CXXVI. A NOTE ON THE NITROGEN OF THE POLYSACCHARIDE COMPLEX FROM *CHONDRUS CRISPUS*.

By MARGARET RUTH BUTLER.

From the Department of Biology, Dalhousie University, Halifax, Nova Scotia.

(Received March 16th, 1935.)

DURING the investigation of some biochemical properties of the polysaccharide extract of the alga, *Chondrus crispus*, varying quantities of nitrogen were found in different samples. The amount of nitrogen present in the extracts as prepared by a standard method [Butler, 1934] was found to differ from one collection of plants to another. In all samples prepared from the same collection of plants, however, it was constant. This indicated that the variations of nitrogen in the extracts depended on variations in the samples from which they were prepared. The total nitrogen was, therefore, determined in a number of collections and in the standard extracts from these same collections. The results (Table I) show that the differences occurring from one collection of plants to another are great; also that in general the nitrogen content of the extract varies as that of the collection from which it is made.

Table I. *Percentage of nitrogen in plants and extracts.*

Sample	% nitrogen in plants (dry weight)	% nitrogen in extract (dry weight)
S	0.74	0.26
N	1.75	0.39
H*	4.05	2.75
C	0.99	0.24
B	0.93	0.29

* It is interesting that Sample H, which shows the greatest deviation from the average nitrogen content, is the only one collected during the winter. Though this is but an incidental observation, it has suggested further investigations, now being carried on regarding seasonal variations.

In an attempt to purify the polysaccharide extract by reprecipitation, the effect on its nitrogen content was noted. As shown in Table II, the change effected was negligible.

Table II. *Percentage of nitrogen in re-precipitated extracts.*

Sample	% nitrogen in extract (dry weight)	% nitrogen in re- precipitated extract (dry weight)
T	0.85	0.86
P	1.21	1.10
D	1.43	1.18
S	0.26	0.30
F	3.55	2.25
M	0.81	0.62
B	0.28	0.29
C	0.24	0.24
C*	0.27	0.27

* 3 times precipitated.

In order to obtain some idea of how the nitrogen is distributed in the plant, it has been determined, as % of dry weight, in various extracts and residues (Table III).

Table III. *Percentage of nitrogen in fractions of Chondrus.*

Sample	% nitrogen in dry weight
Whole plant	0.93
Aqueous extract (not precipitated)	0.64
Residue from aqueous extract	2.06
Crude precipitate	0.28
Alcohol-soluble fraction of aqueous extract	1.13
Purified precipitate (Standard Extract)	0.29
Residue of plants freed from all water-soluble constituents	3.85
Alcoholic extract	0.71
Residue from alcoholic extract	0.82

The highest percentage of nitrogen per dry weight is found in the residue from plants washed under running water for a week or ten days. This is doubtless due to the great decrease in carbohydrate which makes this fraction itself small. Thus, while the percentage of nitrogen per dry weight is high, expressed as % of total nitrogen it would be very small. Because of the colloidal and highly viscous nature of the extract, the difficulties involved in manipulation are great. For this reason it is impossible to make a truly quantitative fractionation of the plants, but an approximation is given in Table IV.

Table IV. *Fractionation of plants.*

Fraction	% whole plant
Aqueous extract	65-67
Residue from above	33-35
Alcohol precipitate of aqueous extract	50-54
Alcohol-soluble aqueous extract	13-15

In Table V is given the percentage distribution of nitrogen as calculated by combining Tables III and IV.

Table V. *Percentage distribution of nitrogen in plant fractions.*

Fraction	% total nitrogen
Whole plant	100
Aqueous extract	37
Residue from above	63
Alcohol precipitate of aqueous extract	13
Alcohol-soluble aqueous extract	15

Identification of samples.

Sample	Place of collection	Month of collection
S	South Shore, P.E.I.	August
N	Souris, P.E.I.	August
H	Halifax, N.S. (Pt. Pleasant)	February
T	Halifax, N.S. (McNab's Island)	August
D	Halifax, N.S. (Devil's Island)	June
P	Peggy's Cove, N.S.	August
C	Cavendish, P.E.I.	August
B	Borden, P.E.I.	August
F	Details of collection missing. Extract made without any washes of absolute alcohol	—
M	Mixture of extracts from different samples dissolved together and precipitated	—

DISCUSSION.

If the nitrogen were simply an impurity one would expect that it might be removed, or at least appreciably reduced, by repeated precipitations. There is, however, no indication of such reduction. On the other hand, there is no evidence that the nitrogen content can be brought to a constant figure for all samples, as might be expected if it were chemically united with the polysaccharide complex (Table II).

No doubt there is protein material in the plants, but in the extract from only one sample, *viz.* H, could a positive biuret test be obtained. The protein nature of the nitrogen in the polysaccharide extracts, therefore, seems doubtful. Furthermore, no nitrogen was detected by the Van Slyke amino-nitrogen method.

Haas [1921] and Russell-Wells [1922] reported positive protein tests for their extracts of *Chondrus*. More recently, Haas and Hill [1933] have reported a sample of *Chondrus* with a total nitrogen content of 2.33 %, as giving a negative biuret test.

Russell-Wells [1922] found that nearly half of the 0.56 % of nitrogen in the extract she used occurred in an ammonium radical. In the present investigation none was identified as such.

From a consideration of these results no suggestion can be offered as to the form in which the nitrogen present exists. It is possible, however, that the nitrogen which accompanies the carbohydrate complex through repeated precipitations does so because it is adsorbed on the colloidal particles.

SUMMARY.

The percentage of nitrogen in the polysaccharide complex extracted from *Chondrus crispus* by a standard method is found to depend on the amount of nitrogen originally present in the plants.

The percentage of nitrogen in this complex is not reduced by repeated precipitations.

The amount of nitrogen associated with this carbohydrate material is calculated to be approximately 13 % of the total nitrogen in the plants.

The nature of this nitrogen is still undetermined.

REFERENCES.

- Butler (1934). *Biochem. J.* **28**, 759.
Haas (1921). *Biochem. J.* **15**, 469.
— and Hill (1933). *Ann. Bot.* **47**, 55.
Russell-Wells (1922). *Biochem. J.* **16**, 578.