CVII. AN EXAMINATION OF THE METABOLIC PRODUCTS OF CERTAIN FUCOIDS.

I. SUGAR.

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For some time past the attention of the authors has been drawn to the sea weeds, and the fact that at Blakeney in Norfolk there exists an extensive formation of the free growing fucoid *Pelvetia canaliculata forma libera* [Baker, 1912; Baker and Blandford, 1915] growing in association with *Salicornia herbacea* and *Suaeda maritima*, suggested a comparison of the metabolic products of this plant with that of *Pelvetia canaliculata* in order to see whether such an essentially sub-aerial plant has a metabolism divergent from that of its parent species living in its normal habitat, the sea. For it will be remembered that the sea weeds show many peculiarities which are not shared with terrestrial plants: the presence of ethereal sulphates in the cell-wall constituents, the lesser amount of cellulose, the presence of alginic acid, the common occurrence of mannitol, the presence of obscure carbohydrates such as laminarin, together with other peculiarities, indicate a type of metabolism different from that of the normal terrestrial plant.

The habitat of *P. canaliculata forma libera* is such that it is submerged in sea water only at the high spring tides. During the inter-tidal periods it is desiccated to a degree which varies with the humidity of the atmosphere, which is dependent on temperature, rainfall and other factors. It therefore may happen, especially in summer, that desiccation may be excessive, indeed often the plants are quite dry and more or less brittle for they merely rest on the surface of the marsh. The formation of sexual organs is extremely rare; reproduction is effected by vegetative means, disjected portions of thallus; the many thousands of plants of an area are thus portions of a few individuals. The parent plant is *P. canaliculata* which also grows on the Blakeney area on the harbour side; but its distribution and abundance are somewhat restricted, for which reason restraint was necessary in its collection—for biochemical investigations generally require much material—else its continued existence might have been menaced and possibly that of its form *libera*. At Blakeney, *P. canaliculata* is normal: it grows attached to pebbles and produces an abundance of conceptacles. It is possibly exposed to more tides than is its form *libera*, but vertically it is probably higher than when growing on an exposed coast-line and is never subjected to wetting by spray; it is therefore exposed to prolonged periods of drought. In passing it may be remarked that these plants are of thallus structure, and the whole plant is engaged in season in photosynthesis. In the present investigation this is an advantage in that difficulties inseparable from a more complex morphology, diffusion of carbon dioxide, translocation of elaborated compounds, etc., are eliminated.

In general terms the metabolism of green plants is based on sugar. Does this also hold for the Fucaceae? According to Kylin [1915] this is so, although the amounts of sugar he finds seem too small to warrant such a conclusion. For his investigation he used air-dried plants and he gives no information regarding the time of collecting, and the state of the tide. These circumstances, together with the interval of time between collection and extraction, may have much significance in the amount of free sugar especially in those fucoids of the upper part of the emerging zone. In order to reduce any possible loss from such a cause we examined fresh material collected after a period of insolation and dropped it immediately into boiling alcohol.

Procedure.

In the present investigation the dates of collection were selected so that all the plants had been submerged for several hours by a day tide; the conditions were therefore favourable for photosynthesis.

The extraction of *P. canaliculata* and its form *libera*, owing to the facility of a laboratory near by, took place immediately after collection, so that the possible loss of sugar by respiratory processes was negligible. On the other hand, *Fucus serratus*, and *Ascophyllum nodosum*, which do not grow at Blakeney in sufficient amount or are absent, were collected from previously selected localities at Folkestone and were brought to London in sea water: the period between collection and extraction was about 2 hours so that the possible loss by respiration could not have been great.

The plants were rinsed in fresh water two or three times in order to remove adhering salts, superficially dried with towels and dropped into boiling absolute alcohol. After boiling for 20 minutes the plants were removed from the alcohol, drained and dried first in air and then in the steam-oven. They were then finely ground in a mill, in order to facilitate extraction, and the powder was extracted by boiling 95 % alcohol on a water-bath for 20 minutes under a reflux condenser. The extract was filtered through calico on a Büchner funnel, the solid residue was returned to the flask and again extracted with boiling alcohol. If necessary, a third extraction was made after which the alcoholic extract was almost colourless and gave little or no residue on evaporation.

All the filtrates were combined and, if the preparation could not be proceeded with immediately, mixed with a little toluene and kept in closed vessels in the dark. The extract was reduced to a small bulk by evaporating off the alcohol under reduced pressure; the resulting residue was deeply coloured and contained a certain amount of fat. The fat and much of the pigments were removed by extraction with ether in a separating funnel; the process being repeated once or twice. The aqueous layer was then a clear pale reddish brown liquid. The combined water extracts were freed from ether, mixed with finely powdered calcium carbonate and evaporated to smaller bulk on a water-bath. The liquid was then filtered and treated with basic lead acetate. The precipitate was filtered on a Büchner funnel, and then ground with water and refiltered. The combined filtrates were treated with hydrogen sulphide to remove the excess of lead; after filtration, solid calcium carbonate was stirred into the solution which was then reduced to a small bulk on the water-bath. On evaporating a portion of the filtrate a dark amber coloured syrup remained.

In order to reduce the ash content of this the syrup was extracted repeatedly with 95 % alcohol, the extract being evaporated over a water-bath to a clear light-brown syrup.

For example: 2 kg. of *Pelvetia canaliculata* required for complete extraction 7850 cc. of alcohol. After the first extraction, the dried plant residue weighed 435 g.; the final yield of syrup was 35·63 g. most of which was extracted on the initial boiling with alcohol. Similarly 2 kg. of the form *libera* required 8560 cc. of alcohol; the dry weight of the plant material after the first extraction was 300 g. and the weight of the final syrup was 43·3 g.

The syrup thus prepared contains in addition to mannitol, inorganic salts together with a small amount of a nitrogenous compound[1] which is not removed by treatment with phosphotungstic acid.

Examination of the syrup.

The following table sets out the qualitative reactions of the syrup.

Table I.

Plant	Date of collection	Rotation	Colour reactions: Molisch's	Thomas's	Bial's	Initial reduction (Fehling)	Reduction after hydrolysis
P. canaliculata	June 1928	.	+	+	+	+*	+ +
P. canaliculata	Sept. 1927	−	+	+	+	+†	+ + +
f. libera	Jan. 1928	+	−	−	−	−	−
	June 1928	+	+	+	+	+†	+ + +
Fucus serratus	Oct. 1927	0	+	+	+	−	+‡
	Nov. 1927	−	+	+	+	−	+‡
Ascophyllum nodosum	Oct. 1927	0	+	+	+	−	+‡

* Very weak. † Weak. ‡ Just detectable.

[1] It was subsequently found that this substance is precipitated by mercuric nitrate; on decomposing the precipitate with hydrogen sulphide and evaporating the carefully neutralised solution there resulted a product which gives the biuret reaction, and is readily hydrolysed by hydrochloric acid yielding ammonium chloride. With potash the compound evolves ammonia in the cold. From these facts it is concluded that the substance is an amide but its complete characterisation awaits further investigation.

The following observations on these tests may be made.

1. *Rotation.* Most of the preparations showed slight optical activity, but this is without significance in that the final preparation contains mannitol which is optically active in relatively strong solution; the observed rotation therefore is the resultant of the activity of this alcohol and the amount of free sugar, when present. To this fact we ascribe the different results obtained with the form *libera* collected at different seasons.

2. *Colour reactions.* The colour reactions are concordant and show that a pentose is present. It should be noted that the colour reactions were given both by the syrup itself and by a dialysate prepared from the syrup. Kylin apparently did not test his preparations for pentose and he concluded that hexose was the significant sugar. In our observations it will be seen that the form *libera* gathered in January gave negative results throughout. We can give no definite explanation of this; possibly the amount of sugar formed in the conditions of short periods of illumination of lower intensity coupled with low temperatures is so small as to be immediately converted into some other compound.

3. *Reduction.* The form *libera*, with the exception of the plants gathered in January, gave the largest initial reduction of Fehling's solution; in no instance was the amount of reduction large enough for accurate determination. After hydrolysis the syrups of all the plants examined gave an obvious and immediate reduction of Fehling's solution but the amount, estimated as pentose by Bertrand's method, was very small. Thus in *Pelvetia canaliculata forma libera* the amount of material available was only sufficient to give a titration figure corresponding to 20 mg. of copper, the minimum quantity provided for in Bertrand's tables. This corresponds to 0·85 % of pentose in the syrup, or 0·018 % of the wet weight of the weed. In *Pelvetia canaliculata*, *Ascophyllum nodosum* and *Fucus serratus* the titration figures were too small to justify the calculation of percentage amounts of pentose.

Attempts to estimate the amount of pentose by the micro-method of Youngburg [1927] were unsuccessful owing to the frothing and charring of the material, a difficulty which was not experienced in control experiments with pure xylose solutions.

It should be noted that Table I gives the results of experiments upon fresh material killed by dropping into boiling alcohol. Rather different results were obtained with material which had been dried and kept for some time before analysis, as may be seen from Table II.

Table II.

Plant	Rotation	Colour reactions: Molisch's	Colour reactions: Thomas's	Colour reactions: Bial's	Initial reduction	Reduction after hydrolysis
P. canaliculata f. libera	0	–	–	–	–	–
Fucus serratus	–	Very faint	–	–	Very faint	.

SUMMARY.

1. There is present in *Pelvetia canaliculata* and *P. canaliculata forma libera*, but not in *Ascophyllum nodosum* and *Fucus serratus*, plants of a lower level of the emerging zone, a small amount of free reducing sugar, probably a pentose.

2. In addition, in *Pelvetia* there is a dialysable pentose complex which only reduces Fehling's solution after hydrolysis. This substance may be a disaccharide or a pentose constituent of the cell-wall. The latter is unlikely owing to its dialysability and to the method employed in its isolation. In view of the fact that plants air-dried for some time and extracted in the same way as the freshly gathered material do not give any sugar reactions, the suggestion that the non-reducing constituent is a transient labile substance, possibly a disaccharide, rather than a reserve material, is not unreasonable and this would account for the small amount found.

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