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INTRODUCTION

SEVERAL interesting studies have been made in the past on the nitrogen metabolism in seaweeds. Haas and Hill (1931) and Haas, Hill and Karstens (1935) isolated water-soluble peptides from the brown alga *Pelvetia canaliculata* and explained their presence as due to lack of metabolic balance traceable to either desiccation or low illumination. To obtain further evidence Haas, Hill and Russel-Wells (1938) examined the calcareous algæ *Corallina squamata* Ellis, *Lithophylum incrustans* Foslie, *Amphiora capensis* Aresch. and *Galaxaura subverticillata* Kjell. A number of unencrusted algæ were also examined, but so far among the latter group peptides were found only in the two species, *Pelvetia canaliculata* and *Griffithsia flocculoses.* It was found that the encrusted forms contained crude peptides to the extent of 0·05 to 0·29% of the dry weight.

Mazur and Clarke (1938) determined quantitatively the amino acids obtained by hydrolysis of formic acid extracts of a number of brown, red, green and blue-green algæ. Channing and Young (1952) attempted the partition of nitrogen in the Laminarialls, *L. saccharina, L. cloustoni* and *L. nodusum* and also in *P. canaliculata,* as volatile-N, nitrate-N, residual Kjeldahl-N and amino-N and reported values ranging from 0·07 to 0·24%, 0·03 to 0·92%, 0·58 to 1·8% and 0·58 to 1·3% respectively on the dry material. These authors also analysed the hydrolysates of the above algæ for the qualitative assessment of their amino acid composition. The major constituents found are aspartic acid, glutamic acid, glycine, alanine, valine, leucine, isoleucine, and other acids like serine, threonine, phenylalanine, lysine and arginine in traces. They could not find any difference between the amino acid composition of the two orders of the algæ studied. These authors (1953) again studied the amino acid composition of the acid hydrolysates of the brown algae, *L. saccharina, A. nodusum, P. canaliculata* and *Rhodymenia palmata.* Coulson (1953 *a*) has studied the nitrogen occurring

in free amino as well as in peptide forms in a number of seaweeds and reported differences between the various algæ examined. He (1953 *b*) also conducted qualitative studies on the amino acid composition of the proteins isolated from a number of marine algæ inclduding *Enteromorpha intestinalis*.

Takagi (1950) studied in detail the protein isolated from the Sea Lettuce (*Ulva pertusa* Kjell). The partition of the protein into water-soluble and alkali-soluble proteins was attempted and the arginine, histidine, lysine and cystine content of both fractions estimated. In another study (Takagi, 1951 *a*) the amino acids of *Ulva pertusa*, *Enteromorpha linza*, *Enteromorpha linza* var. *crispata*, all marine green algæ, were quantitatively studied by the two-dimensional chromatographic technique. The same author (1951 *b*) gives the nitrogen distribution in the various kinds of *Porphyra* during the different seasons. It is observed that the water-soluble nitrogen reaches a maximum in December with minimum in June. The author gives sufficient data to prove that there is considerable variation in the distribution of nitrogen in the various species of *Porphyra*, *viz.*, *Porphyra tenera*, *P. umbilicalis*, *P. pseudolinearis*, *P. Okamurai*, *P. crispata* and *P. suborbiculata*. Takagi and Susuki (1952) estimated the sulphur-containing amino acids in a number of brown, red and green marine algæ and they found the largest amounts in the browns, next in the greens and least in the reds. No methionine was found in any of the species studied. Takagi (1952) separated adenine, histidine and lysine as monopicrates from *P. pseudolinearis*. Dewar (1952) refers to the work of Young on the amino acid constituents of *L. saccharina* and *A. nodusum*. The amino acids, glycine, leucines, aspartic acid, glutamic acid, serine, threonine, proline, phenylalanine and a trace of arginine were identified in the hydrolysates of the last two algæ. In a more recent investigation Ogino (1955) has studied the variations in the total, protein and non-protein fractions in a number of Chlorophyceæ, Rhodophyceæ and Phæophyceæ. He has shown that these fractions vary not only with the species and seasons but also with the age of the plants. Non-protein nitrogen fractions of these species have been shown to contain about 23 amino acids in the free state—the mono amino acids, glutamic acid, aspartic acid, alanine and cystine being the most common in most of the species while in certain species like *Prasiolo japonica* the diamino acid arginine was more conspicuous.

Besides these some amount of work has been done on the inorganic forms of nitrogen present in different types of algæ. Nitrates have been recognised in the growing apices of phæophyceæ by Suneson (1933).

Similarly in the Rhodophyceæ also nitrates have been detected by many workers (Kylin, 1915; Suneson, 1932).

In the light of the studies mentioned in the foregoing review on the nitrogen metabolism in seaweeds investigations were conducted on 11 species of seaweeds common to the Indian Coast. The species taken up for study, the place of their collection, the manner in which the samples were prepared for analyses, etc., have been dealt with in an earlier communication (Krishna Pillai, 1956). In the present account the data relating to the nitrogen constituents of the different seaweeds are presented. An attempt has been made to follow the seasonal variations in the oxidised inorganic nitrogen, volatile nitrogen, protein and non-protein nitrogen, and total and water-soluble organic nitrogen in the seaweeds. The amino acid composition of the acid hydrolysates of two sets of collections of the different algæ representing two distinct stages of growth has also been presented. The data bring to light the various changes taking place in each form of nitrogen during different growth stages of the plants as well as during different months. The knowledge is fundamental to understanding the physiology of the seaweeds.

Methods of analyses

Oxidised inorganic nitrogen.—Two grams each of the sun-dried seaweed were accurately weighed into a mortar and ground well with acid-washed sand. Nitrogen-free distilled water was added little by little during the process of grinding so that the entire material was converted into a colloidal mass. This was transferred to a beaker with the distilled water washings. The material was continually stirred with the aid of an electric stirrer for about an hour and finally centrifuged. The clear extract was taken in a conical flask and acidified with 10 c.c. of conc. hydrochloric acid. About 0·2 g. of Davarda's alloy was introduced, the flask loosely covered with a cork and left for a few hours and the contents were stirred occasionally. The solution was then filtered and the nitrogen estimated by the micro-kjeldahl method.

Total nitrogen.—Ten milligrams of the air-dried material were weighed into a kjeldahl flask and digested with 1 c.c. of conc. sulphuric acid in the usual manner using a mixture of copper sulphate and potassium sulphate as catalytic agent. After cooling the residue was taken up with distilled water and the ammonia estimated in the micro-kjeldahl. For the estimation of the protein-nitrogen quantities ranging from 10 to 20 mg. of the air-dried seaweed were used.

Water-soluble organic nitrogen.—One gram of the air-dried sample was ground well with acid-washed sand in a mortar and made into a fine paste with nitrogen-free distilled water. The pasty material was transferred to a beaker and stirred for an hour continuously. The mixture was centrifuged and the clear solution digested in the usual manner with sulphuric acid and estimated.

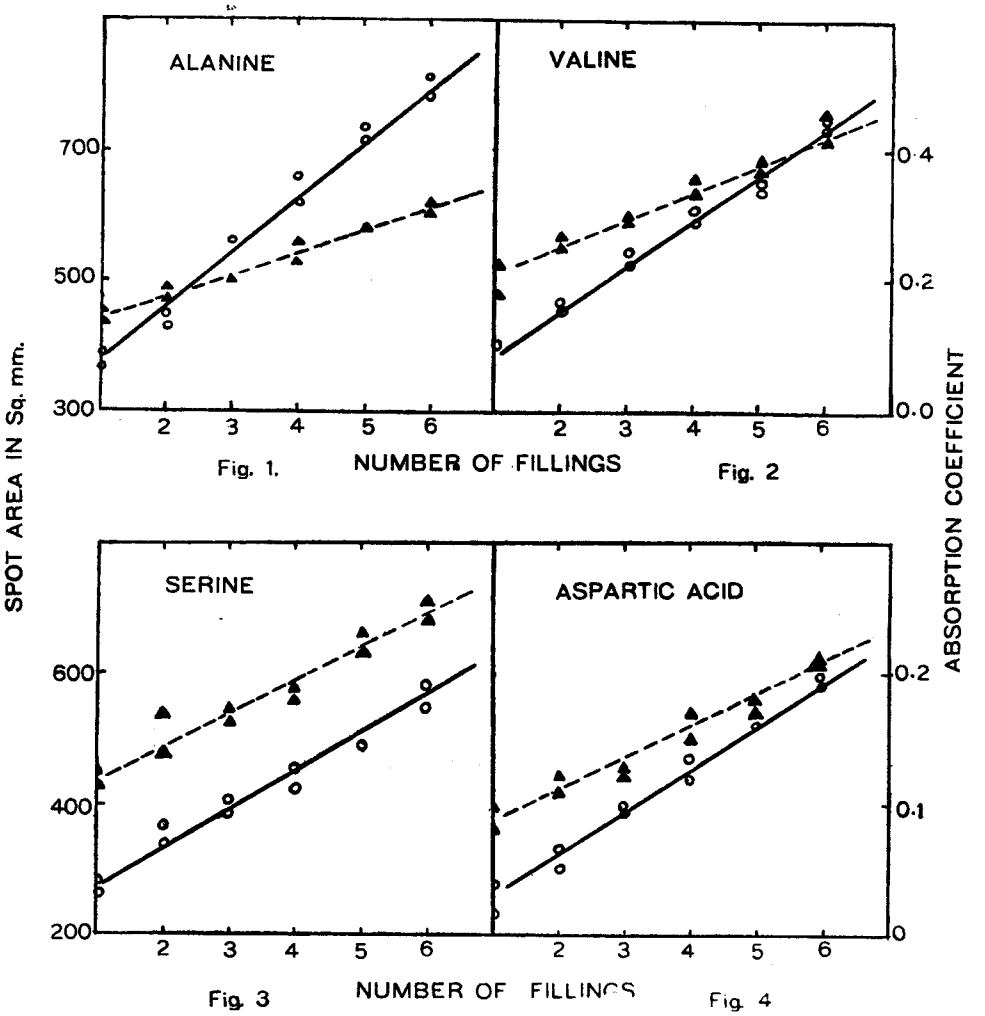
Volatile nitrogen.—Two grams of the air-dried sample were ground well with acid-washed sand. The pasty material was taken up with a few c.c. of water. The solution and washings were distilled in the micro-kjeldahl with alkali and the liberated gases were estimated by titration of the boric acid solution against N/70 sulphuric acid.

Amino acid composition of the seaweeds.—Two sets of seaweeds collected during August and December 1952 were examined for their amino acid composition. In the collection made in August the specimens were mature and fruiting while the samples obtained in December were young and non-fruiting. However, it could not be definitely said whether both collections belonged to the same parent stock, especially since some of the forms were of the floating type. But in spite of this defect the analyses give some idea of the differences in the amino acid composition of both the young and mature specimens of the different seaweeds.

Preparation of the hydrolysate for chromatography.—Two grams each of the air-dried sample were ground well with acid-washed sand and hydrolysed for 24 hours with 10 to 15 c.c. of 6 N hydrochloric acid. The hydrolysate was partially neutralised with sodium hydroxide solution and then concentrated in vacuum to a definite volume. The volume of all the hydrolysates is brought down to this level before microvolumes were spotted on chromatograms.

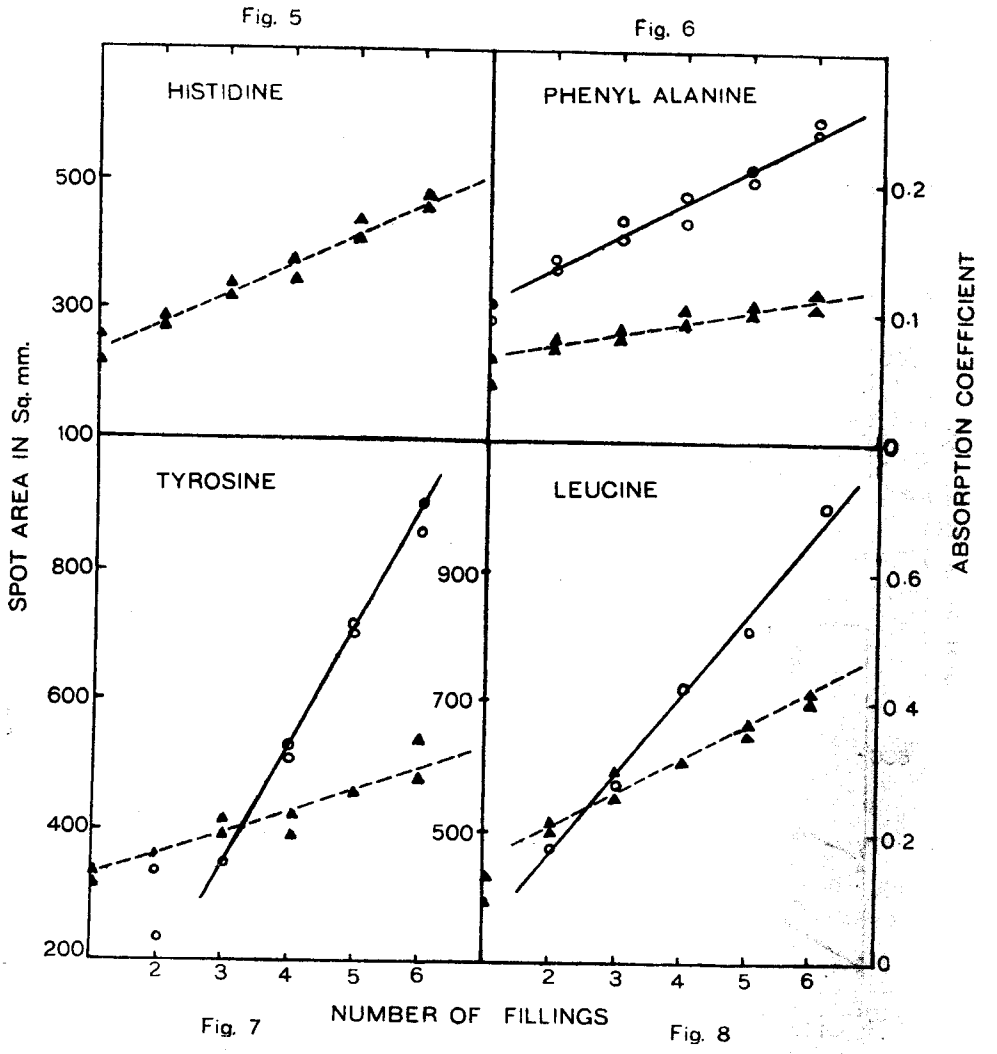
The chromatographic apparatus employed for this purpose was the one described by Krishna Pillai (1953). For the quantitative estimation both the area method (Viswanathan & Krishna Pillai, 1955) and the colorimetric method (Giri, Radhakrishnan & Vaidyanathan, 1953) were employed. Calibration curves for the spot area as well as the intensity of colour in presence of Cu^{++} ions on the spot content were separately drawn for each of the amino acids (Figs. 1 to 13). For determining the accuracy of estimation from unknown solutions standard mixtures of known amino acids were spotted and the corresponding amounts read off from the calibration curves for the spot area and the intensity of colour. It was found that the method (area method) is applicable to concentrations of amino acids between 20 and 100 micrograms and that the error is only within $\pm$ 10%. It is also

noticed that the area method is quite comparable to that of the method of Giri *et al.* where the colour of the spot is estimated.



FIGS. 1–4

For the estimation of the constituent amino acids of seaweed hydrolysates microquantities were spotted on Whatman No. 1 filter paper, developed in the solvent system, *n*-Butanol-acetic acid-water (4 : 1 : 5), and sprayed with ninhydrin solution. Standard mixtures of amino acids were also run side by side with the hydrolysates for purposes of easy identification. The spot areas were determined and the intensity of colour of the spots from a



FIGS. 5–8

duplicate set estimated in the absorptiometer. The results of analyses of the two sets of collections are given in Tables V and VI.

The monthly variations in the inorganic nitrogen content of the different species of seaweeds are given in Table I. Similarly the variation in the protein-nitrogen, water-soluble nitrogen and volatile nitrogen are given in Tables II, III and IV respectively. The actual values of total nitrogen, protein nitrogen, water-soluble nitrogen and volatile nitrogen of the different species are plotted in Figs. 14–22.

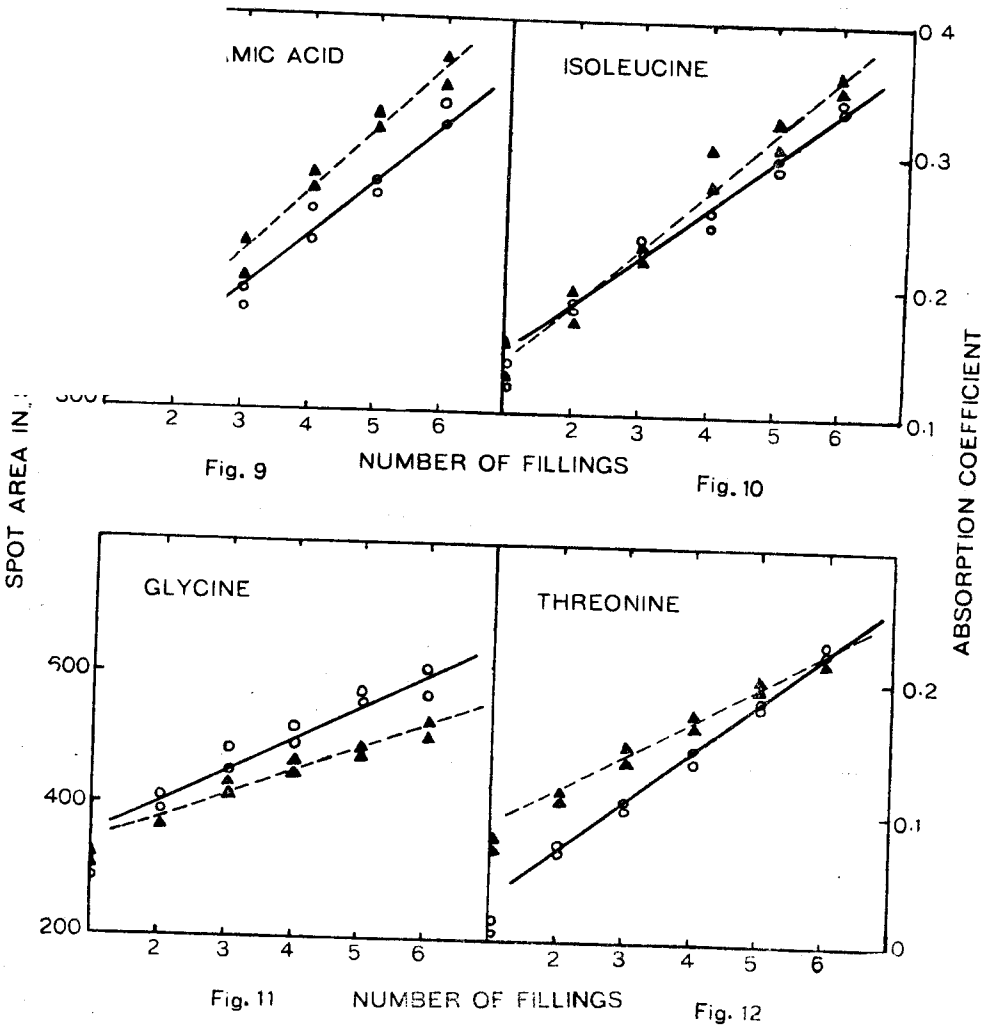


Fig. 9 Fig. 10

Fig. 11 Fig. 12

FIG. 9-12

DISCUSSION OF RESULTS

Oxidised inorganic nitrogen.—From the results it is evident that the maximum amount of inorganic nitrogen is found in the collections made in November, the period corresponding with the actively growing season of the algæ. This observation probably means that nitrate is present in greater quantities in the young fronds than in the older plants. The quantity of inorganic nitrogen decreases thenceforth at a slow rate until in some of the collections made in March–June the value falls to zero. A

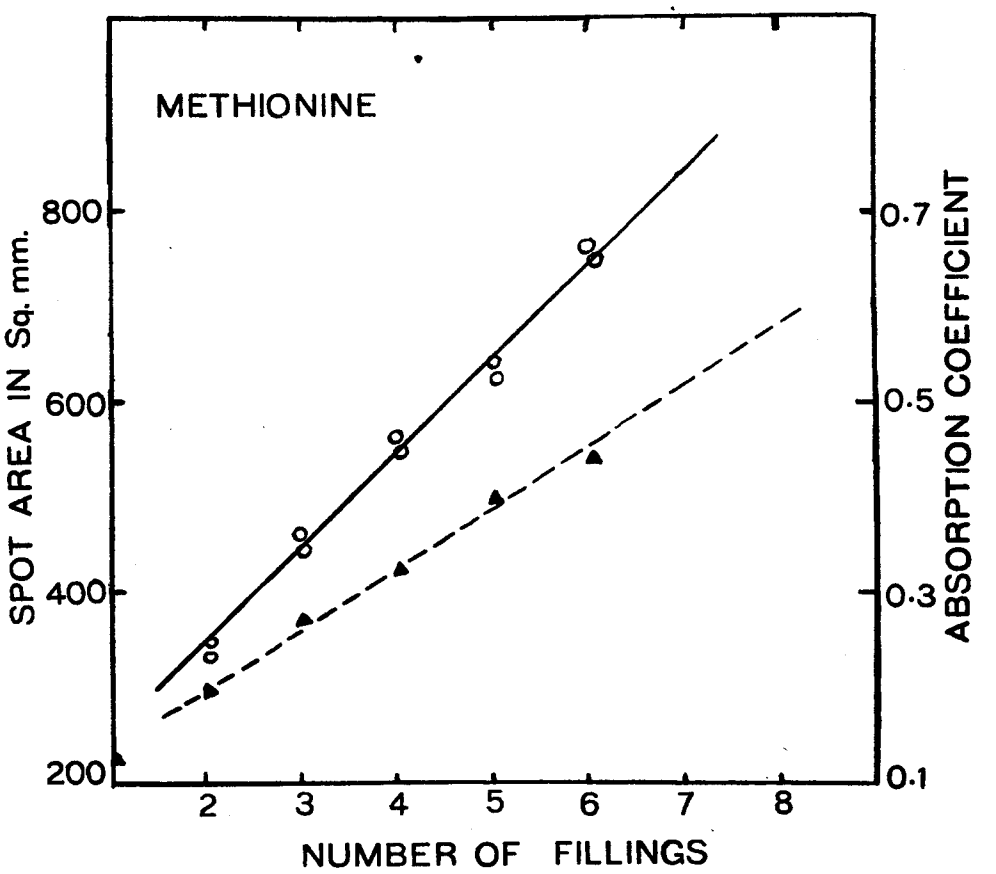


FIG. 13

FIGS. 1–13. ▲- - - - -▲ Absorption coefficient. O———O Spot area in sq. mm.
(1 filling of the micro-pipette contains 18·75 mg. of amino acid)

possible explanation for this phenomenon is that in the young plants nitrogen is absorbed from sea-water in the inorganic form and that as the plants grow the rate of nitrogen absorption from the water becomes less and less. The maximum value of inorganic nitrogen obtained is 0·047%N in *Sarconema furcellatum* in November, while other species examined give varying amounts (Table I). However, these values are small when compared with the figures reported by Channing and Young (1952) for *Laminaria* spp. and also with the values given by Suneson (1932) who reports 1·5% nitrate-nitrogen in *Ceramium rubrum*. With *A. spicifera* and *L. papillosa* another peculiarity is noticed. The inorganic nitrogen content in these two species never goes below 0·02%, whereas in most of the other species the minimum falls to

TABLE I

Variations in the inorganic nitrogen content in seaweeds

(Values expressed as percentage of dry matter)

Name of species	1952									1953		
	A	M	J	J	A	S	O	N	D	J	F	M
G. linum ..	..	..	..	..	..	0·018	0·021	0·030	0·020	0·020	0·019	0·013
G. lichenoides ..	Nil	Nil	0·009	0·010	0 010	0·011	0·019	0·021	0·014	0·015	Nil	Nil
C. dasyphylla ..	Ni	0·010	0·014	0·029	0·021	0·027	0·027	0·027	0·026	0·022	0·015	0·012
E. intestinalis ..	0·012	0·017	0·018	0·021	0·010	0·014	0·021	0·031	0·020	0·019	0·016	Nil
A. spicifera ..	0·023	0 022	0·020	0·021	0·022	0·031	0·036	0·036	0·031	0·030	0·021	0·020
L. papillosa ..	0·013	0·010	0·020	0·023	0·022	0·020	0·040	0·037	0·031	0·025	0·020	0·020
S. furcellatum ..	Nil	0·008	..	Nil	0·009	Nil	0·021	0·047	0·028	0·020	0·013	0·009
H. musciformis ..	0·014	0·000	0·009	Nil	0·015	0·012	0·017	0·018	0·014	0·016	0·009	Nil
R. intricata ..	..	..	..	..	..	..	0·041	..	..	..	Nil	..
P. australis ..	..	..	..	Nil	Nil	Nil	0·024	0·030	0·025	0·021	0·012	0·010

TABLE II

Variations in the protein-nitrogen in seaweeds

(Values expressed as percentage of total nitrogen)

Name of species	1952									1953		
	A	M		J	A	S	O	N	D		F	M
G. linum ..	33·7	74·6	64·5	78·7	63·4	55·6	37·5	50·7	36·1	42·9	..	..
G. lichenoides ..	69·5	64·2	67·6	77·1	98·1	68·2	78·9	60·2	25·4	32·4	58·3	41·9
C. dasyphylla ..	71·0	64·5	65·4	58·8	51·1	90·9	76·3	29·9	18·9	36·9	43·5	61·9
E. intestinalis ..	22·5	22·6	48·9	21·3	63·7	77·2	36·1	.	18·9	86·9	78·9	37·3
A. spicifera ..	63·2	48·1	48·3	59·5	72·9	92·4	76·9	..	20·6	28·6	..	66·1
L. papillosa ..	46·1	48·2	39·7	57·4	85·5	83·9	80·9	33·3	17·0	..	51·0	59·2
H. musciformis ..	35·6	42·1	63·4	63·8	64·5	89·4	83·3	59·3	20·4	24·1	43·6	60·0
S. filiforme ..	..	..	76·3	..	..	..	77·0	..	..	..	36·3	..
S. furcellatum ..	35·	40·6	71·1	68·3	68·3	83·3	70·3	49·9	27·0	42·2	54·5	63·3
R. intricata ..	..	30 4	87·5	..	..	..	80·2	..	..	..	38·0	..
P. australis ..	..	27·0	45·1	43·4	59·9	85·4	79·8	67·9	75·5	68·7	60·1	44·6

TABLE III

Variations in the water-soluble nitrogen in seaweeds

(Values expressed as percentage of total nitrogen)

Name of species	1952									1953		
	A	M	J	J	A	S	O	N	D	J	F	M
G. lichenoides ..	16·3	17·6	21·6	37·3	68·9	40·0	62·8	60·0	42·7	41·1	37·1	58·1
C. dasyphylla ..	0·2	29·3	30·7	41·9	48·4	43·7	59·2	57·7	29·0	25·9	25·9	38·9
E. intestinalis ..	38·6	..	49·6	21·3	21·0	26·1	18·7	28·0	28·4	29·6	29·6	62·7
A. spicifera ..	67·7	61·6	55·4	53·3	52·8	36·0	32·3	..	28·8	29·0	..	33·9
L. papillosa ..	23·0	19·3	37·2	31·0	52·6	47·6	63·5	79·7	23·4	..	29·0	40·8
H. musciformis ..	40·7	24·0	35·2	38·0	55·6	43·6	65·5	69·1	29·2	24·0	19·4	40·0
S. furcellatum ..	14·9	27·1	..	18·4	58·9	54·6	72·7	80·2	33·7	37·6	29·2	36·7
P. australis ..	..	..	..	31·8	56·5	51·9	49·4	53·9	57·6	43·2	39·7	55·4

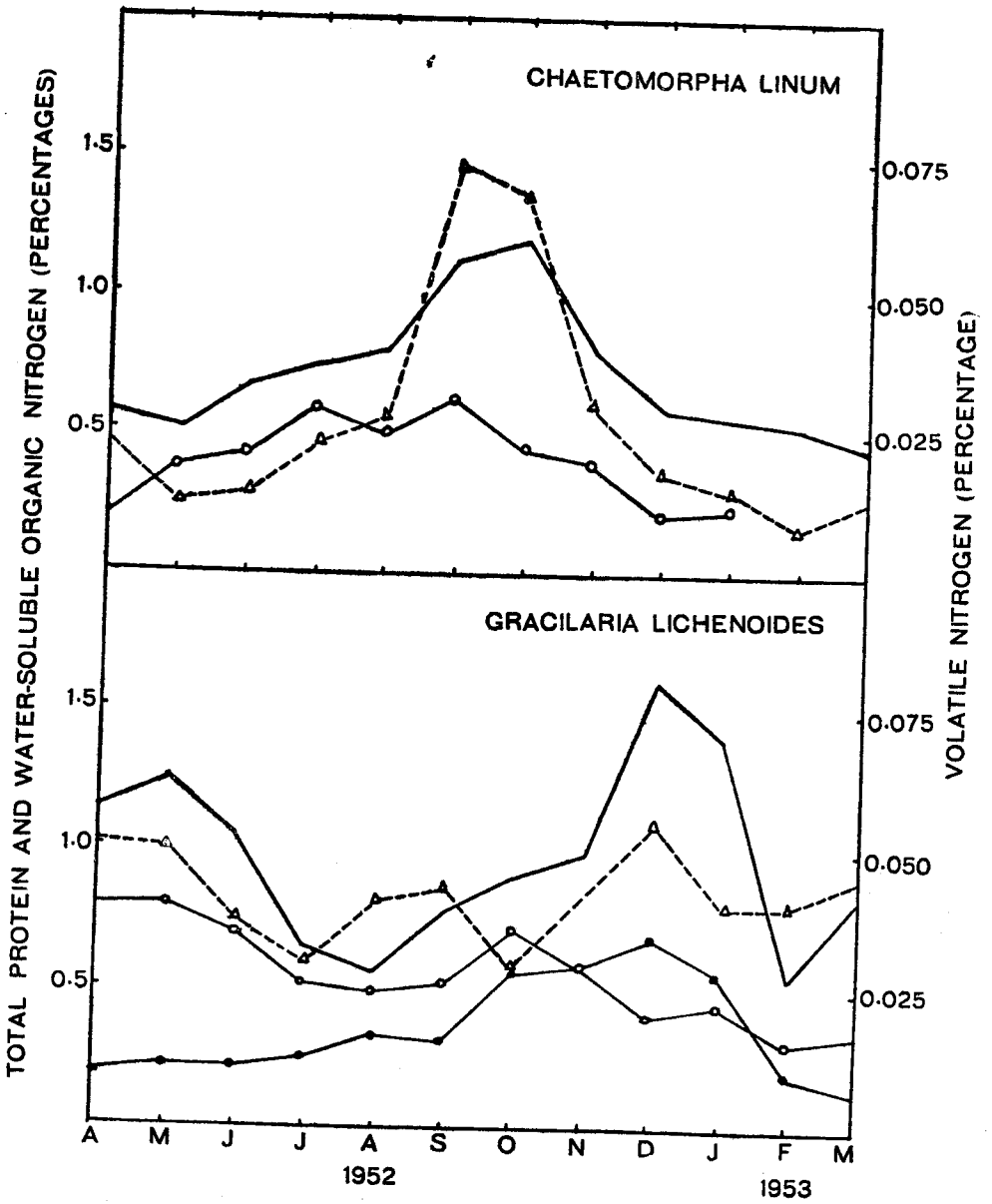
TABLE IV

Variations in the volatile nitrogen in seaweeds

(Values given as percentage of total nitrogen)

Name of species	1952									1953		
	A	M	J	J	A	S	O	N	D	J	F	M
C. linum ..	4·1	2·5	3·0	3·1	3·4	6·5	5·6	3·7	3·0	2·6	1·6	3·2
G. lichenoides ..	4·4	4·0	3·5	4·4	8·1	5·5	3·2	..	3·4	2·9	7·4	5·4
C. dasyphylla ..	4·1	3·4	3·7	2·8	2·5	5·0	4·0	2·4	2·5	2·5	2·7	2·6
E. intestinalis ..	4·6	6·8	5·7	3·8	5·2	5·3	3·3	3·1	3·1	5·6	5·2	1·8
A. spicifera ..	4·4	4·1	4·1	3·5	5·1	6·7	4·8	3·3	4·1	..	..	2·5
L. papillosa ..	5·3	4·9	5·5	3·1	5·2	5·0	2·7	2·7	3·8	..	2·5	3·6
H. musciformis ..	4·7	5·2	5·9	4·3	4·3	3·9	3·1	..	3·8	2·4	1·9	2·1
S. furcellatum ..	3·5	4·3	6·8	6·1	5·2	7·5	4·0	5·3	5·1	6·7	6·6	5·4
R. intricata ..	..	7·1	8·5	..	..	..	8·2	..	..	..	2·0	..
P. australis ..	5·7	5·6	5·7	5·4	4·3	5·0	4·8	5·6	7·9	..	3·0	3·3

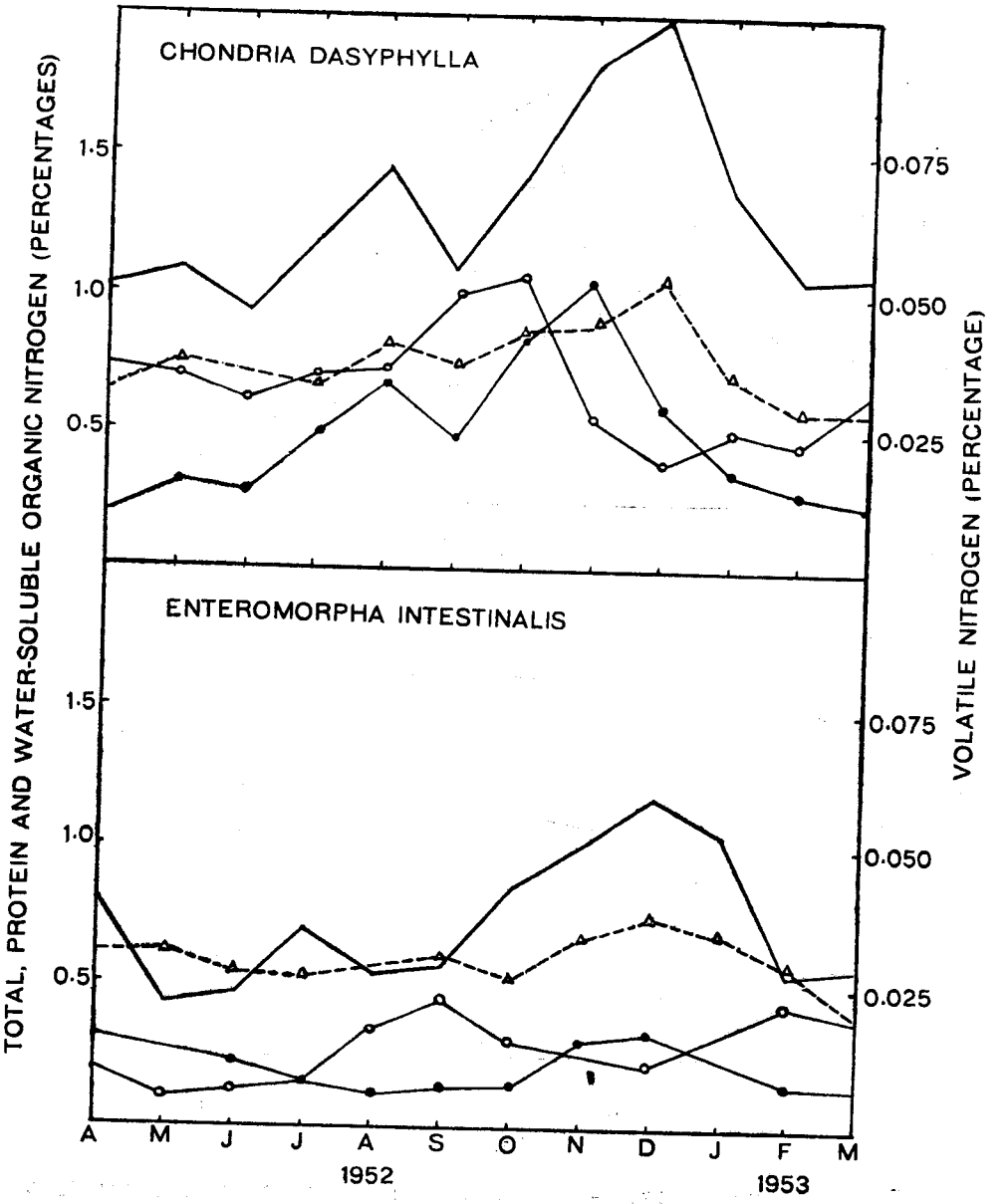
zero. This is an indication that in these two species the conversion of nitrogen to organic form proceeds only within certain limits.



FIGS. 14–15

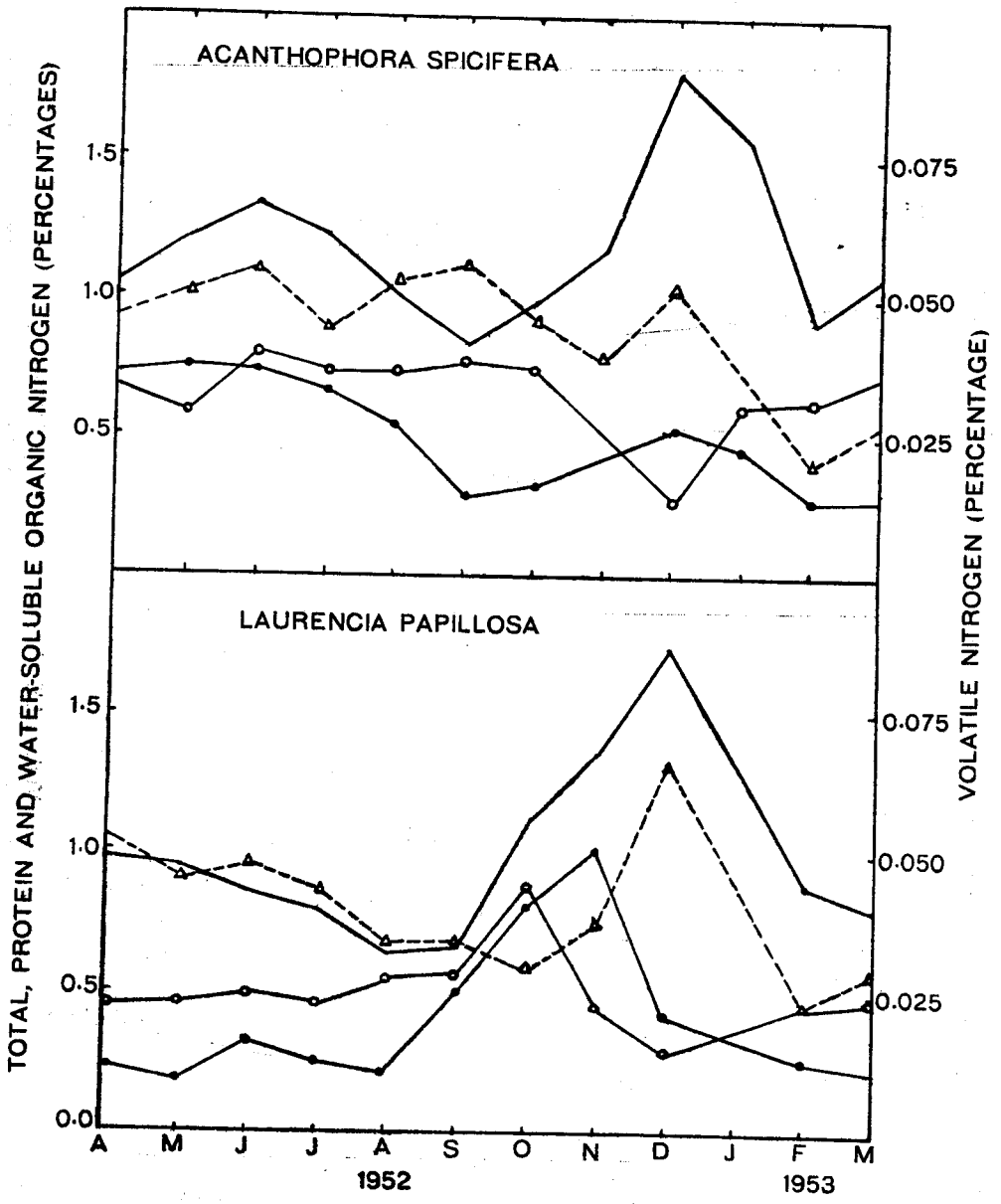
Total organic nitrogen.—The total organic nitrogen content shows wide variations reaching maximum values in October–December in most

cases. The minimum values are found in the months of June, July and August. The highest value 2·04% is recorded in *Chondria dasyphylla* (Fig. 16). In species like *G. lichenoides* the value for total organic nitrogen goes down to 0·5% in July–August. The usual variation in the total



FIGS. 16–17

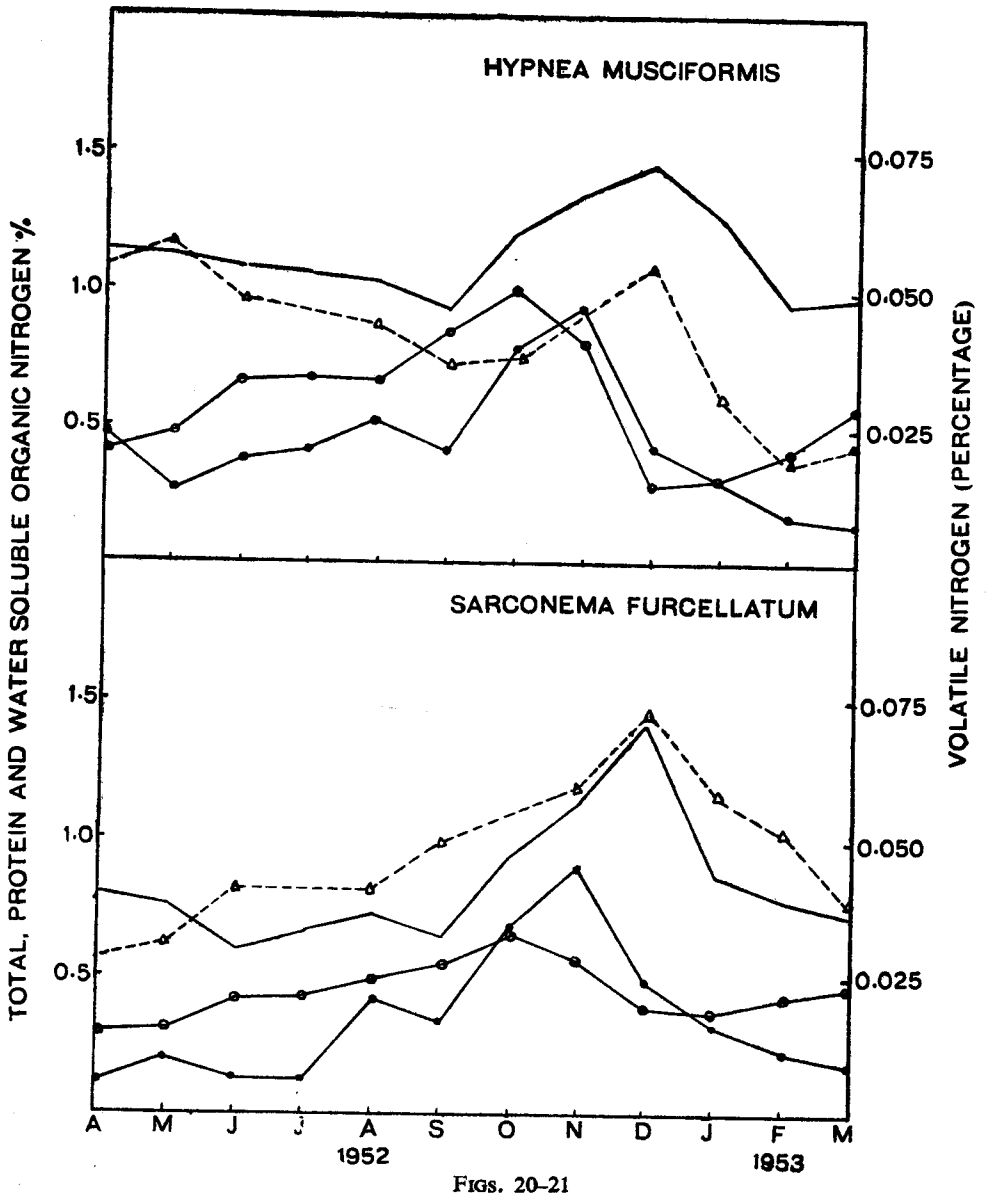
organic nitrogen content is between 0·5% and 1·5% of the dry matter. These figures are in perfect agreement with the values given by Channing and



FIGS. 18–19

Young though they have no comparison with the nitrogen values for *Porphyra* which contains about 8% total nitrogen. It may be noted that

the total organic nitrogen of the brown species is comparatively lower than that of the red, though maximum values of 1·02% and 1·29% respectively were obtained for *R. intricata* and *P. australis.*



FIGS. 20–21

Protein-nitrogen.—It can be seen from Table II that in the young plants collected in December when the total organic nitrogen is maximum the

protein-nitrogen is at a minimum constituting only 17 to 30% of the total nitrogen (an exception being *P. australis*). Probably at this stage the greater part of the organic nitrogen goes into the formation of chlorophyll in the rapidly growing algæ. Afterwards there is a slow increase in the protein nitrogen until it constitutes nearly about 70 to 90% of the total nitrogen in July, August and September. One striking characteristic is that the protein nitrogen is at a maximum when the total nitrogen is at a minimum (*vide* Figs. 14 to 22).

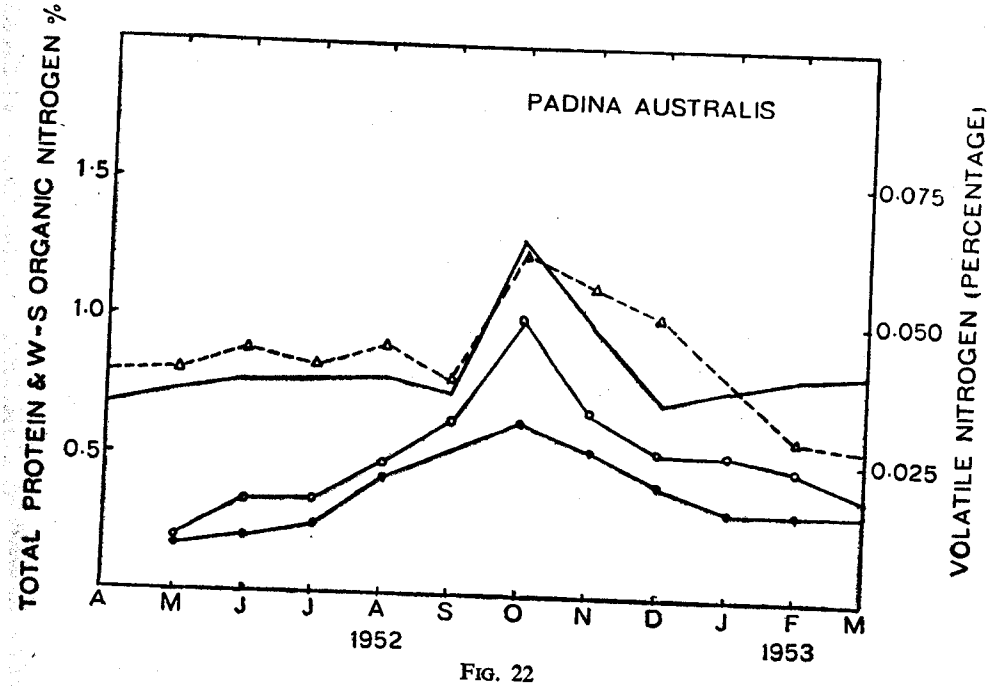


FIG. 22

FIGS. 14 to 22. ——— Total nitrogen. ●——● Water-soluble nitrogen. O——O Protein-nitrogen. △——△ Volatile nitrogen.

Volatile nitrogen.—From the figures plotting values of volatile nitrogen as percentage of dry matter content it can be seen that the amount changes with the total nitrogen giving maximum in October–December and minimum in June–July–August. But when the actual percentage of the volatile nitrogen on the total organic nitrogen as given in Table IV are considered it is seen that the maximum values are obtained when the protein is maximum.

Water-soluble nitrogen.—Water-soluble nitrogen also nearly follows the seasonal changes in the total organic nitrogen showing a maximum of about 50 to 80% of the total nitrogen in November in most cases (Table III). It

may be noted that the water-soluble nitrogen in the collections taken after December is equal or sometimes more than the protein-nitrogen especially in the case of the agarophytes. This probably means that the first stage of conversion of inorganic nitrogen is the water-soluble organic compounds. As the algæ grow the proportion of protein-nitrogen increases and the water-soluble nitrogen decreases thereby showing that in the mature plants the water-soluble nitrogen is converted to water-insoluble proteins.

Amino acids.—The seaweeds analysed for their amino acid content are: *G. lichenoides*, *C. dasyphylla*, *H. musciformis*, *A. spicifera*, *S. furcellatum* and *P. australis*. Table V gives the results of analyses done on the mature

TABLE V

Amino acid composition of the hydrolysates of mature specimens of seaweeds

(Values given as percentage of total nitrogen)

Amino acid	*G. lichenoides*	*C. dasyphylla*	*A. spicifera*	*S. furcellatum*	*H. musciformis*	*P. australis*
Phenylalanine	5·3	5·8	..	5·6	5·0	4·8
Leucine (*s*) ..	1·6	..	3·2	3·6	3·0	4·3
Tyrosine ..	2·5	4·0	..	3·2	..	2·8
Valine ..	4·2	3·2	4·6	4·0	4·0	4·0
Alanine ..	3·3	3·6	1·8	2·8	2·0	3·0
Glycine ..	1·8	2·0	2·0	2·0	1·8	2·0
Aspartic acid	1·5	1·3	1·6	1·5	1·5	1·6
Glutamic acid	3·2	3·4	2·0	3·0	2·8	2·4
Serine ..	1·8	1·2	2·0	1·8	1·6	1·2
Threonine ..	2·3	2·0	2·7	2·0	2·4	2·3
Arginine ..	7·4	7·0	6·6	7·6	7·0	7·4
Histidine ..	1·2	1·6	1·0	1·2	1·2	1·0
TOTAL	36·1	35·1	27·5	37·3	32·7	36·8

specimens and Table VI gives that of the young specimens. It may be seen that there is not much difference between the six different species of seaweeds in regard to the number of amino acids or in the proportion in which they

are present. Even though considerable difference has been noticed in the protein-nitrogen of the young and mature specimens of the various seaweeds the amino acid composition remains almost identical. This shows that the composition of the protein in both the young and mature plants is the same and does not undergo much change during growth.

TABLE VI

Amino acid composition of the hydrolysates of young specimens of seaweeds

(Values given as percentage of total nitrogen)

Amino acid	*G. lichenoides*	*C. dasyphylla*	*A. spicifera*	*S. furcellatum*	*H. musciformis*	*P. australis*
Phenylalanine	3·0	2·3	..	3·6	2·0	3–2
Leucine (*s*) ..	2·2	1·4	2·8	2·3	3·0	3·0
Tyrosine ..	1·0	..	..	1·0	1·0	1·8
Valine ..	2·3	1·8	2·0	2·3	1·6	2·6
Alanine ..	1·5	1·3	1·5	1·4	1·0	2·8
Glycine ..	1·0	1·7	1·0	1·0	1·0	2·7
Aspartic acid ..	1·0	0·8	1·2	0·8	1·0	1·8
Glutamic acid	2·4	2·0	..	2·2	2·2	4·3
Serine	1·0	0·8	1·0	1·0	0·8	2·3
Threonine ..	1·2	0·7	1·2	1·2	1·0	2·7
Arginine ..	4·3	3·7	4·0	4·8	3·8	6·3
Histidine ..	Nil	..	..	..	..	1·4

SUMMARY

An attempt has been made to study the chemical partition of nitrogen in eleven species of seaweeds belonging to the three major groups, Chlorophyceæ, Rhodophyceæ and Phæophyceæ. The seasonal variations in the total organic, water-soluble, volatile, protein and non-protein nitrogen contents were followed by analysing regular monthly collections of the algæ. The importance of each of the fraction in the metabolism of the algæ has been discussed. It is observed that the seaweeds are poor in their total nitrogen content, the values never exceeding 2% on the dry basis. In species

like *G. lichenoides* the total nitrogen content shows an inverse ratio to that of the agar content. Protein nitrogen is less in young plants, while in the mature plants it accounts for more than 75% of the total organic nitrogen. Water-soluble and volatile nitrogen content also follows the total nitrogen giving maximum values in September–December and minimum in July.

The amino acid composition of two sets of collections representing two distinct growth stages has also been studied quantitatively. It is observed that although the protein-nitrogen content varies substantially between the young and the mature plants the amino acid composition remains almost identical.

The chromatographic method employed in the quantitative estimation of the amino acids has been outlined.

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