CCXXVI. A NOTE ON THE PHOSPHORUS CONTENT OF MARINE ALGAE.

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IN a previous communication [Haas and Russell-Wells, 1923], dealing with the occurrence of ethereal sulphates in marine algae, it was stated that in *Chondrus crispus* evidence for the presence of phosphoric acid in organic combination had been obtained. This was shown by the fact that while no ionised phosphate could be detected before hydrolysis, its presence could be readily established after.

The occurrence of small quantities of phosphorus in sea weeds has long been known, but the actual amounts quoted in the literature are based upon ash analyses of the unwashed and undialysed weed. Such analyses give no indication as to the form of combination in which the phosphorus occurs in the weed or whether any of it is in organic combination and whether any such organic compounds, if present, are water-soluble. The present note is intended to throw some light on these questions. The weeds selected were *Chondrus crispus*, *Polysiphonia fastigiata* and *Dilsea edulis*, the two former because we had previously examined them from other points of view and the latter because of its relatively high phosphorus content.

In each case determinations of the total phosphorus content of the untreated weed and also of the dialysed aqueous extracts of the three weeds were made. Portions of the same extracts were hydrolysed with sulphuric acid under pressure and the ionised phosphoric acid thereby released was estimated; it was found that the whole of the phosphorus could be accounted for in this way, thus showing that the entire phosphorus content of the aqueous extracts was combined in the form of an ester of phosphoric acid. Furthermore a number of extracts obtained from samples of weed collected at different times of the year were examined for seasonal variation.

METHODS.

The extraction in the case of *Chondrus* was carried out according to the method previously described [Haas, 1921] whereby two extracts known respectively as C.E. and H.E. were obtained.

The extracts of *Polysiphonia* and *Dilsea* were prepared by heating the weeds with distilled water over a water-bath and dialysing the resulting solutions until free from chloride and phosphate. Previous to analysis the dialysed product was tested for ionised phosphate, in all cases with negative result.

Preliminary experiments having shown that the phosphorus content of the weeds was very small, it was decided to employ a macrochemical method of estimation, since a larger quantity of material could then be used for analysis, thereby reducing the experimental error; Pemberton's volumetric method of analysis was selected. Of the various modifications which have been suggested by different authors we have found Richards and Godden's [1924] for biological

material to be best adapted to our requirements. Etheridge's [1931] observation that Richards and Godden's [1924] instruction to boil out ammonia is unnecessary was confirmed by our experience and this operation was consequently omitted. The exact details of the method of analysis employed are given below. An amount of material corresponding to just under 2 g. dry weight was dried to constant weight in a steam-oven; it was then transferred to a litre round-bottomed flask and gently heated with 10 ml. of concentrated sulphuric acid and 10 ml. of concentrated nitric acid over a gauze until brown fumes were no longer evolved. If the liquid was not colourless at this stage more nitric acid was added and the heating continued. When quite colourless the contents of the flask were washed into a large beaker and made just alkaline with ammonia (sp. gr. 0.880), which in most cases brought about the formation of a gelatinous precipitate of silica; the solution was accordingly filtered, made just acid with concentrated nitric acid, treated with 40 ml. of 50% ammonium nitrate and heated to 70–75°: the phosphate was then precipitated by the addition of 30 ml. of Mathews's [1925] molybdate solution to which 1.5 ml. of concentrated nitric acid had been added. After standing overnight the solution was filtered through asbestos on a Gooch crucible; the precipitate was then washed twice with 10% nitric acid, four times with 2% ammonium nitrate and twice with cold water; it was then washed together with the asbestos into the original beaker by means of cold water and dissolved in a known excess of carbonate-free $N/2$ NaOH, the excess being titrated back with $N/2$ sulphuric acid using 6 drops of phenolphthalein as indicator. The factor 1 ml. $N/2$ NaOH $\equiv$ 0.000596 g. P was used for the calculation.

For the estimation of the inorganic phosphate set free by hydrolysis of the ethereal phosphate in the aqueous extract the requisite amount of material was heated with 75 ml. of 5% sulphuric acid in an autoclave at 105° for 4 hours: the resulting brown solution was filtered and precipitated as above. The results obtained are summarised in Table I.

Table I.

The figures give the mean % P calculated on the dry weight of the material analysed.

	Total P by oxidation		P by hydrolysis Extract
	Extract	Weed	
<i>D. edulis</i>	0.12	0.41	0.11
<i>C. crispus</i>	C.E. 0.030	0.22	C.E. 0.038
	H.E. 0.056		H.E. 0.050
<i>P. fastigiata</i>	April 0.075	0.22	April 0.073
	May 0.036		
	Aug. 0.045		
	Sept. 0.055		
	Oct. 0.022		

The agreement between values obtained for total phosphorus by oxidation and total phosphorus by hydrolysis is well within the limits of experimental error and accordingly justifies the conclusion that the entire phosphorus content of the extracts is present as an ester of phosphoric acid. Furthermore the figures given for the total phosphorus content of *Polysiphonia* extracts from weed collected in different months indicate that there is a slight, though not pronounced, variation in the ethereal phosphate.

SUMMARY.

1. The total phosphorus in three algae, *Chondrus crispus*, *Polysiphonia fastigiata*, and *Dilsea edulis*, has been determined.

2. The total phosphorus in aqueous extracts of the above has likewise been determined.

3. The phosphorus in the said extracts has been shown to occur in the form of esters of phosphoric acid and to vary slightly in amount with the month in which the weed was collected.

REFERENCES.

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