

CLX. A PRELIMINARY NOTE ON THE NITROGEN METABOLISM OF SEAWEEDS. GLUTAMIC ACID PEPTIDE.

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IN the course of an examination of the carbohydrate metabolism of certain brown seaweeds [Haas and Hill, 1929] it was found on testing concentrated extracts for sugars by means of Fehling's solution that the solution acquired a mauve tint. On investigation this was shown to be due to the presence of a substance in the extracts which gave a biuret reaction. The isolation of this substance has now been carried out on a somewhat larger scale than was at that time possible.

1 kg. of dried ground *Pelvetia canaliculata*, previously extracted with light petroleum, was covered with boiling water and kept warm over a small flame for about an hour; the weed was then strained through a fine wire sieve and treated a second time as before. The combined filtrates from the two extractions were then passed through calico and poured into an equal volume of absolute alcohol; the mucilaginous brown precipitate thus produced was strained through silk, and the liquid passing through was precipitated with basic lead acetate. After removing the precipitate the colourless solution was freed from lead by means of sulphuric acid, and the solution, while still acid with acetic acid, was treated with a slight excess of mercuric acetate and left to stand overnight. The precipitate A was then filtered, washed free from acid and decomposed with hydrogen sulphide; after removing the lead sulphide the solution was evaporated to small bulk under reduced pressure and finally dried in a vacuum. Thus prepared the product was a light yellow transparent syrup which gradually hardened to a resinous deliquescent solid (P). This substance was insoluble in organic solvents but was readily soluble in water to give an acid solution which on neutralisation however gave no precipitate with silver nitrate.

As the solid showed no tendency to crystallise after repeated attempts had been made to induce it to do so, it was decided to hydrolyse it, more especially as its general character suggested that it was a polypeptide.

Hydrolysis of the peptide (P).

To this end 1.8 g. of the substance was dissolved in 10 cc. of water and heated with 20 cc. of concentrated hydrochloric acid for 3 hours at 105°; the solution was evaporated to dryness over a water-bath and the residue taken up with water, filtered from some black solid and once more evaporated to dryness, when it weighed a little over 2 g. This material, dissolved in the least volume of concentrated hydrochloric acid, yielded 1.7 g. of crystalline solid which on analysis proved to be glutamic acid hydrochloride.

	C %	H %	N %	Cl %
Found	32.53	5.30	7.28	20.41
Calc. for $C_5H_9O_4N.HCl$	32.69	4.90	7.63	19.89

The mother-liquor from the glutamic acid hydrochloride weighing about 0.6 g. contained ammonium chloride; it was treated with freshly precipitated silver carbonate and, after filtering and saturating with hydrogen sulphide, evaporated to dryness in a vacuum. The resulting syrup on drying yielded a colourless resinous mass which was now free from chloride but still yielded ammonia in quantity on addition of dilute caustic soda in the cold and gave an immediate crystalline precipitate with platinic chloride, showing it to be an ammonium salt. It contained 12.72 % of nitrogen but owing to the limited amount of material available it could not be further identified.

Characterisation of the peptide (P).

That the substance was a peptide was shown by the fact that its amino-nitrogen content was increased eightfold after hydrolysis, from which it may be concluded to be an octapeptide.

Determinations of total and amide nitrogen gave the figures 15.07 % and 3.62 % respectively. In addition it was found that the substance had a strong acid reaction which was not increased by the addition of a neutral solution of formaldehyde. Heating over a boiling water-bath with excess of sodium hydroxide and titrating back the excess of alkali gave an equivalent of 209.2.

These figures coupled with the fact that after hydrolysis there remains, in addition to glutamic acid, a certain amount of material which has not as yet been identified make it unsafe to speculate further upon the probable constitution of the peptide, more especially as it has not been found possible as yet to effect any real purification, and the substance is known to contain a trace of ash. It is however hoped to subject this material to further study when more is available.

Occurrence of the peptide in other algae.

On extending the search for this peptide to other brown seaweeds it was found that most of those examined gave the biuret reaction but that the intensity of the reaction fell off with the depth of immersion of the weed, as

may be seen from the following table, in which the number of + signs indicates approximately the relative strength of the biuret reaction obtained.

<i>Pelvetia canaliculata f. libera</i>	++
<i>Pelvetia canaliculata</i>	++
<i>Fucus vesiculosus</i>	+
<i>Fucus serratus</i>	+
<i>Ascophyllum nodosum</i>	+
<i>Halydris siliquosa</i>	Weak
<i>Himantalia lorea</i>	Absent
<i>Laminaria</i> spp.	Absent

Extracts prepared from the following green and red weeds all failed to give the biuret reaction: *Enteromorpha intestinalis* (both fresh water and sea water), *Ulva lactuca*, *Chaetomorpha* sp. (fresh water), *Bostrychia scorpioides*, *Chondrus crispus*, *Polysiphonia fastigiata* and *Rhodomenia palmata*.

Physiological significance of the peptide.

In attempting to account for the physiological significance of this substance it is worthy of note that polypeptides, with the possible single exception of glutathione, are generally regarded as transition products which only occur occasionally as intermediate products under conditions of somewhat abnormal metabolism, whereas the evidence so far obtained indicates that it is a stable substance of normal occurrence.

It may be recalled that asparagine and glutamine, which also give the biuret reaction, have been found in seedlings belonging to a large number of widely diverging families, but in such plants these substances appear to owe their presence to conditions of carbohydrate starvation, due to insufficient illumination or other conditions tending to reduce the supply of carbohydrate. Whether the conditions of intermittent desiccation to which the brown seaweeds are exposed and the consequent interruption in photosynthesis are sufficient to account for the appearance of this substance it is not as yet possible to say, though the diminution of the intensity of the reaction with increasing normal depth of immersion of the weeds appears to support this view. It is intended to investigate this matter.

Occurrence of trimethylamine.

The filtrate from the precipitate A (p. 1472), after being freed from mercury with hydrogen sulphide and from sulphuric acid by means of baryta, was evaporated under reduced pressure. The resulting syrup was rendered alkaline with sodium hydroxide and steam-distilled; the distillate was collected in hydrochloric acid and then evaporated to dryness. The residue after drying in a desiccator was extracted with chloroform, and the extract on evaporation left a small quantity of material which gave a crystalline precipitate with a solution of iodine in potassium iodide in the presence of hydrochloric acid; in addition, it gave a brown crystalline precipitate with gold chloride solution containing sodium bromide, tests which, coupled with the characteristic smell

given off on the addition of sodium hydroxide, establish the presence of trimethylamine.

SUMMARY.

1. A preliminary survey of the seaweeds growing round the coast of Britain has shown that the brown weeds exhibit certain peculiarities in their nitrogen metabolism which distinguish them from the green and red weeds growing above and below them.

2. Aqueous extracts of certain brown seaweeds, after appropriate treatment, give a marked biuret reaction, the intensity of which diminishes with the depth of immersion at which the weeds grow.

3. The material responsible for this reaction has been isolated and proves to be an octapeptide of glutamic acid.

4. No similar product has been found in the red or green weeds.

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REFERENCE.

Haas and Hill (1929). *Biochem. J.* **23**, 1000.