gibberellins, or at least gibberellin-like substances, are universal in the plant kingdom and might play a vital role in the control of plant growth (*10*).

Further work with those tissue cultures which produce the greatest amount of activity is being completed (*10*).

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New Maintenance Medium for Cell Culture

Although animal serum has been a necessary constituent of most media for continuous cell cultures (*1*), many sera contain "inhibitors" to a wide range of viruses (*2*). It would be desirable to replace serum with another substance which does not contain nonspecific inhibitors or antibody and which would sustain cells in a condition sensitive to viral effects during the necessary observation periods (*1*).

Having observed that boiled skim milk contributed to the maintenance of fully grown LAC cells (*3*), we undertook a study to determine the nutritive value of skim milk in cell culture maintenance media.

Skim milk was prepared by dissolving Pet instant nonfat dry milk in distilled water, according to instructions on the container, in order to reconstitute original volume. The milk was either boiled for 5 minutes or autoclaved at pressure of 7 lb. in.^{2} for 15 minutes. The *p*H was adjusted to 7.2 with sterile 5-percent sodium bicarbonate, and 150-ml aliquots were frozen at – 20°C. This material was designated as 100-percent skim milk.

Basal medium [medium 199 (*4*), or Eagle's basal medium (*5*), at *p*H 7.3 to 7.6] was mixed with varying amounts (0 to 100 percent final concentration) of skim milk and fed to LAC cells and HeLa cells (Gey). Maintenance was very satisfactory in the presence of skim milk (10 to 40 percent) in either basal medium. Final concentration of 20 percent of skim milk in medium 199 was selected for further experimental use. Cell strains monkey heart (Salk) and KB (Eagle) and rhesus monkey kidney cells were maintained in this medium for periods of up to 4 weeks when fed at 7-day intervals. Rhesus monkey kidney may also be maintained in Earle's balanced salt solution containing 20 percent skim milk and either 3 percent Travamin (*6*) or 0.5 percent lactalbumin hydrolyzate (*7*). A twofold concentrate of any one of these media may be mixed with agar for plaque overlay of rhesus monkey kidney cells. As with serum-containing maintenance medium, the degree of cell support by basal medium containing skim milk varies with different cell culture lines and different cell culture strains. Rounded cells and debris may accumulate, especially over the densest areas of cell cultures. Exceptionally dense areas of tube cultures may actually degenerate, leaving a wide margin of clear viable cells, which are suitable for observing viral effects.

The activity of skim milk appears to be associated with one or more of the milk proteins; the activity is not dialyzable and not sedimentable at 78,000g for 1 hour. A material of this type which retains its cell nutrient capacity after exposure to high temperature is rather unusual. Thus, skim milk is the first material which, at least partly, replaces the serum protein necessary for culture of HeLa cells, KB cells, and other continuous cell lines (*5*).

Skim milk medium is turbid, due to suspended casein (*8*), and it is rather striking to observe clearing of the milk medium under certain conditions. Trypsin, stool suspension, and some bacterial contaminations will clear the medium. Monkey kidney cell cultures also clear the medium after 3 to 5 days' incubation at 36°C, while HeLa, LAC, and KB cells do not, in the absence of serum. Proteolysis of the suspended casein is thought to be the mechanism of clearing.

The viral sensitivity of a cell culture in a maintenance medium is relative to its sensitivity in the presence of an established medium on the same cell culture line. In comparative titrations, 4- to 100-fold serum-inhibition of poliovirus was observed when calf-serum-containing maintenance medium was compared with serum-free medium on monkey kidney cells. A number of lots of heated skim milk were tested in this manner, at 20- and 30-percent concentration, without detection of decreased sensitivity. Similar determinations were made with monkey-kidney adapted Coxsackie B1, B2, B3, B4, B5, A7, A9 and vaccinia virus (*9*). There was no evidence that skim milk medium decreased cell culture sensitivity to these viruses. Preliminary studies indicate that HeLa cells in the presence of Ginsberg's medium are tenfold more sensitive to adenovirus 3 and 4 than HeLa cells in the presence of skim milk medium.

To determine whether the heating of skim milk would completely destroy any "antibodies" in milk (*10*), 1 ml of human serum containing antibodies to all three types of poliovirus was added to 9 ml of skim milk, and a 5-ml aliquot was autoclaved in the manner used to sterilize skim milk. The autoclaved aliquot was free of poliovirus inhibitors, demonstrating that the heating process completely destroyed the activity of the added poliovirus antibodies in skim milk. These results indicate that skim milk medium provides an environment which allows relatively full sensitivity to most of the viruses tested. Additional advantages are simplicity of preparation, low cost, and reduction in requisite frequency of feeding the cells.

Skim milk maintenance medium appears applicable as a standard medium for (i) comparative assay of a variety of viruses on a number of different cell cultures, (ii) safety testing of virus vaccines, (iii) isolation of viral agents which were not previously cultured, due to neutralization by serum-containing maintenance media, and (iv) detection of proteolytic activity of cell cultures.

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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-