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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 seawater 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_1, in the early intermolt period; the mean for this stage is significantly higher than the means for stages B_2 or C_3, at a probability level of 0.5 percent on the basis of the *t* test.

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_2	B_1	B_2	C_1	C_2	C_3	C_4	D_1	D_2	D_3
				Normal						
No.	6	4	10	7	6	12	8	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			
				Eyestalkless						
No.			3	7	2	8	19		1	
Mean			0.56	1.87	5.03	1.20	1.22		1.21	
Standard error				0.35		0.25	0.23			

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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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 $Sr(OH)_2$, tagged with Sr^{85}. 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 μeq of strontium.

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

brane) the H-membrane will sorb dissolved strontium. Transfer of strontium from one membrane to the other via solution is very slow. Less than 0.05 percent of the strontium moves in 2000 hours. If the Sr-membrane is brought into intimate contact with the H-membrane, 42 percent of its strontium crosses the boundary within 48 hours.

Alfalfa seedlings, 16 days old, with a shoot length of 3 cm, were rinsed in distilled water for 4 hours. Forty-five plants, weighing 104 mg in an oven-dry state, were tied to the Sr-membrane strip in such a way that only the roots were in contact with it. The root-strip assembly was then immersed vertically in the equilibrium solution, which was aerated and stirred. Roots of an equal number of plants (102 mg), tied in a bundle, were also inserted into the solution. These roots never touhced the membrane. Strong artificial illumination was provided in all experiments.

After 18 hours both sets of plants were assayed for Sr^{85} (sets *A* in Table 1).

The uptake of Sr by contact was twice the uptake from the equilibrium solution, in spite of the fact that only a small portion of the root surface actually touched the membrane.

In set *B* there were 40 plants, 6 days old and weighing 69 mg, oven-dry. The membrane, as in *A*, was in a horizontal position, and the roots were pressed upon the strip with a 300-g weight. Between roots and weight was a layer of cotton. It seems fair to conclude that the higher activity of the *B* plants is due to more intimate contact of roots and strip caused by external pressure.

One cannot invoke the CO_2 theory as an alternate mechanism to contact-uptake, for the release of strontium from Amberplex during an 18-hour period is greater in distilled water freed from CO_2 than in water saturated with CO_2. If high concentrations of CO_2 near the root surface were crucial, the roots in contact with the membrane should sorb rather less strontium, not more, than the solution plants.

Related experiments were conducted with small beads of cation exchanger Chempro C-20 (*4*). This had an exchange capacity of 4.9 meq/g of oven-dry material. To 34.26 g of moist resin (19.1 percent H_2O) was added 122 meq of tagged $SrCO_3$ in 700 ml of solution. At equilibrium this solution contained 2.2 μeq of strontium, also in equilibrium with air.

The strontium-resin was scooped into a short vertical Lucite tube, the open bottom-end of which was covered with nylon gauze. The tube was lowered into the equilibrium solution in such a way that the upper level of the resin fill was horizontal with the level of the solution. The roots of 60 washed alfalfa seedlings, 10 days old and weighing 84 mg, were carefully packed into the resin slurry. Outside the tube, roots of an equal number of plants (98 mg) were inserted into the equilibrium solution, which was continually aerated.

After 18 hours the roots of the contact plants were rinsed in distilled water to free them from adhering resin beads. According to radioassay (Table 2), contact absorption again proved vastly superior to solution absorption. The plants in solution removed only 12 percent of the dissolved Sr.

One might contend that contact effects play no role whatever, that instead the roots excrete chelating substances which free the strontium from the resin particles, and that the strontium chelates then enter the root via free space.

To test this possibility, two strontium-resin columns, R-a and R-b, and two sand columns, S-a and S-b, were arranged in such a way that R-a was above S-a and R-b was above S-b. Alfalfa seedlings were inserted into the sand columns and into R-a but not into R-b. By means of a pumping arrangement, equilibrium solution was brought to the top of R-a. It percolated through the resin into sand column S-a and into a beaker, all by gravity. From the beaker the solution was pumped again on top of R-a, and percolation started anew. Circulation was continuous. The same process operated independently with R-b and S-b.

If the plants excrete chelating compounds, the resulting strontium chelates should, in considerable part, be leached from the resin into the sand. Plants in S-a should accumulate much more strontium than plants in S-b. But in duplicate experiments, each lasting 4 days, no significant differences between sand columns were found. The plants in resin accumulated 16.4 times more strontium than the plants in sand. Of course, if the resin should strongly adsorb the chelate, the chelate would not pass into the sand column, but neither would it diffuse into the free space of the root.

Although dissolved strontium can enter the root through free space, strontium adsorbed on resin cannot. We visualize a transfer of strontium from resin surface to root surface by contact exchange (*5*). Strontium may then migrate into the interior along negative surfaces of cell-wall constituents. We determined diffusion coefficients of strontium in Amberplex membrane ($D = 3 \cdot 10^{-8}$ cm^2/sec) and calculate that adsorbed strontium traverses a distance of 1 μ (corresponding to the thickness of cell walls) in about 1 minute. But ion-exchange migrations could not account for the appearance of substantial amounts of strontium in the leaves in 18 hours. Presumably, inside the root the adsorbed strontium is released into free space, or is acquired by carrier substances, or both, and is subsequently transported to the leaves.

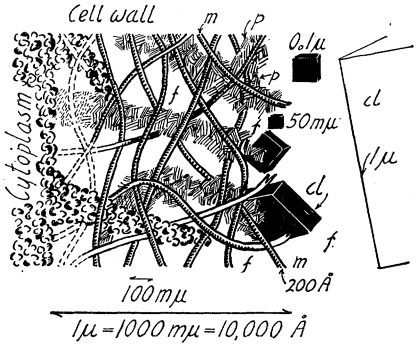

Fig. 1. Model of boundary region of root surface and growth medium. (*m*) cellulose microfibrils; (*p*) pectic substances; (*f*) free space; (*cl*) resin grains or clay particles of various sizes.

Table 1. Uptake of strontium by alfalfa seedlings (in microequivalents per gram of oven-dry material).

	Plants in solution A	Plants in contact with Amberplex	
		A	B
Leaves	0.005	0.023	0.071
Stems	0.086	0.053	0.105
Roots	0.298	0.641	1.043
Entire plant	0.059	0.104	0.174

Table 2. Uptake of strontium by alfalfa seedlings (in microequivalents per gram of oven-dry material).

	Plants in equilibrium solution	Plants in resin slurry
Leaves	0.0	3.6
Stems	4.2	15.5
Roots	16.9	107.1
Entire plant	2.8	17.8

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