters and were able to explain the difference as resulting from an increased tissue utilization of glucose in eyestalkless animals. Kleinholz and Little (1) and Abramowitz *et al.* (10), however, could find no such decrease in crabs.

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### References and Notes

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## Modes of Entry of Strontium into Plant Roots

Cell walls of roots consist of a framework of microfibrils (Fig. 1). Spaces between them may function as "free space" (1), or they may contain metabolic products, particularly pectic substances (2). Nutrient ions enter the root via free space or, as demonstrated below, by way of surface migration.

Cation-exchange membranes of the Amberplex type (3) were converted to H-membranes by leaching with normal HCl. Acid in the pore space of the membrane was removed by prolonged dialysis in distilled water.

To a wet Amberplex strip, 7.4 cm long, 1.5 cm wide, and 0.8 mm thick, was added 1.128 milliequivalents (meq) of Sr(OH)<sub>2</sub>, tagged with Sr<sup>85</sup>. This solution saturated the H-membrane to 80.0 percent. When it was immersed in 700 ml of distilled water, the strip released, at equilibrium, 0.084  $\mu$ eq of strontium.

If an H-membrane of identical size is suspended in this solution (which is agitated and which contains the Sr-mem-

Table 1. Mean values (in milligrams per 100 milliliters), and standard error of the means, for blood glucose of crabs (*Hemigrapsus nudus*) in relation to the intermolt cycle and to removal of eyestalks.

| Item           | Stage          |                |                |                     |                |                |                |                |                |                |
|----------------|----------------|----------------|----------------|---------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                | Postmolt       |                |                | Intermolt           |                |                |                | Premolt        |                |                |
|                | A <sub>2</sub> | B <sub>1</sub> | B <sub>2</sub> | C <sub>1</sub>      | C <sub>2</sub> | C <sub>3</sub> | C <sub>4</sub> | D <sub>1</sub> | D <sub>2</sub> | D <sub>3</sub> |
| No.            | 6              | 4              | 10             | <i>Normal</i>       |                |                |                | 3              | 1              | 2              |
| Mean           | 1.55           | 1.23           | 1.12           | 2.55                | 1.85           | 1.28           | 1.70           | 2.45           | 0.75           | 2.11           |
| Standard error | 0.33           | 0.21           | 0.36           | 0.17                | 0.43           | 0.16           | 0.29           |                |                |                |
| No.            |                |                | 3              | <i>Eyestalkless</i> |                |                |                |                | 1              |                |
| Mean           |                |                | 0.56           | 1.87                | 5.03           | 1.20           | 1.22           |                | 1.21           |                |
| Standard error |                |                |                | 0.35                |                | 0.25           | 0.23           |                |                |                |