chrome *c* in solution is reduced very rapidly by a number of reducing agents, e.g. ascorbic acid which reduce the cytochrome *c* in the heart-muscle preparation much more slowly (Slater, 1949*b*). Thus the various 'cytochrome reductases' which have been isolated may be the appropriate diaphorase, which when reduced, can be reoxidized either by methylene blue or cytochrome *c* in solution, but which *in vivo* is not oxidized by cytochrome *c* but by another catalyst, which may be a haematin compound like cytochrome *c* (Slater, 1949*c*).

SUMMARY

1. The Co I H_2 -cytochrome *c* reductase activity of heart-muscle preparation is about the same as its succinate-cytochrome *c* reductase activity at room temperature.

2. The concentration of oxidized cytochrome *c*

does not fall exponentially with time during the course of the reduction. The rate of reduction of cytochrome *c* is approximately a rectangular hyperbolic function of the oxidized cytochrome *c* concentration.

3. The rate of reduction of the endogenous cytochrome *c* of the heart-muscle preparation, calculated from the rate of the aerobic oxidation of Co I H_2 , was 1200 times that of the same concentration of cytochrome *c* added to the heart-muscle preparation.

4. The Co I H_2 -cytochrome *c* reductase activity of heart-muscle preparation is inactivated by treatment with BAL.

5. It is concluded that the Co I H_2 -cytochrome *c* reductase in heart-muscle preparation consists of two or more enzymes.

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On Certain Peptides Occurring in Marine Algae

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In an earlier communication Haas, Hill & Russell-Wells (1938) expressed their intention of investigating the water-soluble peptide occurring in the red alga *Griffithsia flosculosa* Batt. (*G. setacea* C. Ag.). The outbreak of war, however, interrupted the work, and further collaboration became impossible. The present communication is an attempt to amplify the earlier work on *G. flosculosa*, and to provide certain additional data concerning the peptides contained in two species each of the brown alga *Pelvetia* and the encrusted alga *Corallina*, belonging to the Phaeophyceae and Rhodophyceae respectively.

METHODS

The freshly gathered weed was spread out to dry and worked up as required, drying having been found to have no effect on the nature of the extract. For extraction the dried weed was immersed in 4-5 times its weight of water and extracted for about 1 hr. at 60-70° with occasional stirring; after straining the weed, a second extraction was made and the combined extracts were filtered through paper pulp and precipitated by basic Pb acetate; the filtrate from this was freed from Pb by saturation with H_2S and the resultant acid solution was concentrated under reduced pressure at about 60°. The resulting solution was made alkaline with Na_2CO_3 , freed from NH_3 by a rapid current of air, and after neutral-

izing was precipitated by mercuric acetate with alternate addition of Na_2CO_3 to neutralize any acidity. The precipitated material was repeatedly washed by decantation and centrifuging and finally decomposed by H_2S . The resulting filtrate was evaporated to dryness under reduced pressure.

RESULTS

The peptide of Griffithsia flosculosa

It was previously suggested (Haas & Hill, 1933) that the occurrence of peptides of this kind might be due to restricted photosynthesis resulting in the production of insufficient carbohydrate for normal metabolism of intermediate products in the synthesis and breakdown of protein. Such conditions would seem to apply to *Griffithsia*, which normally grows in shaded pools. At Parke's Beach, Lymington, Hants, from which locality practically all the material for examination was collected, it was growing below the *Laminaria* zone and was only exposed to full daylight for a couple of hours at fortnightly intervals of low spring tides, a circumstance which considerably restricted opportunities for collecting material.

Records of the occurrence of the peptide, as indicated by the biuret reaction, are given below:

	March	April	July	August	September	December
Biuret reaction	+	+	Faint	Absent	Absent	+

It will be observed that the biuret reaction is either faint or absent during the summer months,* an observation which lends some support to the suggestion that lack of light may be a factor contributing to the occurrence of the peptide at other times of the year.

Tests for certain constituent amino-acids in the samples of peptide extracted at the various seasons revealed no significant difference in composition throughout the year; in all cases the same four—glycine, alanine, arginine and histidine—were found.

It is fully recognized that the interpretation of the results here presented requires considerably more evidence than is offered in this communication, but lack of opportunity for securing further evidence by the collection of other similarly situated algae and the length of time required for studying seasonal variation prevents continuation of the investigation and for this reason the observations so far made are published despite their incompleteness in the hope that they may be of interest to future investigators.

The peptides of Pelvetia canaliculata, Pelvetia canaliculata forma libera, Corallina officinalis and Corallina squamata

The above algae, though belonging to different families and growing under entirely different conditions, share the common characteristic of containing peptides. In the case of the two species of *Pelvetia*, reduced photosynthesis could be accounted for by prolonged periods of intertidal desiccation which, on the salt marsh at Blakeney Point, extends to about a fortnight between spring tides for the free floating *P. canaliculata forma libera*.

The occurrence of peptides in calcareous algae such as the two species of *Corallina* could again be

attributed to reduced photosynthesis due to restricted penetration of light through the encrusting calcareous sheath.

No systematic attempt was made to investigate the question of seasonal variation of peptide in these weeds, the results recorded applying only to isolated examples.

Pelvetia canaliculata was collected in Plymouth and *P. canaliculata f. libera* was collected at Blakeney Point, Norfolk.

Of the two species of calcareous algae *Corallina officinalis* was obtained from Plymouth in February

Table 1. *Qualitative composition of peptides from marine algae*

	Glycine	Alanine	Arginine	Histidine	Aspartic acid	Glutamic acid
<i>Griffithsia flosculosa</i>	+	+	+	+	—	—
<i>Pelvetia canaliculata</i>	+	+	+	+	—	+
<i>P. canaliculata f. libera</i>	+	+	+	+	—	+
<i>Corallina officinalis</i>	+	+	+	+	+	—

It must therefore be concluded that the peptide is not a constant constituent of *Griffithsia flosculosa*, but that when it does occur its qualitative composition does not appear to vary, at any rate in so far as the four simple amino-acids are concerned; there is, however, no evidence to show whether it is an up-grade or downgrade product of metabolism.

* A specimen of *Griffithsia* collected in September in an entirely different locality, namely Poole Harbour, likewise contained no peptide.

1948, and *C. squamata* was gathered at Dancing Ledge, Dorset, in May 1948. This latter sample was found to be free from peptide, though material collected from the same locality at other periods of the year gave the biuret reaction, thus suggesting that this weed is likewise subject to seasonal variation.

Table 1 shows the qualitative composition of the peptides examined. Tyrosine and tryptophan were notably absent from the peptides in all cases.

Properties of the peptides

The various peptides were very similar in appearance, being pale-yellow or light-brown semi-solids, which when quite dry were almost brittle like resin, but were deliquescent and readily soluble in water and diffusible through a cellophan membrane. They were not precipitated by basic lead acetate, copper sulphate, picric, tannic or trichloroacetic acids, but they were precipitated by mercury salts. The ordinary colour reactions for arginine, histidine, tyrosine and tryptophan were carried out on the intact peptide, but for the detection of alanine and glycine an adaptation of methods suggested by Alexander & Seligman (1945) and Alexander, Landwehr & Seligman (1945) were employed on the hydrolysed product prepared by heating the peptide for some hours with 20 % (w/v) aqueous hydrochloric acid and evaporating to dryness; the method employed depends upon the reaction of the amino-acids with ninhydrin and the identification by colour tests of the formaldehyde or acetaldehyde produced from glycine or alanine respectively.

To this end 5 ml. of 0.1 % (w/v) solution of the hydrolysate was distilled with 2 ml. of phosphate buffer pH 5.5, 1 ml. of 1 % (w/v) aqueous ninhydrin and a glass bead, in a short-necked round-bottomed flask; the latter was connected to a 4 mm. diam. condenser tube bent to reach the bottom of a 10 ml. graduated cylinder, containing 2 ml. of 1 % (w/v) aqueous NaHSO_3 standing in ice water. The liquid was distilled briskly so that about 7 ml. were collected in as many minutes and the distillate was tested as follows.

Glycine. To 5 ml. were added in portions 4 ml. of conc. H_2SO_4 with repeated shaking and cooling. When cold, 3 drops of 5 % (w/v) aqueous chromotropic acid were added and the mixture was warmed in a boiling water bath. If glycine is present a deep red-violet colour develops almost at once and increases in depth as heating is continued.

Alanine. 1 ml. of the ice-cold distillate was carefully poured into 8 ml. of conc. H_2SO_4 cooled in ice. One drop of 5 % (w/v) aqueous CuSO_4 and 0.2 ml. of *p*-hydroxydiphenyl (1.5 % (w/v) in 0.12N-NaOH) were then added. With alanine, an immediate white precipitate results which, on vigorous shaking, gives way to a clear blue colour. This can be intensified by immersing in a boiling water bath for a minute or two.

The two colour reactions do not interfere with each other, and the same solution can be used for the detection of either or both of the amino-acids concerned.

DISCUSSION

The examination of certain water-soluble peptides extracted from a small selection of marine algae leads to the conclusion that these substances are not of constant occurrence throughout the year. While the observations here recorded are hardly sufficient to justify drawing any definite conclusions, it may at least be said that they are not at variance with the hypothesis that the peptides may be intermediate products of protein metabolism resulting from deficient photosynthetic activity caused by unfavourable conditions of growth, either as regards illumination or moisture.

In view of the possibility of these peptides having antibiotic properties, samples were submitted for testing against various bacteria, but only the peptide from *Corallina officinalis* exhibited any activity in a concentration of 1 mg./ml. at which it inhibited *Streptococcus pyogenes* Richards. In view of this no detailed or quantitative analysis of these peptides was undertaken.

SUMMARY

1. Evidence is supplied in support of the suggestion that the occurrence of water-soluble peptides in *Griffithsia flosculosa* and *Corallina squamata* is subject to seasonal variation.

2. The isolation and properties of such peptides are described.

3. The peptide obtained from *Corallina officinalis* alone showed any antibiotic activity.

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Note added 21 March 1950. Dekker, C. A., Stone, D. & Fruton, J. S. (1949, *J. biol. Chem.* **181**, 719) have recently established the composition of the peptide contained in *Pelvetia fastigiata*, a closely related species of *P. canaliculata*.

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