CXCI. SEASONAL VARIATIONS IN *CHONDRUS CRISPUS*.

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PREVIOUS study of certain chemical properties of *Chondrus crispus* indicated that seasonal variations might be significant in the understanding of its metabolism [Butler, 1935]. With a view to obtaining such knowledge the present work was undertaken, the intention being to follow the changes noticeable at monthly intervals during the year.

In order to eliminate ecological variations other than those which were seasonal, a series of monthly collections was made from the same location, situated on MacNab's Island in Halifax Harbour [MacFarlane & Bell, 1933]. Collections were commenced in January 1931, and continued at monthly intervals until September when it was impossible to obtain another sample from that location. It was decided to begin collections at the same location in September of a year when no *Chondrus* had been collected previous to that month.

It was impossible to collect in December of that year, but collections were obtained in September, October and November 1934, and in January 1935; a collection was also made in November 1935 and has been used by way of check. Reference to the figures, in all of which the two January levels are close, indicates that the hiatus in collecting has had no serious effect on the results.

Treatment of collections.

The *Chondrus* plants were picked from spots scattered over the whole area mentioned above (approximately 750 sq. ft.). As the plants are small, any one collection contained a sufficient number to eliminate individual variations. When brought into the laboratory they were spread on tables and allowed to dry at room temperature. The plants were then carefully picked to free them of all foreign matter, both plant and animal, and when thoroughly cleaned the air-dried plants were ground in a grain mill. Samples were dried in an electric oven at approximately 80° for 6 hours or more.

Preparation of polysaccharide extracts.

From each of the monthly collections described above the polysaccharide extract was prepared according to a standard method previously described [Butler, 1934]. These are the extracts referred to throughout this paper.

COMPARISON OF WHOLE PLANTS OF *CHONDRUS* AND THEIR EXTRACTS.

Chondrus. From January to April the plants are dark in colour, a reddish or greenish black; from May till August the colour becomes lighter; whilst from September to January the colour gradually becomes dark again.

Extracts. The extracts are light and fibrous in texture, and are always sufficiently brittle to make it possible to powder them by grinding in a mortar.

Such slight variations as were noticeable in the appearance of extracts seemed, in general, to follow the natural colour variations of the whole plants.

Ash determinations (Fig. 1). Duplicate ash determinations were made on the monthly collections of *Chondrus* and on the extracts prepared from them. Ashing was carried out in an electric muffle furnace at approximately 600°. The results of the ash determinations on about 1 g. of the extracts were very satisfactory, only in one case was there a difference greater than 0.6% between duplicates. The results for ash of the whole plants were not so constant as in the case of the extracts. Quite large differences occurred between duplicates, and many determinations were repeated. The maximum difference for any of the duplicates accepted for Fig. 1 was 1.6%.

Fig. 1¹. Ash in *Chondrus*.
• Whole plants. ○ Extracts.

Fig. 2. Nitrogen in *Chondrus*.
• Whole plants. ○ Extracts.

In general the plants and extracts show the same series of fluctuations, the changes becoming apparent slightly later in the extract.

The percentage of ash is especially significant in the extracts, where only combined ash material is present. As has been pointed out previously [Butler, 1934] the standard extract is not a single chemical substance but is a mixture of closely related ethereal sulphates.

Any one, or all, of at least the following three types of sulphate esters may be present in the standard extracts:

- Type I. $[R(OSO_2O)_2Ca]_n$.
 Type II. $[(ROSO_2O)_2Ca]_n$.
 Type III. $[HOSO_2O.R(OSO_2O)_2Ca]_n$.

Within any one of these types *R* may vary in complexity, in composition or in both; and *n* may vary, as well as the cation.

On ashing, types I and II break down in such a way as to retain in the ash as $CaSO_4$ one-half of the total sulphate; in type III, however, only one-third of the total sulphate will be retained in the ash.

It is suggested that in esters of these types a low complexity of carbohydrate will be associated with a high percentage of ash and from variations in the latter

¹ In each figure, the August and September points have not been joined because of the break in collecting at that time. Also, the November 1934 and January 1935 points have been connected with dotted lines because of the absence of the intermediate point. The extra point in each represents November 1935.

it might be possible to obtain some indication of the complexity of the carbohydrate radicals. The results obtained will be discussed later in relation to these considerations.

Nitrogen.

The percentages of nitrogen in the monthly collections of *Chondrus* and in the extracts from each were determined by the Kjeldahl method. The results are expressed graphically in Fig. 2.

It is readily seen that the percentage of nitrogen in the extracts depends directly on that present in the whole plants. This observation had been previously made on extracts prepared from samples collected at widely separated places, as well as at different seasons [Butler, 1935].

Carbohydrate in whole plants and extracts.

Since the amount of fatty material in *Chondrus* is negligible, the percentage of carbohydrate has been calculated as the difference between 100 % and the sum of the percentages of crude protein and ash.

Fig. 3 shows these results graphically, and it is evident that the general trend of variations is similar in plants and extracts.

From the foregoing data, the assumption that variations in the extracts are expressive of variations in the plants themselves seems justified. From this point, therefore, attention has been directed to a more detailed study of the changes taking place in the extracts, as being significant of those in *Chondrus* itself.

Fig. 3. Carbohydrate in *Chondrus* by difference.
○ Whole plants. • Extracts.

Fig. 4. Ash and sulphate in *Chondrus* extracts.
• Total sulphate. ○ Ash.

Variations in the extracts.

Sulphate. The percentage of total sulphate in each extract was estimated, after hydrolysis in 5 % HCl, by precipitation with 10 % BaCl₂. A weighed sample (approximately 0.5 g.) of the extract was boiled for 5 hours under a reflux condenser in 250 ml. of 5 % HCl. The hydrolysed solution was then made up to volume again and 25 ml. aliquots were used for the sulphate determination. Aliquots of this same hydrolysate were used for estimating the percentage of reducing sugars present.

The total sulphate results, given in Table I, col. (1), are averages of closely agreeing duplicates.

In Fig. 4 the percentage of total sulphate in the extracts is compared with that of ash.

Table I. *Percentage of sulphate in extracts.*

Collected		(1) % SO ₄ (after hydrolysis)	(2) % SO ₄ calcu- lated from ash	(3) % SO ₄ lost in ashing	(4) % total SO ₄ retained in ash
January	1931	27.97	9.72	18.25	34.76
February	1931	29.00	10.04	18.96	34.61
March	1931	28.53	10.07	18.46	35.28
April	1931	29.57	10.23	19.34	34.58
May	1931	28.13	9.42	18.71	33.50
June	1931	28.04	11.18	16.86	39.88
July	1931	29.70	9.55	20.15	32.14
August	1931	29.25	13.77	15.48	47.09
September	1934	28.21	12.06	16.15	42.75
October	1934	28.07	12.68	15.39	45.15
November	1934	28.31	10.87	17.44	38.42
January	1935	27.49	8.76	18.73	31.87
November	1935	28.56	11.69	16.87	40.95

They are seen to correspond closely until May, after which, until November, when a falling off occurs in both, there appears to be no correlation. The sulphate is a better indication of actual combined inorganic material than ash, because of the uncontrollable factors which may enter into the estimation of the latter. Sulphate was also estimated in the ash from each sample, and the amount present calculated as % SO₄ in the original extract. These figures, also, are given in Table I, col. (2), and, along with them, col. (4), the percentage of total SO₄ retained in the ash.

Carbohydrate as reducing sugar. The reducing sugars present in the hydrolysate described under "Sulphate" were determined in 2 ml. aliquots by the Hanes modification of the Hagedorn-Jensen technique [Hanes, 1929]. The results, expressed as percentage carbohydrate calculated as glucose, are given in Table II, col. (2), which includes also the percentages of carbohydrate obtained by difference, col. (1), corrected for the sulphate lost on ashing.

Table II. *Percentage of carbohydrate in extracts.*

Collected		(1) Carbohydrate by difference (corrected)*	(2) Carbohydrate found calculated as glucose
January	1931	51.31	53.10
February	1931	47.05	50.83
March	1931	47.55	50.88
April	1931	47.39	49.75
May	1931	56.56	52.95
June	1931	59.68	53.84
July	1931	62.31	54.90
August	1931	59.12	53.71
September	1934	61.27	56.64
October	1934	60.94	55.34
November	1934	56.93	54.67
January	1935	49.75	53.10
November	1935	58.04	54.17

* The correction was made by deducting the percentage of sulphate actually lost (see Table I, col. (3)) as well as the ash and protein.

The figures obtained by the two methods may be seen to agree fairly well during the low level of the early part of the year. In May, carbohydrate, calculated by difference, shows an increase; it may be that something is lost in ashing and is being calculated as carbohydrate by difference, though it has no

reducing power. The same sequence of changes is evident in both, even though the level is different, which suggests that relative values are correct although the absolute ones may not be.

In Fig. 5 the variations in carbohydrate (calculated as glucose) are shown graphically, in relation to protein and sulphate.

Fig. 5.¹ Carbohydrate, total sulphate and protein in extracts of *Chondrus*. • Carbohydrate. ○ Total sulphate. ○ Protein.

Fig. 6. Sulphate ratios in *Chondrus* extracts. A, $\frac{\text{Sulphate in ash}}{\text{Total sulphate}}$ •. B, $\frac{\text{Carbohydrate}}{\text{Total sulphate}}$ ○.

For the sake of clarity in the discussion which is to follow, two ratios have been included in Fig. 6; A, % SO_4 in ash/% total SO_4 , and B, % carbohydrate (calculated as glucose)/% total SO_4 .

DISCUSSION.

As suggested in the section on ash determinations, variations in the percentage of ash in the extracts may be some indication of the complexity of the carbohydrate. By reference to Figs. 4 and 5 it may be observed that the experimental results only partially coincide with this assumption. During the early part of the year the percentage of ash is fairly high and that of carbohydrate low. Also, the period of low ash level, May to July, corresponds with the high carbohydrate one. From about October, however, percentages of both fall off together, suggesting the intervention of other factors.

In considering the relation between ash and carbohydrate it should be remembered that for a similar carbohydrate complexity in all three esters, each will leave a different percentage of ash.

Fig. 6, showing graphically the ratio of sulphate in ash to total sulphate, may be considered in interpreting the results, especially with reference to the type of ester predominating. It will be observed that from the beginning of the year until May, this ratio is such as to indicate a predominance of the acid salt (type III), in which it is 0.333. The ratio rises to that required by esters of type I or II (0.500) about the time (August) when the ash rises. From October, this ratio and the percentage of ash both fall off, which, taken together, might indicate a return to the acid salt.

How do carbohydrate variations fit in with these ash and sulphate variations? The low carbohydrate level from January to April may account for the rise in

¹ The scale of percentages in Fig. 5 is not continuous. In order to include the three graphs in the same figure, two breaks have been made.

sulphate percentage which accompanies it. In April when carbohydrate begins to rise, the percentage of sulphate drops, but from June to October they increase and then decrease together. During the time, then, when the previously considered evidence seemed to point to predominance of the acid salt, the percentage of carbohydrate is low. At the same time as it begins to increase, there is, according to the ash-SO₄/total SO₄ ratio, a change to the normal type of salt. Simultaneously with the decrease in percentage carbohydrate (i.e. from October) there is, according to the sulphate ratio, a gradual return to the acid type of salt.

Fig. 6 shows that the ratio is low until April, when it begins to increase along with carbohydrate; from September, it begins to fall, again along with carbohydrate. While the percentages of both sulphate and carbohydrate are increasing there is a drop in the ratio, so that even though the carbohydrate is increasing, it is not doing so in proportion to the sulphate. This drop of ratio in July, therefore, would seem to signify a greater increase of sulphate, such as would be necessary for a change to the normal salts. In July, when the lowest ash-SO₄/total SO₄ ratio point occurs, a high percentage of carbohydrate is reached; but the percentage of sulphate is also high at this time. The steep rise in the ash-SO₄/total SO₄ ratio, which follows this low point, would seem to indicate a more or less sudden change from type III salts to type I or II. Further evidence in support of this change in type is provided by the high level of carbohydrate/SO₄ ratio from September to January. From these considerations then, it appears that the normal salts predominate during the summer months, when the ash-SO₄/total SO₄ and carbohydrate/SO₄ ratios (and ash) are high.

A possible explanation might be that active assimilation beginning about April is responsible for the increase in the carbohydrate/SO₄ ratio. From September, when it may be assumed the reserve of carbohydrate material is highest, there is a gradual decrease of both carbohydrate and sulphate and, according to the sulphate ratio, a simultaneous reversion to the acid salt, as the supply of normal esters becomes finally exhausted in January. From then on until about April there is a decided drop in the carbohydrate/SO₄ ratio. The evidence of the sulphate ratio points to a predominance of the type III ester during this time.

Comparison of the curves for total sulphate and for carbohydrate shows them to be more or less reciprocal until June, after which they fluctuate together until January. During this latter period a high level in their ratio occurs, so that it seems justifiable to assume the carbohydrate complexity to be high during that period, even after the drop of the ash-SO₄/total SO₄ ratio indicates a return to the type III ester.

From January until about April, i.e. during the time of most active catabolism, the carbohydrate/SO₄ ratio drops, possibly as the carbohydrate complexity decreases, whilst during the active anabolic phase, April to September, this ratio increases.

It may be that the acid type of salt is the fundamental basis of the extract, or of the carbohydrate economy of the plant, and is always present. As a hemi-cellulose it can be used in metabolism if required, but the normal esters are the surplus or storage forms which are first used. When the supply of these is exhausted, the ordinary acid ester is metabolized by a reduction in its carbohydrate complexity.

SUMMARY.

A series of monthly collections of *Chondrus crispus*, made from the same location over a year period, is compared with the polysaccharide complex extracted from them in regard to ash, nitrogen and carbohydrate content.

Similar variations in ash content occur in both, though they are apparent later in the extracts than in the whole plants.

The nitrogen present in the extracts is found to depend directly on the amount present in the *Chondrus* plants.

The percentage of carbohydrate is low until March or April when a rise begins and a high level is reached extending from May to October.

The relationships of the sulphate retained in the ash and the total sulphate of the extracts indicate predominance of an acid type of ester from January to May. From May till October esters of the normal type predominate. After October, until January there appears to be a gradual return to the acid type of salt.

The relations of carbohydrate to total sulphate also indicate that the acid salt predominates from January to about May.

The hypothesis is suggested that the supply of the carbohydrate complex synthesized during the summer months is largely of the normal ester types, and that after the supply of these has been used up, the acid type of ester alone remains to be metabolized.

REFERENCES.

- Butler (1934). *Biochem. J.* **28**, 759.
— (1935). *Biochem. J.* **29**, 1025.
Hanes (1929). *Biochem. J.* **23**, 99.
MacFarlane & Bell (1933). *Proc. N.S. Inst. Sci.* **18**, 134.