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Composition of the Cell Sap of Halicystis Ovalis (Lyng.) Areschoug.*

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The cell sap of a species of *Halicystis* which "is cast up on the beaches as clear green, balloon shaped cells" at Bermuda was analyzed by Osterhout and Dorcas[1] and reported to differ strikingly from that of *Valonia macrophysa* Kütz, collected at Bermuda, as determined by Wodehouse[2] and by Osterhout.[3] The contrast seemed all the more striking since the species was at that time erroneously identified as *Valonia ventricosa* J. G. Ag. This identification was subsequently revised by Blinks,[4] who considers the species analyzed by Osterhout and Dorcas to be *Halicystis ovalis* (Lyng.) Areschoug.

Osterhout and Dorcas[1] reported that while cells of *Valonia macrophysa* contain a sap which is very rich in potassium and poor in sodium, precisely the opposite condition obtains in *Halicystis* (which they call *Valonia ventricosa*). The ratios of potassium to sodium in the two forms were found to be 5.72 and 0.0278 respectively. Since the cells which they analysed floated in sea water while normal *Valonia* cells of either species sink,[5] it appeared questionable whether these cells were entirely normal. Osterhout and Dorcas[1] say in this connection: "The writers made every effort to obtain young growing cells. . . . . During the past 2 years a few cells apparently of the same species have been found attached. . . . . Unfortunately there seems to be no prospect of obtaining such cells in sufficient quantity for analysis." The analysis reported by them refers to cells torn loose from their habitat and drifted ashore.

Blinks[6] has recently reported that the electromotive forces developed by cells of *Halicystis* differ strikingly from those previously

* The expense of this research was met in part by a grant from the Board of Research of the University of California, for which grateful acknowledgement is made. I am also greatly indebted for the facilities made available by the Hopkins Marine Station, Pacific Grove, California, and the Division of Plant Nutrition of this University.

[1] Osterhout, W. J. V., and Dorcas, M. J., *J. Gen. Physiol.*, 1925, vii, 633.

[2] Wodehouse, R. P., *J. Biol. Chem.*, 1917, xxix, 453.

[3] Osterhout, W. J. V., *J. Gen. Physiol.*, 1922, v, 225.

[4] Blinks, L. R., *Science*, 1927, lxv, 429.

[5] This has repeatedly been observed by M. M. Brooks and by the writer.

[6] Blinks, L. R., *J. Gen. Physiol.*, 1929, xiii, 223.

observed with *Valonia*. He states: "The sap of the small cells used in these experiments was essentially the same in composition as that of the large floating cells" as previously reported by Osterhout and Dorcas.

It is not made clear whether this statement is based on more than qualitative tests, and it seems probable, in the absence of any definite statement, that the cells used were those cast up on the beaches.

It therefore seems worth while to publish exact determinations of the potassium and sodium content of the sap of undoubtedly normal cells of *Halicystis* collected from their actual positions of growth on *Lithothamnion* incrustations below mean low tide level on the Pacific Coast. These are given below in Table I, together with similar data for *Valonia macrophysa* and *Valonia ventricosa* collected at Tortugas, and those for *Halicystis* as given by Osterhout and Dorcas.[1] The methods used in the new analyses reported here were like those described by Brooks,[7] except that potassium was determined gravimetrically as the chloroplatinate. The analyses were made by Mr. Roy Overstreet.

TABLE 1.

The potassium and sodium content of sea water and sap from various sources expressed as per cent of the total halide content in each case. The sap analyses made by ourselves are those of composite samples obtained by extracting sap from a large representative group of cells.

Solution	K	Na	K:Na	Author
Sea water, (Bermuda)	2.15	85.87	0.0251	Osterhout and Dorcas[1]
Halicystis, Sap, (Bermuda)	2.58	92.80	0.0278	" " "
Sea water, (Pacific Coast)	2.17	30.1	0.0271	Brooks, original
Halicystis, sap, (Pacific Coast)	58.9	39.2	1.50	" "
Valonia macrophysa sap (Tortugas)	79.2	14.3	5.53	Brooks[7]
Valonia ventricosa sap (Tortugas)	92.1	8.7	10.58	Brooks, original

It will be seen that normal *Halicystis* cells of the Pacific coast form, which appear to be morphologically identical with *H. ovalis*,[8] contain a sap much more like that of the 2 species of *Valonia* than like that of *Halicystis ovalis* as reported by Osterhout and Dorcas. It is true that these *Halicystis* plants do not accumulate potassium to quite so high a concentration as do the Valonias, nor contain so little sodium, but they do contain more potassium than sodium (ratio 1.50:1), and accumulate potassium, while in effect excluding sodium. Thus potassium is 28.12 times as concentrated in the sap as

[7] Brooks, S. C., *Protoplasma*, 1929, viii, 389.

[8] See, for example, Collins, F. S., "The Green Algae of North America." Tufts College Studies (Scientific series), 1909, II, No. 3, p. 372.

in the surrounding sea water, while sodium is only 0.517 times as concentrated. The corresponding accumulation ratios (concentration in sap: concentration in sea water) reported by Osterhout and Dorcas for drifted specimens of what is supposedly the same species are 1.2 for potassium, and 1.08 for sodium.

This difference in composition of the sap of plants collected in the places and manners described corresponds with the fact that Osterhout and Dorcas found their *Halicystis* cells to float in sea water, like dead *Valonia* cells, while those collected on the Pacific Coast sink like living cells of *Valonia*.

The significance of these facts is not clear. It is possible that we are dealing with physiologically distinct but morphologically identical forms. It is also conceivable that the cells cast up on the beaches and studied by Osterhout and Dorcas[1] and by Blinks[4, 6] are, while apparently still living, nevertheless abnormal or possibly moribund.

Perhaps the most satisfactory explanation is that we are dealing with but one species, but that the lower temperature of the Pacific Ocean (15° C. in July when the collections were made) as compared with the Bermuda waters (summer temperatures not far from 25°) may result in a decreased permeability to electrolytes.

That permeability of plant cells to water is decreased by lowering the temperature over this range was shown by van Rysselberghe,[9] and quantitative determinations on *Arbacia* eggs by McCutcheon and Lucke[10] show that their permeability to water is about halved by change of temperature from 24° to 15°. Osterhout[11] assigns a somewhat smaller temperature coefficient ($Q_{10} = 1.33$) to the permeability of *Laminaria* thallus to electrolytes.

Decrease in permeability could, on the basis of the theory proposed by the writer,[7] result in an increasingly preponderant intake of potassium as compared to sodium and in that way cause the observed difference in the cell sap. This presupposes that the permeability to sodium is at the higher temperature already quite high, so that the penetrabilities at the higher and lower temperatures would correspond roughly to positions C and B respectively in Fig. 5 of that paper.

Summary: Normal cells of *Halicystis ovalis* (Lyng.) Areschoug were collected on the Pacific coast from their usual habitat, and the potassium, sodium, and chloride contents of the sap determined.

In contrast to similar analyses reported for drifted specimens of

[9] Van Rysselberghe, Fr., *Acad. Roy. Belg., Bull. Cl. Sciences*, 1901, 173.

[10] McCutcheon, M., and Lucke, B., *J. Gen. Physiol.*, 1927, x, 659.

[11] Osterhout, W. J. V., *Bot. Gaz.*, 1917, lxiii, 317.

the same species collected at Bermuda these cells were found to resemble those of all species of *Valonia* so far studied in exercising selective accumulation of potassium, and resisting the entry of sodium.

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Growth and Bone Changes in Rats Injected With Alkaline Anterior Pituitary Extracts.*

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(Introduced by E. M. K. Geiling.)

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Some 175 rats, ranging in weight from 30 to 350 gm. were employed in testing the potency of alkaline anterior pituitary lobe extracts over short periods of time. The method of preparing the extract as described by Evans and Simpson[1] was adopted. In rats weighing between 30 and 100 gm. the presence of the growth hormone could not be detected. However, in animals weighing from 175 to 300 gm., growth stimulating powers were observed. With animals 200 to 225 gm. in weight, after 12 days of injection (the dose being 1 cc. daily intraperitoneally) the experimental rats gained at an average of 2.1 gm. *per diem* over the control rats. After 14 days of treatment with 1 cc. doses intraperitoneally daily, injected animals weighing between 250 and 300 gm. gained remarkably in weight, averaging 2.7 gm. daily over the control animals.

Periosteal bone growth studies of rats were made by including madder in the diet. Madder was shown by Kölliker to be deposited only in zones of osteoblastic activity, staining newly growing bone red. The skulls and mandibles were used as test bones. In young animals periosteal activity was so great that after feeding madder for 2 weeks the bones were diffusely stained pink and made comparative studies impossible. However, in rats over 175 gm., periosteal bone formation was found to be limited to discrete zones of ossification when madder was fed throughout the experiment. The

* After the completion of this work there appeared a communication in this journal by Evans and his associates, Vol. xxvii, Nov., 1929, employing rats weighing between 250-270 gm. His growth results are in accord with ours of the same group.

1 Evans, H. M., and Simpson, M. E., *J. Am. Med. Assn.*, 1928, xci, 1337.