Summary.

Developmental and cytological studies in the Rhodophyceae.

by

Fred Wilkinson Knaggs.

The present studies have been concerned with the ecology, morphology, cytology, and life-history of two members of the genus Rhodochorton; R. floridulum (Dillwyn) Näg., and R. purpureum (Lightf.) Rosenvinge, both of which are of common occurrence on the shores of Britain.

Rhodochorton floridulum.

The general ecology of the species was investigated at two sites: Ardneil Bay (Ayrshire) and Portmahomack Bay (Easter Ross), and compared with that observed at other sites in Scotland and England. These surveys were found to correspond with published information concerning the ecology of the species in other parts of its geographical distribution. It has been observed that the morphology of the species is subject to environmental control and three distinct ecological forms can be recognised: the mound form, typically found on rocks in the mid-littoral region of gently sloping, sheltered sand beaches; the low-lying mat form, commonly found at low water mark and below, as well as on more exposed shores, and the pendant form, confined to vertical rock surfaces in sheltered

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localities.

The species has been found to be more morphologically complex than had previously been thought, and branches of specialised function, responsible for the form and compactness of the plant as well as its ability to bind sand, can be recognised.

The species has been cultured under controlled conditions in order to provide a continuous supply of material suitable for cytological examination and to enable the investigation in vitro of the life-history. It has been observed that under certain conditions of culture which promote vigorous vegetative growth sporangial degeneration occurs, and preliminary experiments have shown that if the cultures are screened with solutions of various dyes this degeneration may be prevented. This suggests that certain inhibitory wavelengths of light are present in the incident radiation, possibly wavelengths in the region of 515mu since eosin yellow which absorbs strongly in this region has been shown to be effective in preventing degeneration.

The germination of the tetraspores has been followed in culture. The microscopic plants which they produce are dioecious and bear sexual organs similar to those of other Florideae. Although these plants remain healthy for many months fertilisation does not appear to occur readily under the conditions of culture and the form of the carposporophyte is consequently unknown. It is of interest that in external form the plants are similar to certain species of Acrochaetium, though the chromatophore is of the type characteristic

of R. floridulum.

Using acetocarmine as a nuclear stain the number of chromosomes in the cells of the tetrasporophyte has been shown to be in the region of 20, and meiosis has been observed in the formation of the spores. The nuclear cycle is therefore diplohaplontic.

The life-history may be of an unusual and unrecorded type involving a diplohaplontic nuclear cycle and a trimorphic somatic cycle. If the discovery of the carposporophyte confirms the existence of such a cycle R. floridulum can be placed close to that group of algae having a similar nuclear cycle together with a diphasic somatic cycle (in which the tetrasporophyte and the gametophyte are morphologically similar). It is possible that either of these life-histories represents the evolutionary precursor of the other.

Rhodochorton purpureum.

R. purpureum has been described by earlier authors as occurring in a number of ecological forms over a wide range of ecological conditions. The present study has confirmed the ecological and morphological adaptability of the species.

The forms studied in the field have been cultured in the laboratory under conditions similar to those developed for R. floridulum, and it has been observed that under constant and identical conditions the forms undergo morphological modification such that the original differences between them are progressively reduced. The result of these changes is such that smaller forms, e.g. forma purpureum and globosa develop characters similar to the

larger forma intermedium. The smaller forms may therefore be considered to be ecologically stunted rather than genetically determined. Since it has been observed that features such as cell length and breadth, branching and the presence or absence of tetrasporangia may be influenced by culture, these features appear to be of little value as taxonomic characters within the group.

The chromosome complement of the somatic cells of the tetrasporophyte has been shown to be in the region of 20, while that of the spores is half this number. It seems likely, therefore, that reduction division takes place in the sporangium and that the nuclear cycle is diplohaplontic.

Only the early stages of spore germination have been seen and consequently the nature of the plants to which they give rise is unknown. It seems likely however that they will be morphologically dissimilar to the tetrasporophyte so that the life-cycle may be similar to that of R. floridulum.

Developmental and Cytological Studies in the Rhodophyceae.

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Contents

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Introduction.

General Introduction.

There are many examples, especially in northern waters, of marine algae which are, or which appear to be, represented by only one phase in their life-history, frequently the tetrasporophyte. Two such algae are the commonly occurring members of the genus Rhodochorton: R. floridulum (Dillwyn) Naeg., and R. purpureum (Lightf.) Rosenvinge.

The present investigation was undertaken in an attempt to elucidate the life-history of these two species by determining: (1) the chromosome number of the tetrasporophyte; (2) whether or not reduction division takes place during the formation of the tetraspores; (3) the nature of the phase produced by the germinating spores.

As Krishnamurthy (1959) has pointed out, the investigation of the life-history of an alga should comprise two sections: firstly information concerning the sequence of morphological or somatic phases, and secondly information regarding the number and sequence of the related nuclear phases. In addition Drew (1955) has drawn attention to the need for information concerning the relation of the plant to its environment.

The present study therefore includes the ecology, morphology and cytology of the tetrasporophytes together with the morphology and cytology of the phase produced by the tetraspores.

The taxonomic status of the genus.

The genus Rhodochorton was established by Naegeli (1862) as a member of the Ceramiales and included only the two species mentioned above. In the same paper he introduced a second genus, Acrochaetium, which he

considered closely related to the fresh-water genus Chantransia. As noted by Papenfuss (1945) and others, the characters which he employed to separate the two genera are now known to be untenable and this has resulted in various rearrangements of classification and specific interchanges between the two. Hamel (1925) was the first to consider that the genera might be closely related and he brought them together in the family Helminthocladiaceae, although he admitted that the correct taxonomic status of Rhodochorton would remain uncertain until the discovery of the female organs of reproduction. Drew (1928) merged the two genera under the name of Rhodochorton because she could find no definite characters on which to separate them. Later Kylin (1944) and Papenfuss (1945 and 1947) split the Rhodochorton-Acrochaetium complex into four genera, but their new genera are not identical. As regards Rhodochorton, Kylin (1944) retained it in the Nemalionales while Papenfuss (1945) on the other hand returned it to its original position in the Ceramiales. Other authors, e.g. Feldmann (1945) and Taylor (1957) place the genus in the Nemalionales.

The identity of the species

(1) R. floridulum.

R. floridulum was first described by Dillwyn (1802-1809) under the name of Conferva floridula and transferred to the genus Callithamnion by Agardh (1828) before being incorporated in Naegeli's Rhodochorton where it remained until Papenfuss (1945) transferred it to Chromastrum Papenfuss (later (Papenfuss, 1947) renamed Kylinia Rosenvinge). In the same year Feldmann (1945) included the species in the genus Rhodothamniella Feldmann.

However, most workers, e.g. Dixon (1961), retain the old name by

which it is probably best known, and for this reason this is the name which has been adopted in the present work.

(2) R. purpureum.

The nomenclature adopted in the present work is that proposed by Papenfuss (1945) subsequently adopted by the majority of later authors e.g. Feldmann (1954), Taylor (1957), Lund (1959), and Jorde & Klavestad (1963). In his paper Papenfuss (1945) showed the specific name 'Rothii' by which the species was originally known, to be invalid since it was applied to a species of Conferva by Turton (1806) who proposed the name Conferva Rothii as a substitute for Ceramium violaceum Roth, which is a species of Polysiphonia, and not for Conferva violaceae Roth (1797), which is a species of Rhodochorton in Naegeli's sense. For reasons which Papenfuss (1945) explain, Dillwyn (1802-1809) believed Conferva Rothii (Turton) to be a substitute for Conferva violaceae Roth and later authors adopted Dillwyn's nomenclature. In renaming the species 'purpureum' Papenfuss (1945), in agreement with Harvey (1846) and other older authors, believed that the plants commonly known as Rhodochorton rothii (Turton) Naeg. and Rhodochorton purpureum (Lightf.) Rosenvinge are the same species. This assumption cannot be verified since as Dixon (1959) has shown, that portion of the Lightfoot herbarium containing the type specimen of R. purpureum (Byssus purpurea, Lightfoot 1777) has not yet been traced. However, to avoid confusion, the current terminology has been adopted in this work.

Other species of Rhodochorton have been considered synonomous with R. purpureum:

R. parasiticum Batters (1896), considered synonomous by Jonsson (1902).

R. intermedium Kjellman (1883), considered synonomous by Jonsson (1902), Borgesen (1902), Rosenvinge (1923-24, 1926), and Lund (1959).

R. islandicum Rosenvinge (1900) considered synonomous by Borgesen (1902).

It may be that R. bisporiferum Baardseth (1941) is also synonomous (p. 92).

PART ONE. *RHODOCHORTON FLORIDULUM*

A. The Ecology of Rhodochorton floridulum

Ecology.

An ecological survey of Rhodochorton floridulum was undertaken in order to provide information concerning possible differences in the ecology, morphology and reproduction of plants from different habitats and geographical locations within the British Isles, attention being also paid to the ecology in other parts of the geographical range as reported in the literature.

In this thesis the term association has been used for a community of algae in which two or more species are co-dominant. The term community has been applied when only one species is dominant.

Geographical distribution.

The species has been recorded from the coasts of the British Isles as far north as Shetland (Holmes and Batters, 1892; Batters 1902; Irvine, unpublished). It is also present on the north coast of France as far east as Cap Gris-Nez (Leblond in Hamel, 1925), and on the west coast as far south as Guethary (Sauvageau in Hamel, 1925; Davy De Virville, 1962).

Outside this area the species has been recorded from one locality only, Tristan da Cunha (Baardseth, 1941).

Previous ecological observations.

Previous authors, notably Cotton (1912), Rees (1935) and Blackler (1951), have described the occurrence of the species in a variety of habitats, both in the littoral and the sub-littoral:

1. As a constituent of the underflora of the dominant Phaeophyceae,

occurring under:

Fucus spiralis

Ascophyllum nodosum

Fucus vesiculosus

Fucus serratus

Himanthalia lorea

Laminaria spp.

2. As a constituent of characteristic associations:

It is a common constituent of several characteristic associations, in particular those of Laurencia-Gigartina, and Corallina-Cladostephus. In rocky localities Laurencia and Gigartina, and Corallina and Cladostephus are co-dominants, but as the situation becomes more sandy Laurencia and Corallina give way to Rhodochorton as co-dominant and finally in favourable situations, such as gently sloping sandy beaches with isolated boulders, Rhodochorton becomes the dominant or only species present.

The Rhodochorton community was first described by Cotton (1912) from Clare Island, but has since been reported as common throughout the British Isles typically occurring on sandy beaches with rock-masses or boulders in moderately exposed to sheltered situations. Under these conditions the species reaches its greatest development, carpeting the rock with its typical mounds, which often attain a height of 6 cms., and collectively stretch unbroken over areas which may be of considerable extent.

Frequently Rhodochorton is the only macroscopic species present, more often others are found, for example Cotton (1912) recorded the occurrence of the following:

Cladophora arcta — Ceramium strictum

Polysiphonia fibrata — Ceramium ciliatum.

3. As a constituent of sand pool vegetation.

Cotton (1912) has described the species as occasionally occurring as a member of a sand pool vegetation, carpeting pure sand between isolated rocks in very sheltered regions.

This is doubtless merely an extension of the Rhodochorton community described above.

4. As a constituent of the sub-littoral vegetation.

Both Cotton (1912) and Rees (1935) have reported that in some localities the species can descend into the sub-littoral.

5. As a constituent of cave vegetation.

Cotton (1912) records the occurrence of the species in marine caves, in one case growing with Cladophora rupestris on a rock surface at a point where fresh water exuded from the roof and walls. In the present investigation this habitat has been met with on a single occasion, at Spiggie on the Isle of Shetland, where a rather stunted specimen of 1 cm. in height was found growing on the walls at the mouth of a cave in association with Rhodochorton purpureum (Lightf.) Rosenvinge.

Present investigations.

During the years 1960, 1961, 1962, ecological investigations were carried out on the following shores:

Scotland:

Millport Bay (including Kames Bay), Keppel Bight, and White Bay, on the Isle of Great Cumbrae which lies in the Firth of Clyde.

Ardneil Bay, Ayrshire.

Gruinard Bay, Wester Ross.

Portmahomack Bay, Easter Ross.

Queen's Bay, Lerwick, and Muckle Sound by Loch of Spiggie, Isle of Shetland.

England:

Marsden Bay, County Durham.

Lulworth Cove, Dorset.

Wembury Bay and Braunton Burrows near Saunton, Devon.

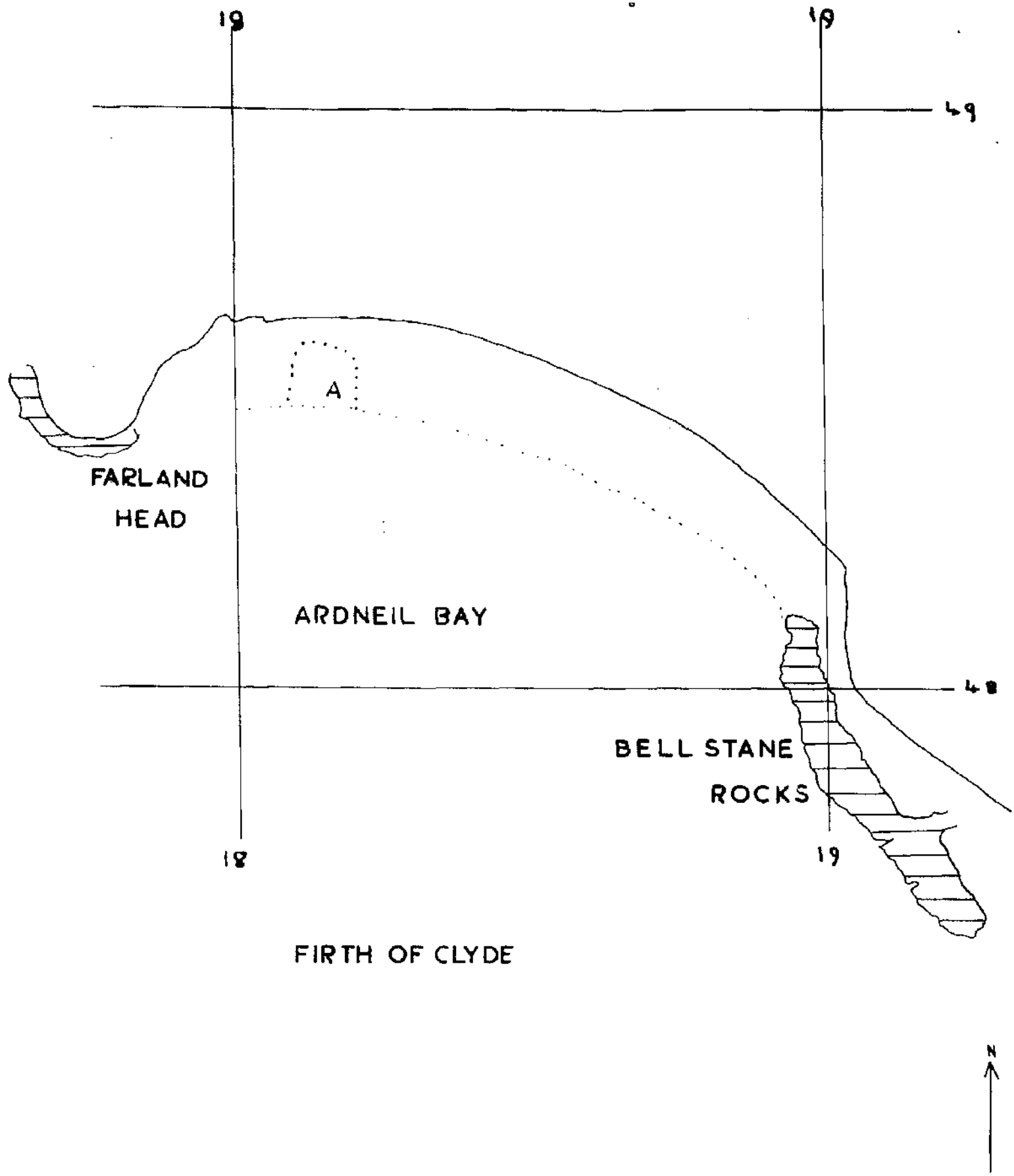
Porthoustock, Kynance Cove, Gwithian Sands, Newquay Bay and Bude Bay, Cornwall.

Of these shores, Ardneil Bay and Portmahomack Bay were examined in greater detail than the others and it was from these localities that the greater part of the material used in the cultural and cytological investigations was obtained.

The ecology of Rhodochorton floridulum in Ardneil Bay (lat. 55° 48.2', long. W. 4° 53.5').

Description of the site.

Ardneil Bay is bounded in the north east by the rocky promontory of


19
19
49
A
FARLAND
HEAD
ARDNEIL BAY
48
BELL STANE
ROCKS
18
19
FIRTH OF CLYDE
N

Farland Head, and in the south west by the Bell Stane Rocks, a sweep of about seven eights of a mile in length.

Rhodochorton is most abundant in the area delimited on the accompanying map (Fig. 1) by a dotted line and marked 'A'.

In this region the upper mid-littoral is composed largely of sand in which occur isolated gravel and boulder patches, while below this level, extending into the sub-littoral, occur rock and boulder fields.

Beyond this area the beach is largely composed of sand, and the boundaries of the boulder patches are in a constant state of flux as sand is deposited and removed by successive tides.

The average gradient throughout the area is 1/46 feet.

Exposure is moderate in the region investigated but increases towards Farland Head.

The algal vegetation.

The dominant alga throughout the littoral region wherever rocks occur is R. floridulum, and the community which it forms extends in places from a little below M.H.W.N. into the sub-littoral.

The number of common associates is not great; Enteromorpha compressa, Ulva lactuca and Porphyra purpureum (Roth) Ag. being the most commonly occurring species between M.H.W.N. and M.T.L., below M.T.L. Fucus serratus is occasional on the larger boulders and becomes more numerous in places.

Near low water mark, isolated patches of a Rhodochorton-Gigartina stellata-Chondrus crispus community occur, in which Rhodochorton is always dominant. Occasionally Cladostephus spongiosus appears in this

community, but its development is never as extensive as is found at the same level in Portmahomack Bay.

Throughout the greater part of the littoral region Rhodochorton forms the typical mounds 2-4 cms. high on low lying rocks, but on the larger boulders its appearance is rather different (Plate 1B).

At and below low water mark, the species occurs beneath Laminaria digitata and Alaria esculanta, and occasionally forms a community with R. purpureum on the sides of large rock masses.

At this level the plants form a compact, low-lying mat quite different in form from the mounds found at a higher level. Similar mats have been observed at other sites and appear to be the result of increased wave action.

The Rhodochorton tufts are generally heavily epiphytised by microscopic algae including Acrochaetium secundatum, Bangia fusco-purpurea, a variety of diatoms, as well as young stages of many algae including Ulva lactuca, Enteromorpha compressa, Porphyra purpureum, Cladophora spp. and Ectocarpus spp.

The tufts also provide anchorage for the majority of the macroscopic species present, the number and types varying according to the time of year.

Apart from those already mentioned, the following have been recorded in late spring:

Chorda filum

Scytosiphon lomentarius

Phyllitis fascia.

Apart from the difference in form of the plants from the upper and lower levels one other difference was recorded; the plants growing above M.T.L. were yellow-red compared with the deeper red of those below this level.

There appears to be no time lag between the onset of tetrasporangial formation in the plants at different levels of the shore.

As noted above, the boundaries of the boulder fields are in a perpetual state of flux as the sand shifts daily under the influence of the waves. This sand movement is responsible for two things:

(1) Areas of the Rhodochorton community may be completely covered by sand up to a depth of several inches, at least for the duration of low tide, and perhaps for even longer periods.

(2) The apical regions of Rhodochorton are rarely completely exposed to sunlight during the period of low water, since they are normally covered with a fine layer of sand.

Cytological examination was made of material from all levels, but the material for cultivation was taken from the lower mid-littoral.

Plate 1.

A. A general view of the lower boulder field at Ardneil Bay, looking towards Farland Head. March 1961.

B. A close-up view of the boulder marked with an arrow in 'A', showing the tufted form of Rhodochorton floridulum.

A

B

Figure 2.

Diagram of Portmahomack Bay.

B is the west wing.

A is the east wing.

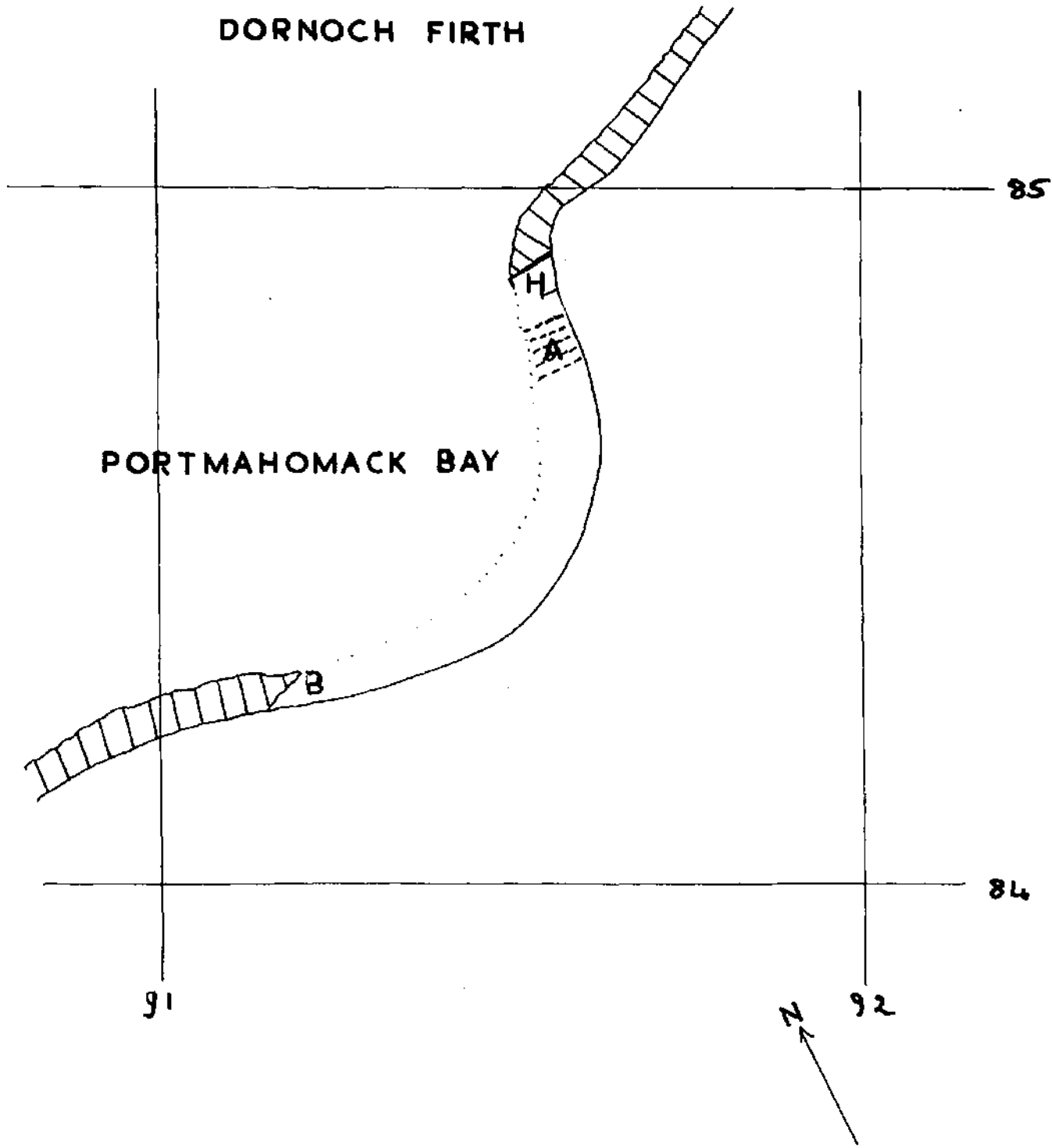
H is the harbour.

The straight lines represent the boundaries of the Ordinance Survey 1 kilometer grid square and are numbered accordingly.

The regions delimited by a continuous black line and crossed by parallel black lines indicate rock masses.

Broken parallel lines indicate a rock mass with parallel ribs.

The area delimited by a fine dotted line represents the region uncovered by the tide consisting mainly of sand.


DORNOCH FIRTH
85
H
A
PORTMAHOMACK BAY
B
84
91
92
N

The ecology of Rhodochorton floridulum in Portmahomack Bay, Easter Ross (lat. 57° 50', long. W. 3° 49').

Description of the site.

The bay is bounded in the north east by the harbour rocks and in the south west by the Balnabruach rocks, a sweep of about five eights of a mile in length (Fig. 2). The greater part of the littoral zone is composed of sand with occasional boulders, but at either end, rock masses and boulders occur between M.H.W.S. and M.L.W.S., stretching in places into the sub-littoral.

The beach is gently shelving with an average fall of one foot in thirty. The exposure is moderate at the Balnabruach and decreasing towards the harbour.

R. floridulum occurs on the rock masses at the east and west wings, on the isolated boulders in the central sandy region, and on rocks in the harbour.

The west wing.

Rhodochorton is the dominant alga on the sandstone ribs which run from above M.H.W.S. to the sub-littoral and divide the sandy bay from the rocky Balnabruach promontory (Plate 2A). The species occurs from a little below M.H.W.N. to the sub-littoral. At the top of its range it is confined to the lower portions of the rocks, the tops of which are capped by a luxuriant growth of Enteromorpha compressa (Plate 2B); below mid-tide level however it covers the rocks with a dense carpet up

Plate 2.

A. A general view of the rock ribs on the west wing, looking towards the Balnabruach promontory.

B. A close-up view of the rock marked with an arrow in 'A', showing Enteromorpha (E) and Rhodochorton (R).

A.

B

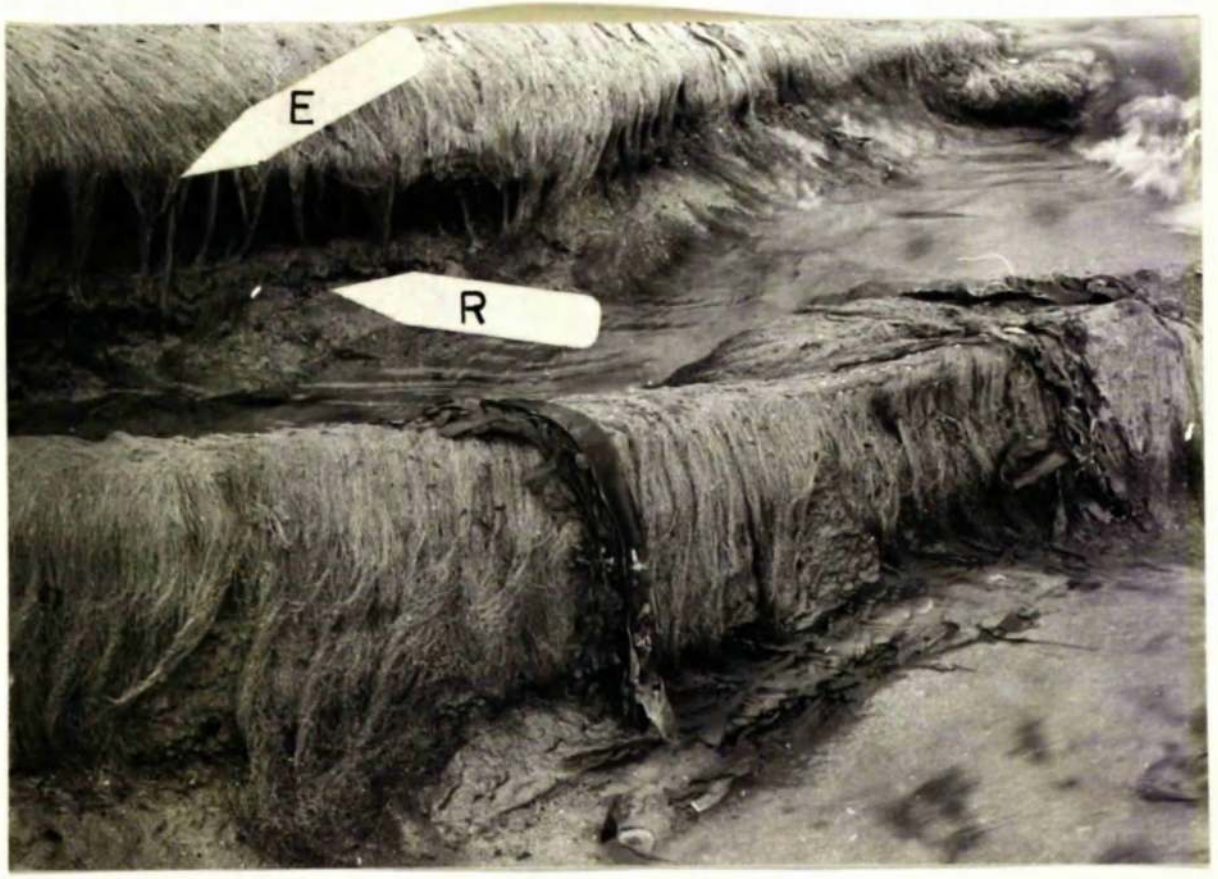


Plate 3.

General view of the Rhodochorton community on the slabs of the Balnabruach promontory showing the low-lying form of the plant. Portmahomack April 1962.

to 4 cms. thick. In this region the only other macroscopic alga is Porphyra umbilicalis f. laciniata which occurs locally. The carpets are however heavily epiphytised by diatoms, blue-greens and Acrochaetium secundatum. At low water Rhodochorton occurs under Laminaria digitata and L. saccharina and goes down into the sub-littoral. At the same level on the flat-topped rocks of the Balnabruach promontory the plant has the low-lying form noted at Ardneil Bay (Plate 3).

The east wing.

While in the west wing Rhodochorton is the dominant species throughout the greater part of its vertical distribution, in the east it is found mainly as a constituent of the underflora of the dominant Phaeophyceae which here occupy the typical zones. The species occurs under Fucus spiralis (Plate 4A), Fucus vesiculosus, and Fucus serratus (Plate 4B and Plate 5 A&B), being particularly abundant under the last named. Frequently at low tide level communities of Rhodochorton-Gigartina, and Rhodochorton-Cladostephus occur in which Chondrus crispus and Rhodymenia palmata are often present, though in small amounts.

On the smaller isolated rocks the Rhodochorton community similar to that of the west wing appears, frequently pure, but often with Enteromorpha compressa, Sphacelaria radicans and Porphyra purpureum as associates. Between these rocks a sand pool vegetation occurs containing R. floridulum in small amount.

At all levels Rhodochorton is heavily epiphytised with diatoms and Acrochaetium secundatum.

Plate 4.

A. shows a portion of a rock with Rhodochorton under Fucus spiralis with Porphyra umbilicalis and barnacles.
Portmahomack July 1961.

Rhodochorton is indicated by the arrow.

B. shows a rock with Rhodochorton in typical mound form with Fucus serratus and Gigartina stellata.
Portmahomack July 1961.

A

B

Plate 5.

A. shows a low-lying Rhodochorton mat dominated by Fucus serratus and Enteromorpha compressa.
Portmahomack July 1961.

B. shows a low-lying mat of Rhodochorton growing on the vertical surface of a rock dominated by Fucus serratus.
Portmahomack July 1961.
Rhodochorton indicated by the arrow.

A.

B.

The harbour.

The substrate in the harbour is one of muddy sand with isolated rocks. Rhodochorton extends from the Fucus spiralis zone to the extensive Chorda filum belt at low water. Rhizoclonium implexum and Vaucheria thuretii are frequent associates. A similar muddy sand community was observed at Bude Harbour, Cornwall.

The material for cultivation was collected from the rocks on the west wing.

Comparison with other sites.

Three of the other sites visited showed a well developed vertical distribution of the species:

(1) Millport Bay (including Kames Bay).

This can be divided into two distinct regions: (a) the rock masses where the typical Phaeophycean zonation is established, and (b) the open sandy regions such as Kames Bay.

The vertical distribution in (a) is the same as in the corresponding region of Portmahomack Bay; Rhodochorton occurs as a constituent of the underflora of the Phaeophyceae from Fucus spiralis down to the Laminarians and beyond into the sub-littoral. In (b) the sandy beach Rhodochorton community occurs with Gigartina stellata, Rhodymenia palmata, Ulva lactuca, Enteromorpha compressa and Porphyra umbilicalis f. laciniata as occasional associates.

Plate 6.

A. A general view of Wembury Bay showing the rock masses and the sandy region.

B. A close-up of the rock indicated by the arrow in 'A' showing: Typical mound form of Rhodochorton (R)

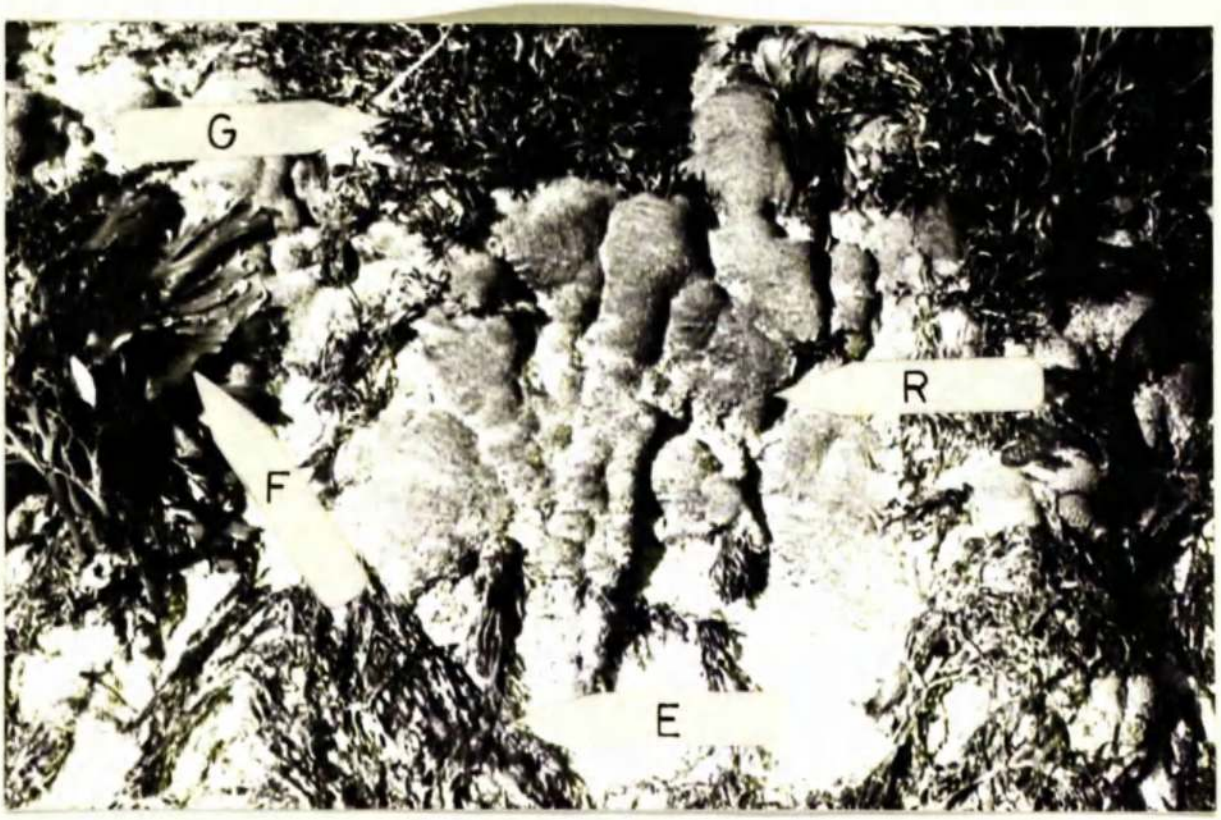
Gigartina stellata (G)

Fucus serratus (F)

Enteromorpha compressa (E)

A.

B.



(2) Wembury Bay.

This too is divided into rock masses and open sandy regions (Plate 6A). In both the distribution is as described for similar sites in Scotland, that is, on the rock masses the species occurs from the Fucus spiralis zone down to low water, while on the open sandy regions it is common on isolated boulders down to low water mark from a little above M.T.L., with Porphyra umbilicalis, Ulva lactuca, Enteromorpha compressa and Gigartina stellata as associates.

(3) Newquay Bay.

The distribution in Newquay bay is similar to that at Wembury, but the development of the Rhodochorton covering is much more extensive, being often several square yards in extent.

General observations.

Rhodochorton floridulum was found to be typical of sheltered or moderately exposed sand beaches, and often occurs on rock masses adjacent to such beaches. At Porthoustock, however, an exception to this was observed; here the beach is composed of loose shingle with isolated boulders and rock masses (Plate 7A), and in the crevices on the larger rocks stunted (1 cm. high) plants of R. floridulum were found. This is the only occasion on which the species was found growing in the absence of free sand on a substratum of non-sandstone rock.

The alga was found to be scarce or absent from shores more than moderately exposed, e.g. Gwithian Sands, Cornwall, where it occurs

Plate 8.

A. A general view of the lower portion of the littoral region at Keppel Bight showing the localised occurrence of the mac form of Rhodochorton typical of exposed areas.

B. shows the Rhodochorton community in close-up. In this localised area it is the only species present.

A.

B.

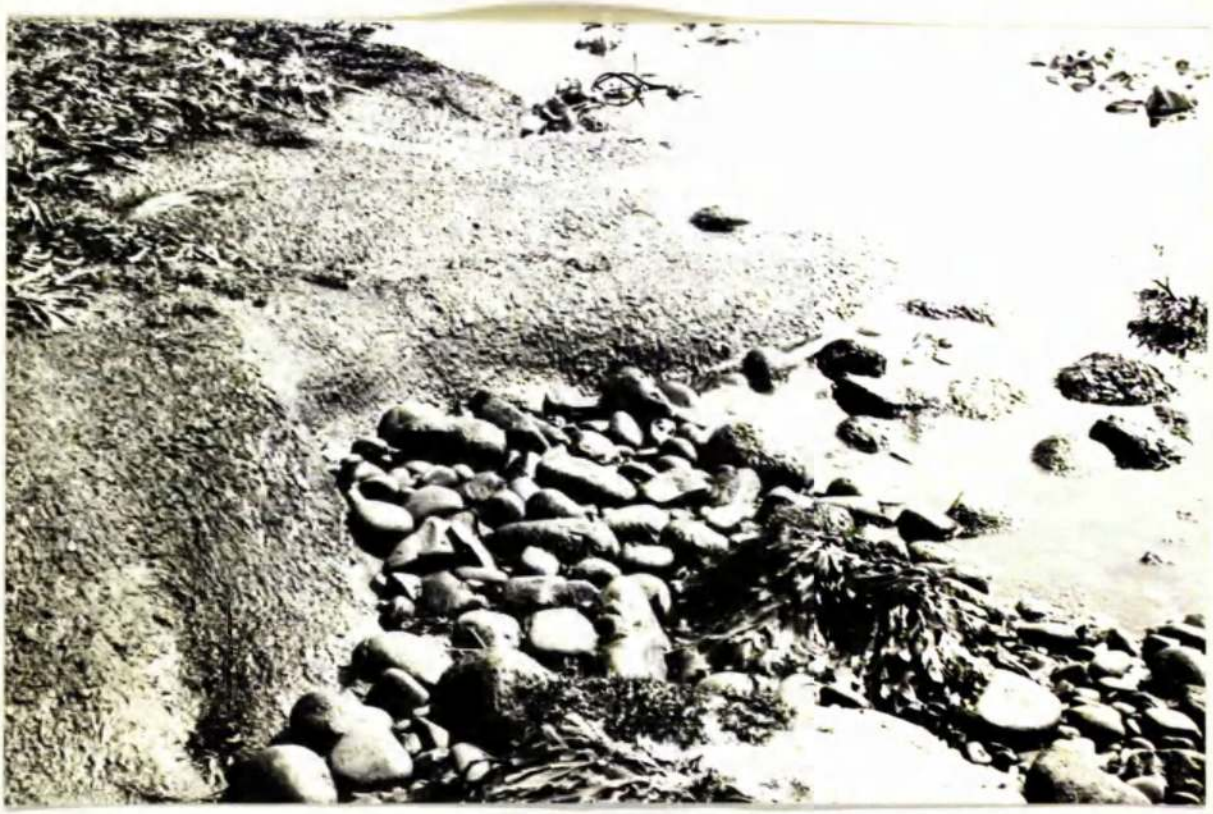
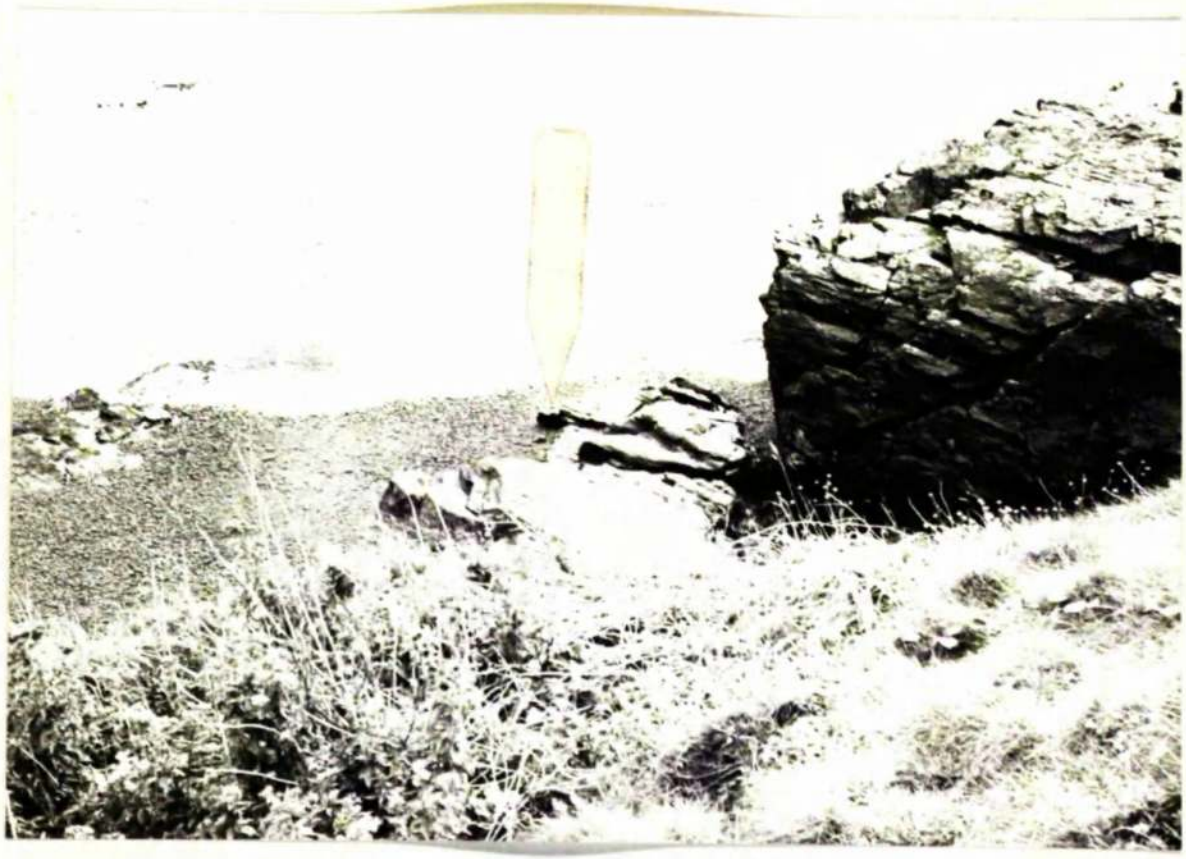


Plate 7.

A general view of a portion of the shore at Porthoustock showing the gravelly nature of the beach. On the rock marked with an arrow a small amount of stunted Rhodochorton was found. September 1962.



infrequently in low mats up to 1 cm. in height on the landward side of large rock masses in the sand. Similarly at Keppel Bight on the Isle of Cumbrae the species is absent from the upper part of the shore, occurring at and below the level of the Fucus serratus zone, although in the more sheltered neighbouring Kames Bay it extends downwards from the Fucus spiralis zone. As at Gwithian Sands, the species has the form of a flat cake, closely applied to the rock surface (Plate 8) and differing considerably from the typical mounds found for example at Wembury (Plate 6B).

This low-lying form was encountered also on the more exposed rocks at the Balnabruach promontory, Portmahomack Bay (Plate 3) as well as at low water in Ardneil Bay.

R. floridulum was found to be absent, at least from the upper part of its normal range, on shores where the insolation is high, except where shade is provided by rock masses or larger algae.

Thus, at Gruinard Bay in Wester Ross, where conditions are similar to Ardneil Bay, the species is absent from almost the entire littoral region, becoming established only at and below low water, while the upper boulder fields are colonised by its normal associates Porphyra and Enteromorpha.

At Kynance Cove in Cornwall (Plate 9A) the plant is present only on those rock surfaces which are shaded for the greater part of the day, there being a well defined boundary between the shade and light sides. R. floridulum is here absent from the smaller rock masses isolated in the sand and is confined to the vertical surfaces of the large rock masses. In this position it hangs down in a loose mat in close contact with the

rock surface (Plate 9B), a form quite different from either the typical or the compact mats of exposed localities.

Conclusions.

The present work confirms previous observations that Rhodochorton floridulum occupies a wide vertical range on the shore. It was found to be absent from the more exposed shores although it can tolerate a certain degree of exposure. The greatest degree of development is reached on rocks in the mid-littoral region of gently sloping sand beaches.

The species was found to be absent from highly insolated shores unless shade was provided by rock masses or larger algae.

The nature of the habitat was found to exert an influence on the form of the plant and previously unrecorded variations from the typical form were observed.

There appear to be no differences in the ecology of the species within the range of its British distribution.

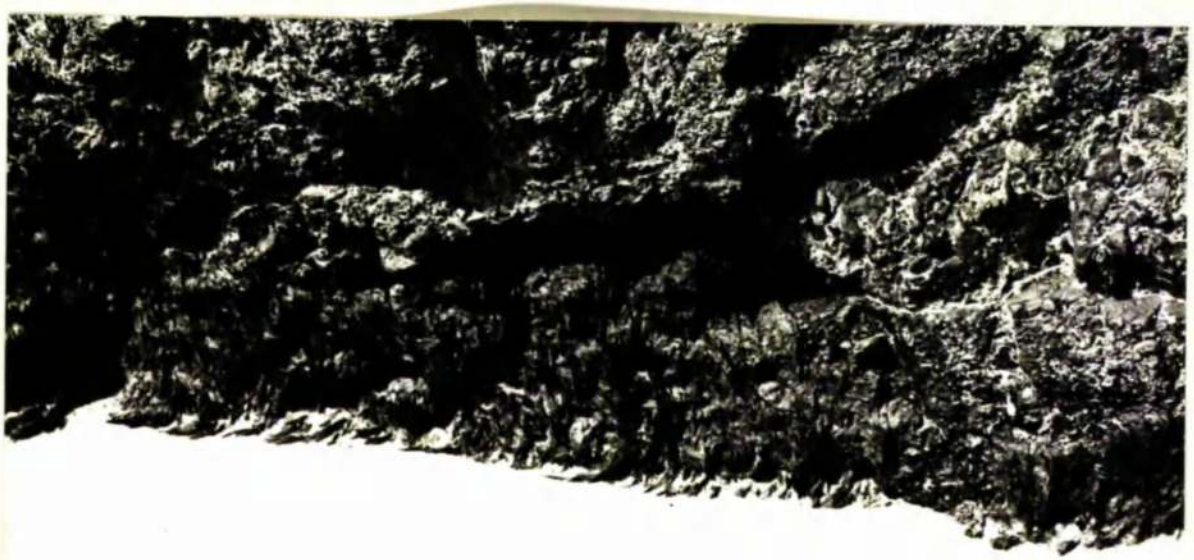
Plate 9.

A. General view of Kynance Cove from the cliff-top showing the form of the rock masses. September 1962.

B. is the portion of 'A' indicated by the arrow, in close-up, showing the rope-like form of Rhodochorton on the vertical rock surface.

A.

B.



B. Morphology.

1. The Morphology of the Tetrasporophyte.

The morphology of the tetrasporophyte.

Rhodochorton floridulum is a simple uniaxial, heterotrichous alga of the type designated by Fritsch (1945, vol. 2) as representing the primitive condition in the Florideae. It typically forms dense globose, fastigiate tufts from 2-6 cms. in height on sand-covered rocks on gently sloping sandy shores.

The prostrate system:

The prostrate system is composed of a mat of interwoven, creeping filaments, sparingly to frequently branched, the branches arising from the middle, occasionally from the anterior or posterior end of the cells.

The size and shape of the cells is extremely variable, frequently they are barrel-shaped, measuring between 18-30u long, by 15-20u broad; often they are cylindrical with dimensions similar to those of the cells of the erect system.

Due to the fact that the cells are in very close physical association with the substrate they are often irregular in form (Fig. 3). The association appears to be entirely physical since there is no evidence to suggest that the cells secrete adhesive substances, although the spores at least have been shown to do so.

The growth of the prostrate filaments is by the activity of an apical cell which remains in close contact with the substratum throughout the life of the plant.

Branching:

Branching occurs most frequently in the region immediately behind the apex, the laterals being initiated from the daughter cells before the nucleus enters the interphase condition. Branching can however occasionally occur in the more mature regions distal to the apex.

The branches produced are of two types: (Fig. 4).

(a) Horizontal.

(b) Erect.

Both may arise from a single cell, although more commonly one type only is produced. A maximum of three branches arising from one cell has been observed, and in such instances only two lie in any one plane.

(a) The horizontal branches.

The horizontal branches are apparently of unlimited growth, and in older specimens cannot be distinguished from the parent axis; they branch in a similar manner and there is no difference in cell size.

(b) The erect branches.

The erect branches are produced at irregular intervals and usually at a slightly later stage than the prostrate branches, although in some instances it has been observed that several erect filaments have been produced before the further initiation of prostrate branches.

The first visible stage in the production of an upright filament is the appearance of a slight conical protuberance in the middle, occasionally from a point nearer the anterior or posterior end, of the dorsal surface of a cell. This protuberance elongates until, when it

Figure 3.

Portion of the prostrate system showing: the distortion of the cells due to contact with the substratum; the varying position of origin of the erect filaments, and the shape of the cells.

'a' indicates the collar formed around the filament following regeneration of the apical cell.

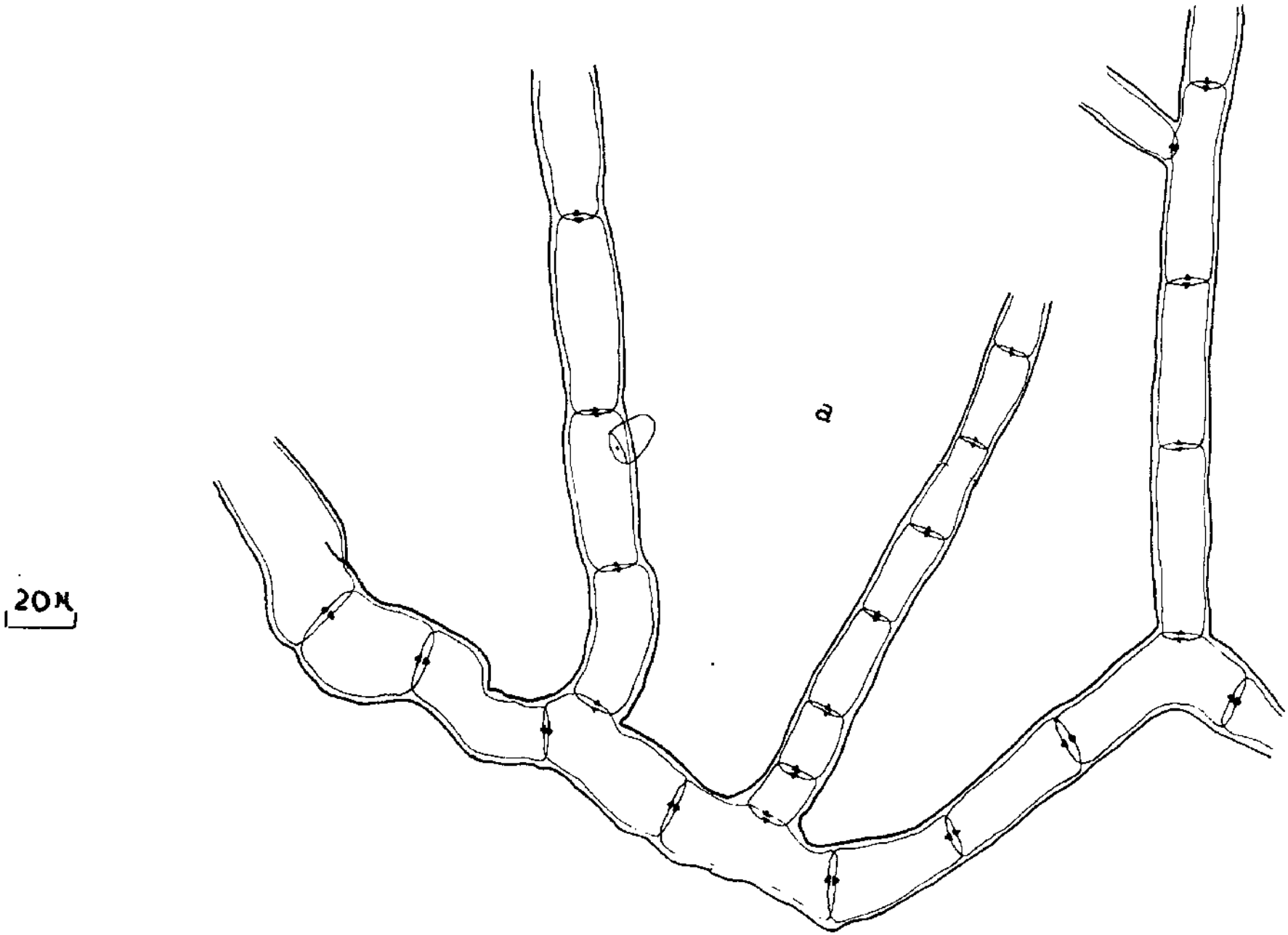
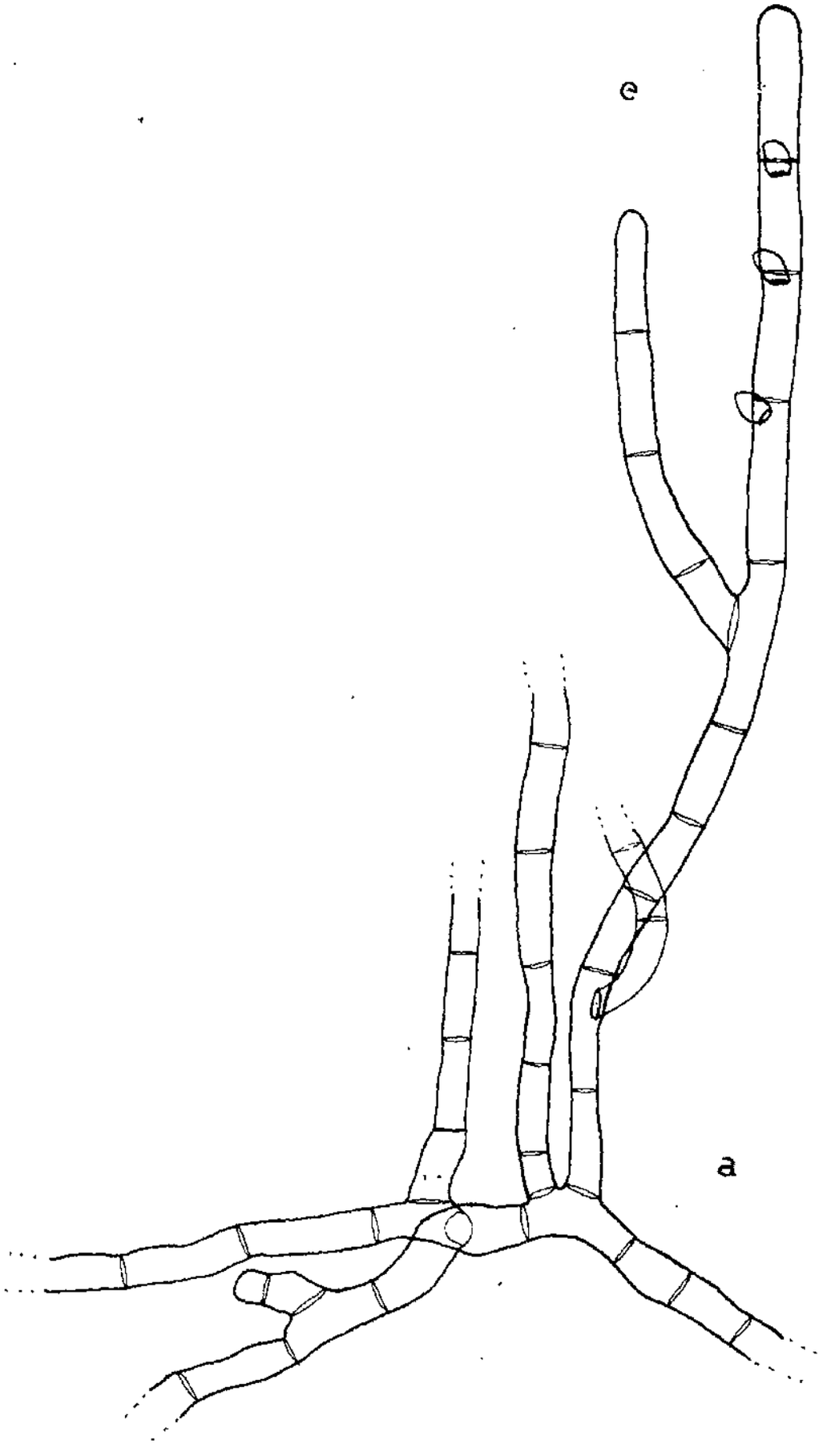

a
20μ

Figure 4.

A small portion of a young plant showing: (1) prostrate and erect filaments; (2) the production of two upright filaments from a single cell (a); (3) the initiation of laterals from the cells immediately behind the apex of the erect filament (e).

Broken lines indicate that portions of the plant have been omitted.



20N

The size of the cells is extremely variable even in a single filament, but they are, with few exceptions, always cylindrical. They vary in length from 38-129u, and in breadth from 15-31u, the ratio of length to breadth normally being in the range of 2-5/1 with the majority of cells having a ratio of over 3/1.

These figures are similar to those published by Hamel (1925) for the species on the coasts of France.

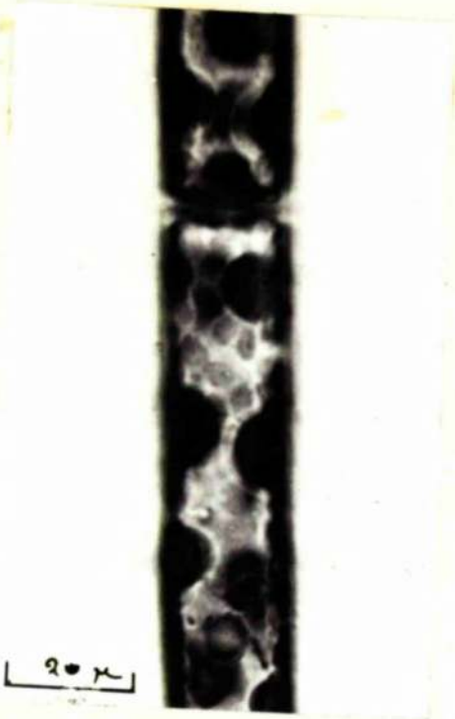
Cell content:

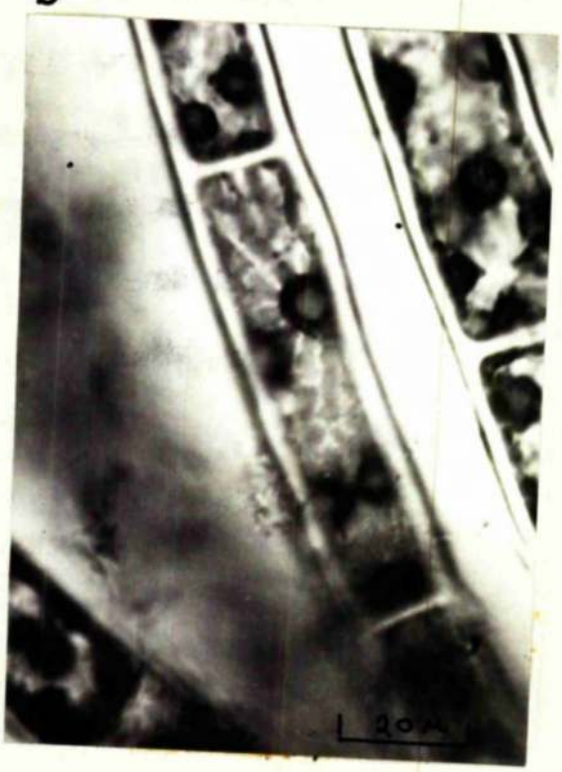
Each mature cell has a large central vacuole surrounded by a layer of cytoplasm in which is embedded a variable number of pyrenoids, each the centre of a star-shaped chromatophore (Kuckuck, 1897).

In wild material the chromatophores of the cells above the level of the compact sand layer are red in colour, while those below the sand, in conditions of permanent shade, are a dull yellow. This latter colour persists in unwashed material in culture, but is converted to the normal red when the sand is removed.

The number of chromatophores (and hence pyrenoids) per cell of the main filaments can range from 5-20. It is not constant even between daughter cells, and bears no strict relationship to the size of the cell.

PLATE 10





Photomicrographs showing variation i

The smaller cells of some laterals however may contain but one. This observation does not agree with that of Papenfuss (1945) who stated that each cell contains four chromatophores.

The shape and the degree of development of the chromatophore rays is variable within the cells of one filament. Plate 10 illustrates three such variations; in (a) the rays are reduced to small disc-like lobes around the margin of the pyrenoid.

in (b) they are greatly elongate, having the form of ribbons with indented margins.

in (c) they are elongated and lobed.

The rays are well developed in shaded material so that the margins lie closely together, whereas in brightly illuminated plants they are much reduced.

Staining fresh material with dilute iodine solution shows that the cytoplasm contains a large number of small granules of Floridean starch. These granules appear to be absent from the chromatophores themselves.

The only other conspicuous content of the cell is the centrally placed nucleus.

Branching of the erect system:

The erect system is generally much-branched, especially in the apical regions of the typical mound form.

As in the prostrate system, the production of laterals is generally confined to the newly formed cells immediately behind the apex, only

rarely are laterals produced from the older cells of the filaments.

The typical erect branches generally arise from a point near the top of the mother cell; whereas the downward-growing laterals arise from the distal end.

The examination of young plants (Fig. 6C) reveals that branching is irregular, sub-secund and pseudo-dichotomous; true dichotomous branching has never been observed in this species.

The original erect filament is soon equalled in length by the erect or erect-adpressed laterals and, since there is commonly no difference in cell dimensions, is indistinguishable from them.

In plants with prolific basal branching a thread of interwoven filaments is commonly formed around the main axis during the upward growth of the laterals, and since they later branch about the same level, the laterals also tend to become interwoven, so that a pseudo-axis with pseudo-laterals is formed.

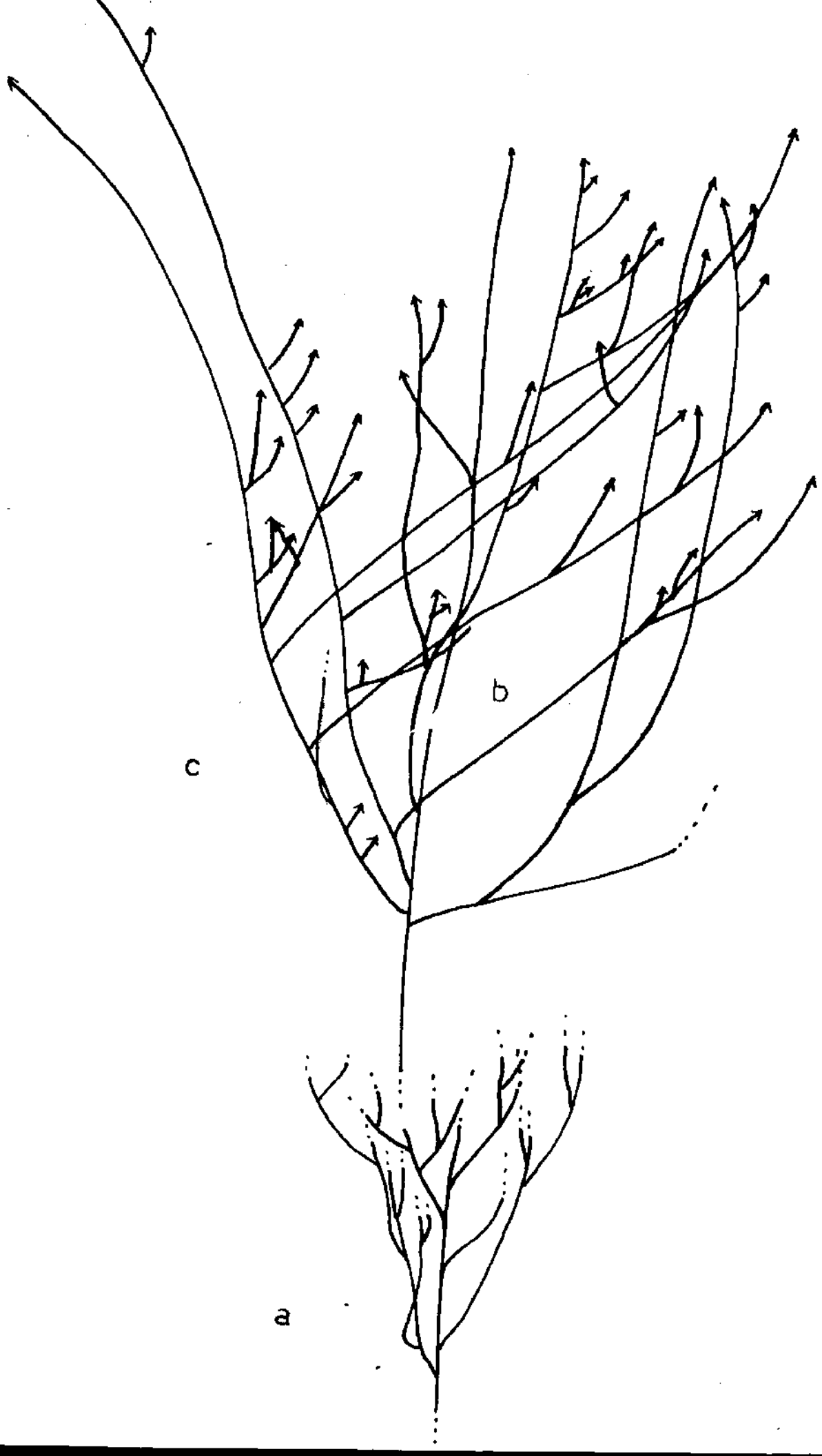
The formation of these threads is aided by the production of laterals which grow downwards, producing both erect and downward growing secondary branches which become entangled with the normal erect laterals produced at a lower level. In contrast, in plants in which basal branching is infrequent, the majority of the erect filaments are free throughout their length.

In the typical form of Rhodochorton floridulum the majority of the filaments, both primary, and laterals of several orders, branch abundantly in the sub-apical regions so that each filament gives rise within a short

Figure 5.

Diagram of the pattern of branching in the apical region of a filament of the typical form of the plant showing the abundant production of laterals and that they almost all lie in the one plane; a, b, and c are examples of those which do not.

Broken lines indicate that a portion of the filaments has been omitted.


c
b
a

distance to a 'fan' of filaments as shown in figure 5. The branches of any one filament are generally confined to one plane, but the plane of branching of neighbouring filaments is seldom similar, so that the branch systems of one filament grow into the systems surrounding it, becoming entangled with them. It is these interlocking branch systems which are responsible for the formation of the mounds and tufts which are typically found on gently sloping sandy beaches. The mounds are made more compact by the formation of special branches which will be described later.

The pattern of branching is different in the plant when it grows under exposed conditions where wave action is heavy. As previously remarked (Ecology), under these circumstances the mounds are absent and the plant instead forms a low-lying compact mat. This is largely due to the absence of abundant and localised apical branching, laterals being produced more or less evenly over the length of each erect primary filament so that a uniform sward is formed. The compactness of the turf is the result of the laterals becoming entangled with each other.

Three types of branches which aid in the process of entanglement have been found:

1. The spreading branch.

Whereas the erect branches which have been described arise from the parent filament at an angle equal to or less than 45°, the angle of initiation of a spreading lateral is greater than 45°, so that the plane of growth is more horizontal than vertical. A similar plane of growth can be achieved if after initiation at a normal angle to the parent

Figure 6.

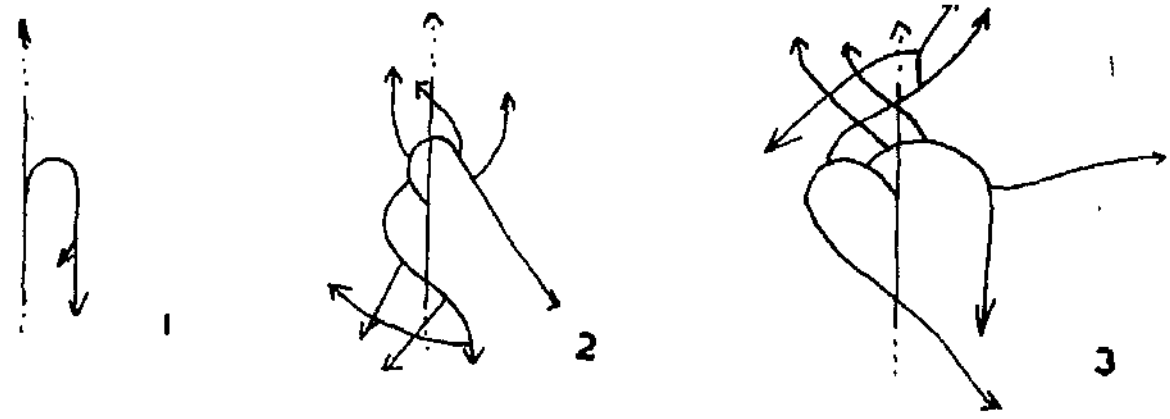
A. 1-3. Hook branching showing several types of secondary lateral.

B. 1-3. H-branching, showing several types of secondary lateral.

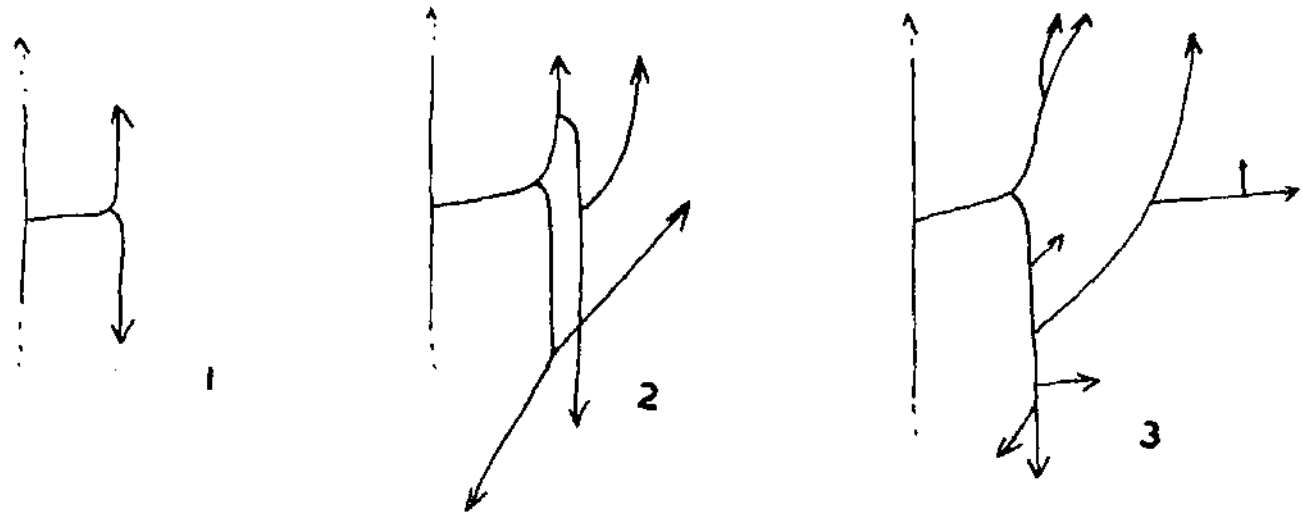
C. Diagram of branch system of a portion of a young plant, showing how the laterals will tend to grow in between one another.

In all cases arrows indicate the direction of growth; broken lines indicate that a portion of the filament has been omitted.

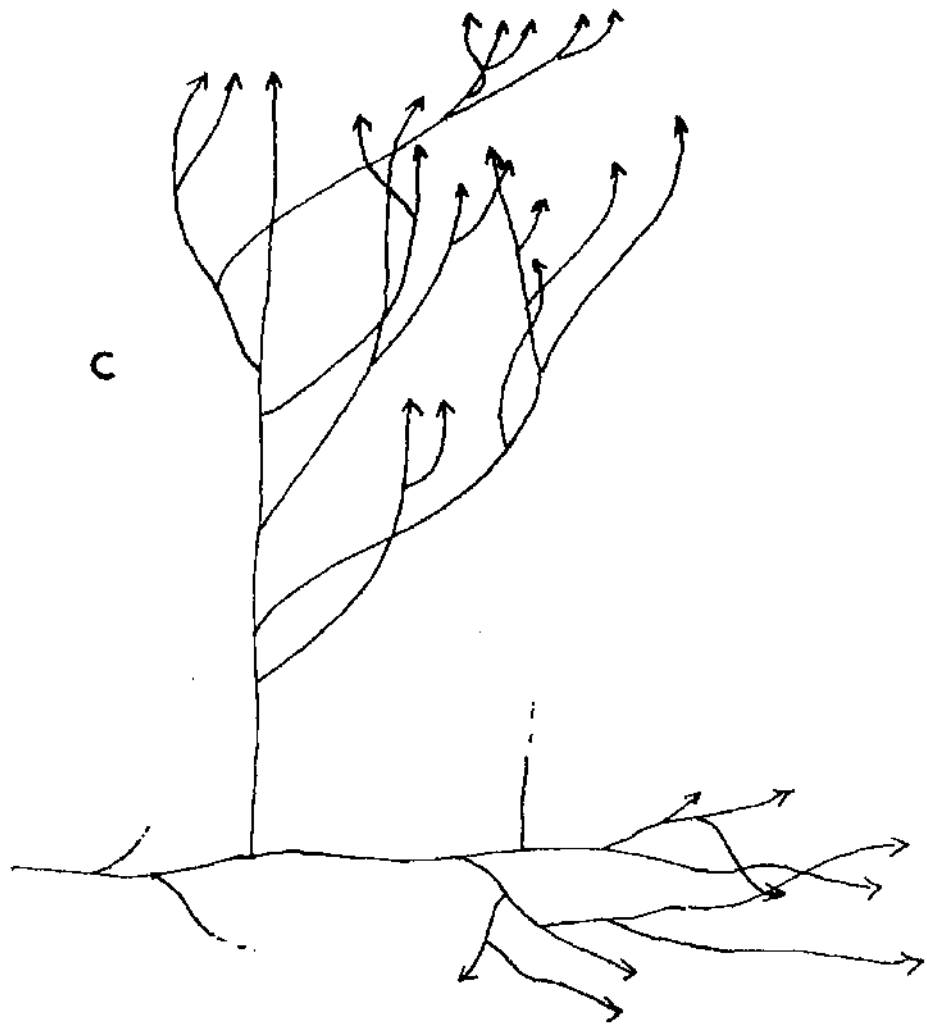
A



B



C



become entangled with them.

2. The hook branch (Fig. 6 A/1, 2, 3).

The angle of initiation of a hook branch is normal, but after a short period of growth in the plane of initiation a strong growth curvature in a downward direction takes place, stronger than in the case of the spreading lateral, so that, depending on the duration of the stimulus, the subsequent growth of the lateral may be away from, parallel to, or across the parent filament.

Hook branches normally bear secondary laterals which may be of several types: (a) Downward-growing.

(b) Erect.

(c) Spreading.

(d) Hook.

3. H-laterals (Fig. 6 B/1, 2, 3).

The angle of initiation of an H-lateral is generally greater than 45°, but after a short period of growth strong growth-curvature in an upward direction takes place, generally within the length of one cell. This same cell also produces a downward-growing lateral, and both filaments grow parallel with the parent axis, at least for a short distance,

so that an H-shape is formed. Both arms of the H normally branch abundantly.

The effect of these branches which, with the exception of 1., are most numerous in the lower regions of the thallus, is to bind the primary upright filaments and their erect branch systems into a compact mass. It is due to their presence that the species can bind sand particles, since without them the complex system of interlocking filaments in which the particles become lodged would be absent.

The closely adpressed filaments also form a very efficient capillary system enabling the plant to retain sufficient moisture to prevent desiccation, even when exposed for several hours to full summer sunlight. This is necessary for the survival of the plant, since Biebl (1952) has shown that the minimum humidity which it can tolerate is 88.0%.

It has been found that these laterals are largely absent from samples of plant collected from vertical surfaces, so that in such plants the erect filaments are largely free.

A fourth type of lateral branch of unknown function has also been recorded from all the sites visited:

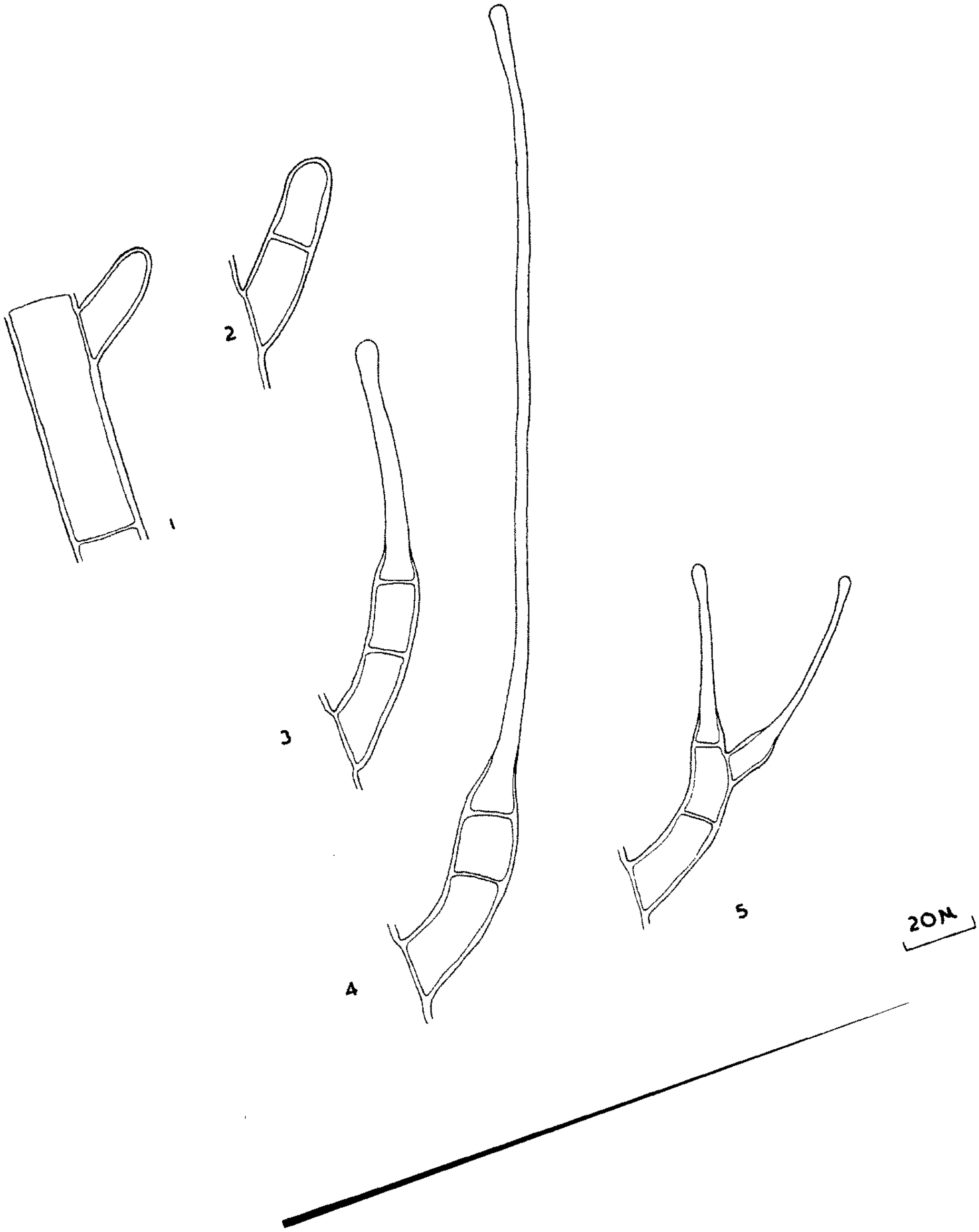
4. Hair-bearing laterals of limited growth.

During the summer, and apparently concomitant with the production of the tetrasporangia with which they are often associated, lateral branches of limited growth are produced in the sub-apical regions of the filaments. They consist of from two to many cells and may be simple or branched. Each apical cell produces one or two colourless hairs of up to 200u in

Figure 7.

1-4 show the stages in the development of a colourless hair.

5 shows an apical cell with two such hairs at an early stage of development.


1
2
3
4
5
20M

length (Fig. 7). These laterals have been recorded in material from all the sites visited and they also develop in culture. Since they are not present at all times of the year, it is assumed that they are eventually shed, in which case the apical cell of the supporting filament may become meristematic.

The tetrasporangial branches.

The onset of tetrasporangial production varies from year to year.

Following a year of good weather with abundant sunshine the formation of tetrasporangia has been found to commence towards the end of June; following a year of poor weather with little sunshine sporangial development has been found to commence in late December. It is not however unusual to find at least a few tetrasporangia present at any time of the year, as reported by Blackler (1955), but there is nevertheless a period of optimum production which varies from year to year and is apparently subject to the influence of the weather.

The development of the tetrasporangia of both R. floridulum and R. purpureum has been described by Harvey Gibson (1891) and a brief description only will be given here:

Prior to the production of the sporangia, the filaments in the upper regions of the plant branch copiously in the manner previously described. These branches then produce short laterals, consisting of one or more cells, from several or all of their cells except the apical cell. The short laterals so produced may bear a solitary sporangium as figured by

Harvey (1846-1851); more often two or more are borne as shown in Figure 8A. Frequently, however, the laterals are longer than one cell and may themselves branch. Eventually further growth is prevented by the production of a sporangium from the apical cell. The formation of the sporangia on the laterals is generally basipetal, and they may be sessile on the cells themselves, or these cells may bear short laterals which may branch again, each branch terminating in a sporangium and bearing sporangia on the adaxial surface of the majority of their cells. Occasionally a cell may produce sporangia on more than one surface so that a cluster is produced. Figure 8B shows a simple cluster.

The development of the sporangia.

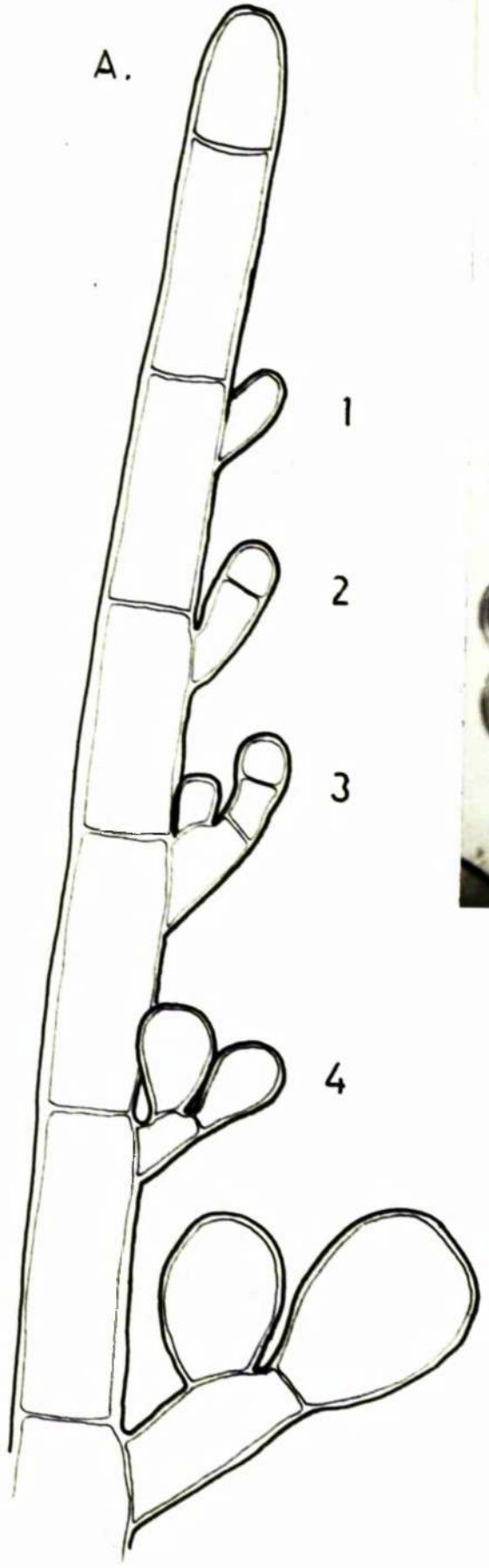
All sporangia with the exception of those borne terminally on a lateral are initiated in a manner identical to that of a vegetative branch, as a conical protuberance arising from a point near the top of the parent cell. This protuberance is cut off by a cross-wall and enlarges to form an ovoid-spherical sporangium, the narrow end of which is directed towards the base. The sporangium enlarges until at maturity it measures about 20-24μ broad and 28-31μ long. It then contains four spores arranged in a quadrate manner.

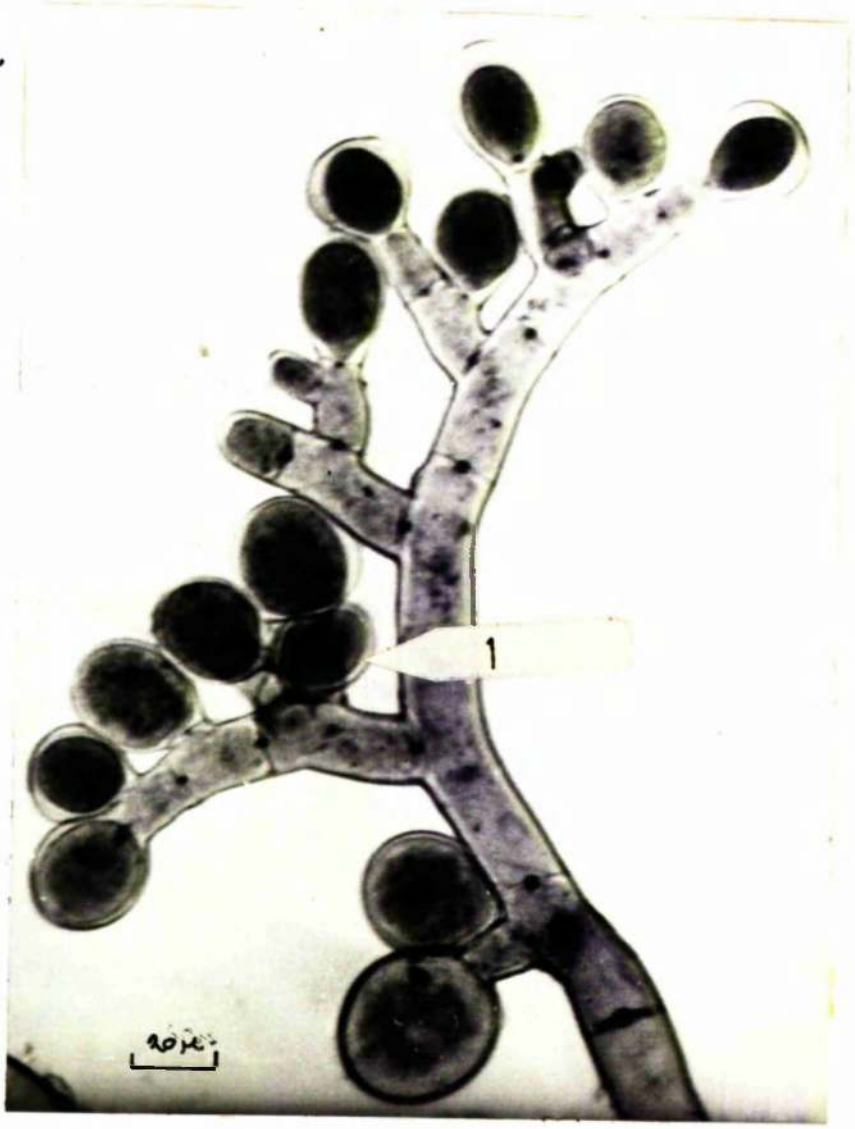
The young uninucleate sporangium is densely filled with cytoplasm, the peripheral layers of which contain a variable number of chromatophores and pyrenoids. Apart from its shape there is therefore little difference between a young sporangium and a vegetative cell, and under certain conditions, e.g. in unscreened cultures, the sporangium can, if the first

Figure 8.

A. 1-5 show early stages in the development of sporangia on simple lateral branches.

B. shows a more complex lateral branch with sporangia lying in more than one plane (1).





20 μ

stage of nuclear division has not taken place, grow out into a vegetative branch differing from the normal only in its swollen base.

The first division of a sporangium is at right angles to its long axis, the septum arising from the inner wall and gradually extending inwards from the periphery. The initiation of the septum may take place before the division of the nucleus so that at late metaphase the nucleus lies in a pore in the septum which is later closed as the daughter nuclei separate. In most cases, however, the initiation of the membrane is delayed until separation of the daughter nuclei has taken place.

The second division of the sporangium may or may not be synchronous in both halves but finally four uninucleate spores are formed which round off and are shed through an apical pore in the sporangium. A second sporangium may be formed within the first, but in general further growth does not take place, so that at least in culture the empty spore-cases remain.

The anomalous development of the tetrasporophyte.

During the examination of tetrasporic material of R. floridulum collected and fixed in acetic-alcohol at Ardneil Bay in March 1961, a single lateral differing from the others was discovered. With the exception of this single example all the laterals examined bore tetrasporangia in the normal manner. In parts this lateral produced similar tetrasporic secondary laterals, but certain cells of the filament, often adjacent to cells bearing tetrasporangial branches, produced a second type of lateral;

these often branched abundantly, producing a 'brush' of filaments of similar length, but were also frequently more simple as shown in Figure 9. The terminal cells of these filaments bore one or two pear-shaped spherical cells, between 4.5-6.0u in breadth.

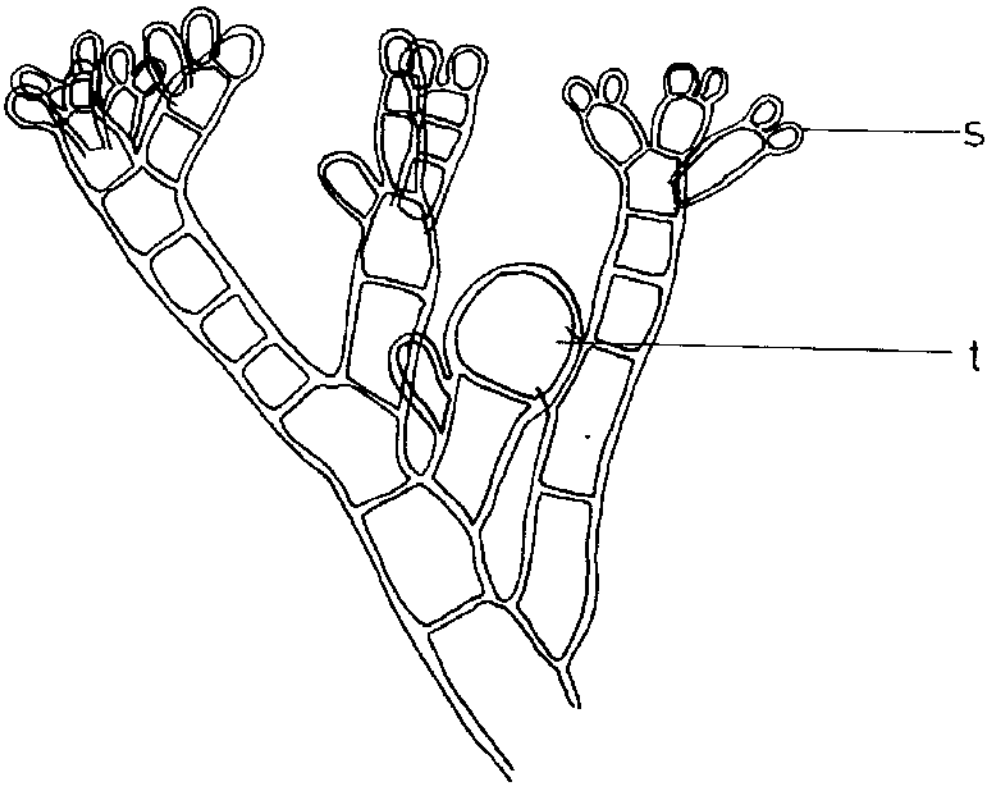
In the larger examples the contents appeared to have rounded off into a single unit, and in some cases empty cells with a terminal pore were observed, suggesting that the contents had been released.

Since the material had been preserved in acetic-alcohol the pigment had been removed from the cells, and for this reason it is uncertain whether or not the 'sporangial' contents were pigmented; in no cases however were these 'sporangia' observed to contain a pyrenoid, and this suggests that in fact the contents did not possess a chromatophore.

It was thought at the time that the male organs of the species had been discovered, since both the form and arrangement of the 'sporangia' are similar to that of the spermatangia of many Florideae, but the subsequent observations of the development of the tetraspores formed after meiosis, coupled with the fact that despite intensive searching over a period of two years, no similar specimen has so far been discovered, suggests that the 'sporangia' are not in fact spermatangia, or if they are, then their occurrence on the tetrasporophyte is anomalous.

The contents of the 'sporangium' may in fact be haploid, but since only resting nuclei were observed this cannot be verified. Neither was it possible to determine the chromosome complement of the tetrasporangia which occurred on the same branch. It may be that the nuclei of certain of the cells of this branch had undergone reduction division and had

FIG. 9



Portion of tetrasporophyte showing a region of anomolous development

s spermatium

t tetrasporangium

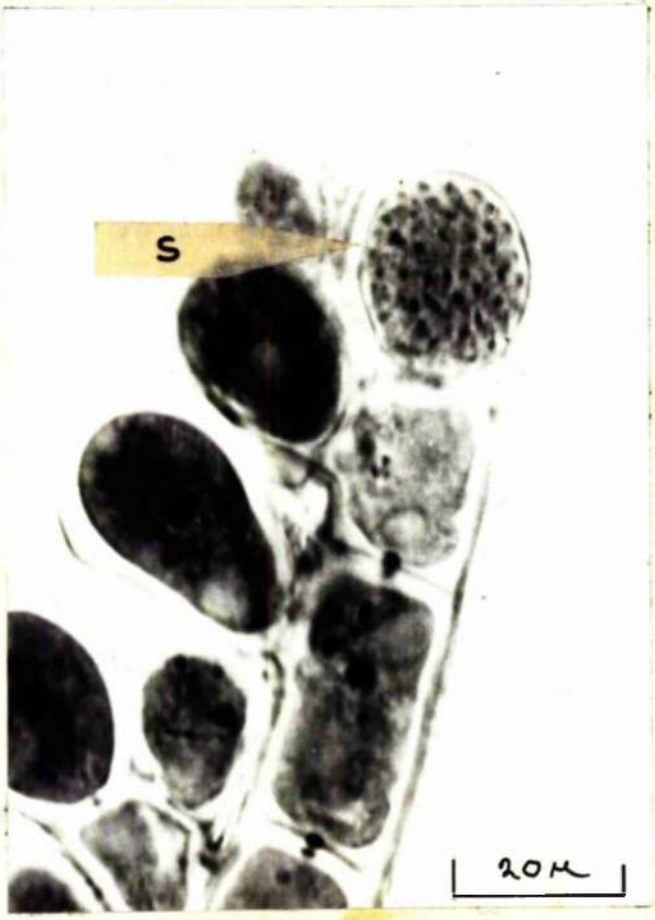
subsequently produced haploid laterals bearing spermatangia. Certainly these branches are very similar in form to the male plantlets produced by the germinating tetraspores, but if reduction division had taken place then it would seem likely that carpogonia as well as spermatangia would have been formed. So it may be that the 'sporangia' are reduced tetrasporangia, although the absence of pyrenoids argues against this.

Although it is not possible to come to any conclusion regarding the nature of these units, it is of interest to note the resemblance they bear, both in form and arrangement, to the spermatial branches described by Rosenvinge (1923-24) in a tetrasporic specimen of R. penicilliforme.

It seems that Rosenvinge saw only one such specimen and no further records of the occurrence of spermatia in the species have been seen. It may be that Rosenvinge's specimen is as anomalous as the present example is taken to be, although this cannot be confirmed without first investigating the cytology and life-history of R. penicilliforme.

Spermatangia have been recorded from only one other species of Rhodochorton - R. violaceum - a fresh water member described by Drew (1935). This is a dioecious species, although antheridial branches may occur on a female plant, and both sexual and tetrasporic plants bear monosporangia. Since nothing is known about the nuclear cycle and the place of reduction division in this species it is not certain if it is closely related to the present species.

Plate 10A



Fungal infections.

Dixon (1960) has reported in considerable detail the types of fungi infecting species of Ceramium, describing the general reactions of the host to the parasite. In the present investigation, infected plants were occasionally found. The regions apparently most susceptible to infection are the apical cells and the developing sporangia. Plate 10 A is a photomicrograph showing an infected sporangium(S). It has not been possible to identify the species responsible for the infection, nor the mode of infection. Dixon (1960) records that the fungi caused hypertrophy of the host but this does not seem to occur in the present species, in which the infected cells are never larger than uninfected ones.

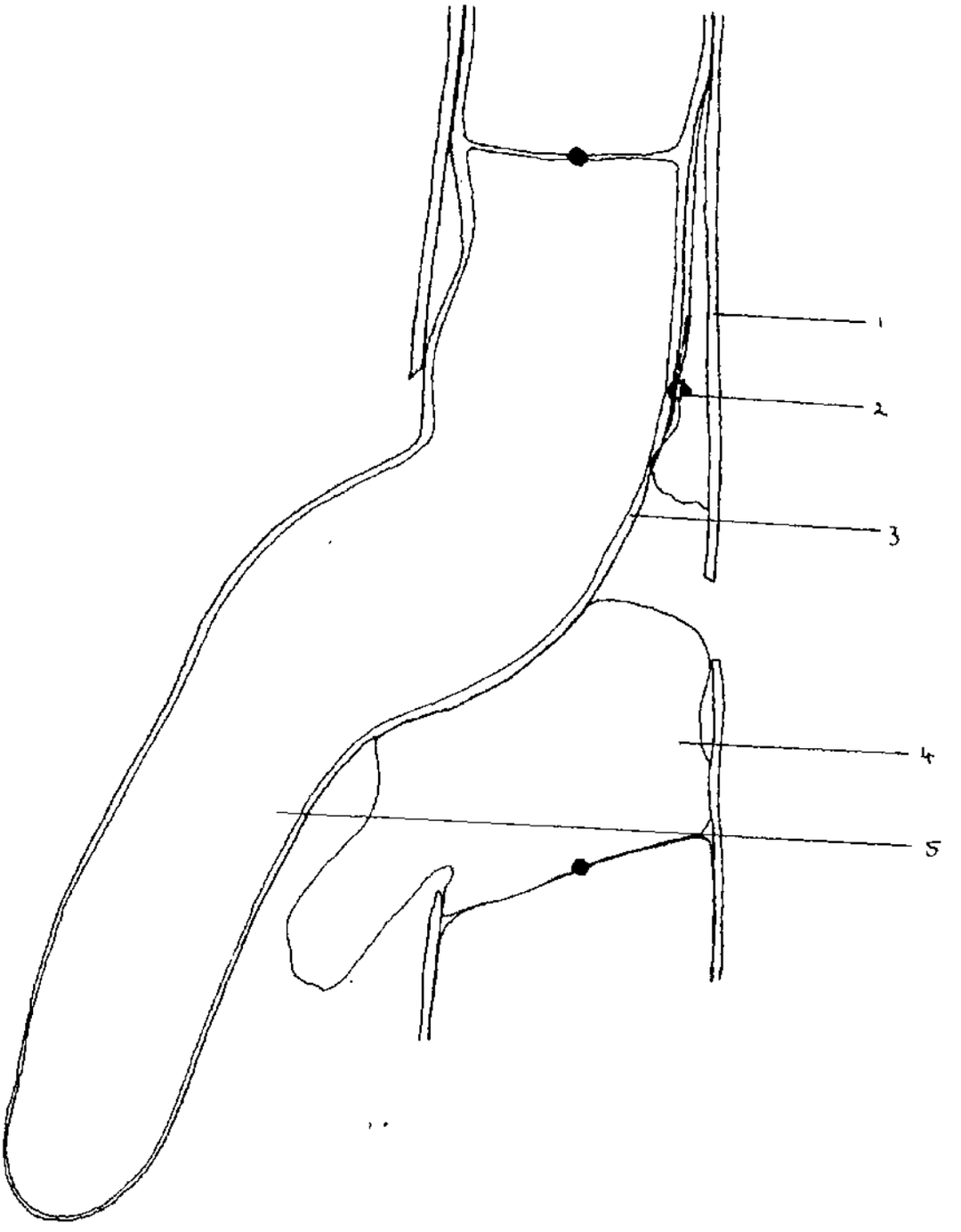
Vegetative reproduction.

Instances of vegetative reproduction in Rhodochorton floridulum are relatively rare compared with R. purpureum. Occasional instances have been met with similar to one type found in the latter species; an intercalary cell, for reasons which are not understood, becomes meristematic and produces a filament which grows into the cell beneath, disrupting its contents in the process. This filament then emerges through the wall of the filament and the upper portion separates from the rest of the plant and becomes established as a separate entity. Figure 10 shows the early stages of this process. After separation, the first entire cell below the level of separation becomes meristematic and produces a new apical cell in the manner described later.

Figure 10.

The drawing shows an intercalary region of regeneration.

1. is the old outer wall.
2. is the pore with a portion of the old septum attached.
3. is the outer wall of the new filament.
4. is a portion of the protoplasm of the cell into which the new filament has grown.
5. is the apical region of the new filament.


1
2
3
4
5

The production of a downward-growing filament from an intercalary cell can also result following the death of the cell below and this is probably the main method of vegetative reproduction.

If the apical regions of actively growing filaments are severed in culture, they will normally produce such downward growing filaments which eventually undergo growth-curvature so that the plane of growth is at right angles to the axis. At the point of curvature one or more laterals are produced, also at right angles to the axis, so that a heterotrichous condition is established. The little plantlets so formed continue to grow and branch in the manner of parent. So far, examples of such plantlets have not been found on the shore and it may be that vegetative reproduction is not particularly important in this species.

Cell division and cross-wall formation.

The process of cell division was followed in the apical cells of the erect system.

After cross-wall formation the newly formed apical cell elongates until it is between 88 and 160u in length and 16-24u broad. In the early stage of elongation the cell is entirely filled with dense cytoplasm, but as the length increases a vacuole is formed in the lower region and this vacuole gradually extends until immediately prior to cross-wall formation it may occupy one third to two thirds of the length of the cell. The upper region remains densely protoplasmic and contains pyrenoids slightly smaller than those of the lower portion (about 3u compared with 5u), which lie in the cytoplasmic lining of the vacuole. The boundary between the

upper and lower regions is sharply defined and convex, the upper portion projecting for some distance into the lower, and it is along this boundary that the cross-wall is formed. The wall of the filament consists of two layers, the inner layer adjoining the cell cavity is said for most of the Rhodophyceae to consist largely of cellulose, while the outer layers are composed of pectic substances (Fritsch, 1945 vol. 2; Dawes et al, 1961). Staining the material with iodine solution after treatment with 80% sulphuric acid gives a blue colouration to the inner wall, staining with Ruthenium red imparts a delicate pink colouration to the outer wall. The chemical structure of the walls is thus similar to that in the majority of the Rhodophyceae. It is from the inner wall only that the cross-wall arises.

The stages in the formation of the cross-wall are best seen in squash preparations since this flattens the apical cell and thus accentuates the convexity of the wall enabling the central portion, which is normally masked by the outer, to be examined.

From the inner cell wall a membrane is formed, growing upward for a time before curving over and growing along the line of separation between the upper and the lower regions. The membrane is initiated simultaneously as a ring round the inside of the cell and as this is added to around its circumference a cone is gradually formed. During its formation a second membrane is initiated from the inner wall at a level slightly higher than the first, and this grows downwards into the mouth of the first formed cone, continuing its growth closely adpressed or fused with it. At the end of this first stage the septum has the form of a double cone, one lying

inside the other, as shown in Figure 11. At the apex of each cone a small ovoid 'plug' is formed lying so close to each other as to appear as one (Plate 11A). As secondary deposition takes place along the membranes the 'plugs' are pushed apart and become distinguishable as separate structures, at the same time increasing in size. They remain in contact by means of a cylindrical, stainable strand which may be an open channel. (Plate 11B) and which appears to be continuous through the body of the 'plug'. At maturity each 'plug' consists of a cone projecting into the cell cavity, a circular plate (average diameter 2-5u), to which the cytoplasm remains attached during plasmolysis, and which is also attached to the membrane, and a smaller cone directed towards the sister 'plug' (Plate 11C). The relative size of the 'plugs' varies and often the uppermost is the larger of the two.

As shown in Plate 11C the 'plug' seems to occupy a perforation in the membrane and is probably an elaborate pore connecting the cytoplasm of adjacent cells. The form of these pores is remarkably similar to pores in the cross-walls of certain Basidiomycete mycelia (Moore and McAlear, 1962) and merit detailed investigation.

Since the greater part of any plant of Rhodochorton floridulum is buried in the surrounding sand and therefore capable of little or no photosynthesis it would seem likely that the plants would possess a system for the conduction of foodstuffs from the cells exposed to light to those which are not, especially to the active apices of the prostrate system, and it seems likely that the pores described are the channels through which such conduction takes place.

Figure 11.

Diagramatic illustration of the cross-wall.

1. The outer wall of the apical cell.
2. The inner wall of the apical cell.
3. The outer wall of the daughter cell.
4. The inner wall of the daughter cell.
5. The top of the cone of the septum of the daughter cell.
6. The cone of the septum of the apical cell (top not shown).
7. The cone of the septum of the daughter cell.
8. The pore.

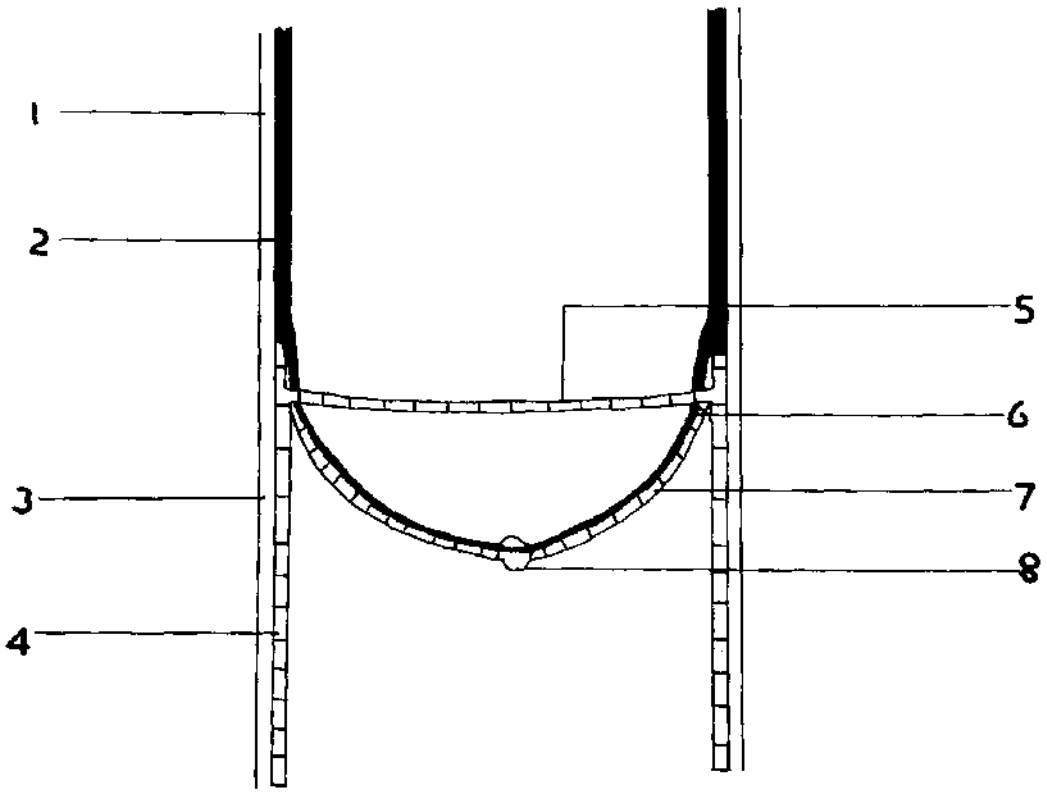

1
2
3
4
5
6
7
8

Plate 11.

A. Photomicrograph of a young pore.

a. is the septum.

b. is the pore. Stain - acetocarmine.

B. Photomicrograph of a mature pore showing the hemispherical dome (c), the ring (b) and the connection between neighbouring pores (a).

See also Plate . Stain - acetocarmine.

C. Photomicrograph of a cell showing:

a. The area of curvature of the inner wall.

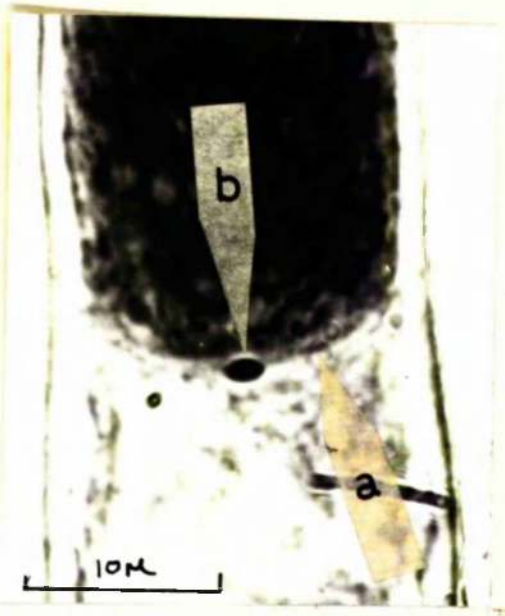
b. The conical portion of the septum of the lower cell.

c. The pore attached by the ring to the septum at a point where the septum appears to be perforated.

d. The inner wall of the cell.

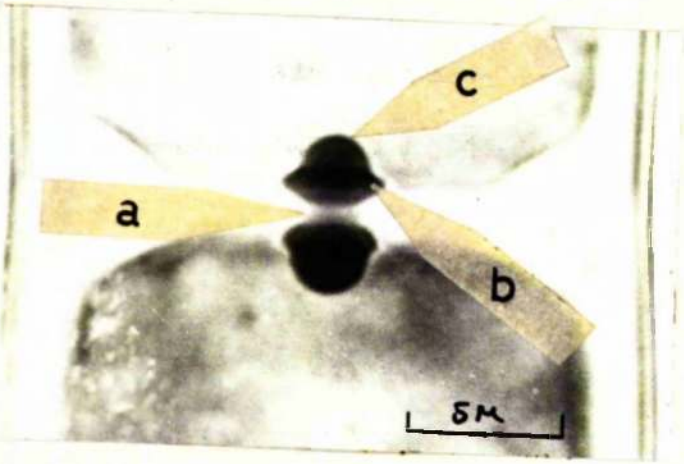
e. The outer wall of the cell.

This preparation was prepared by soaking the material, after staining in acetocarmine, in concentrated lactophenol and then squashing under a cover slip in such a way as to cause the cover glass to slip.

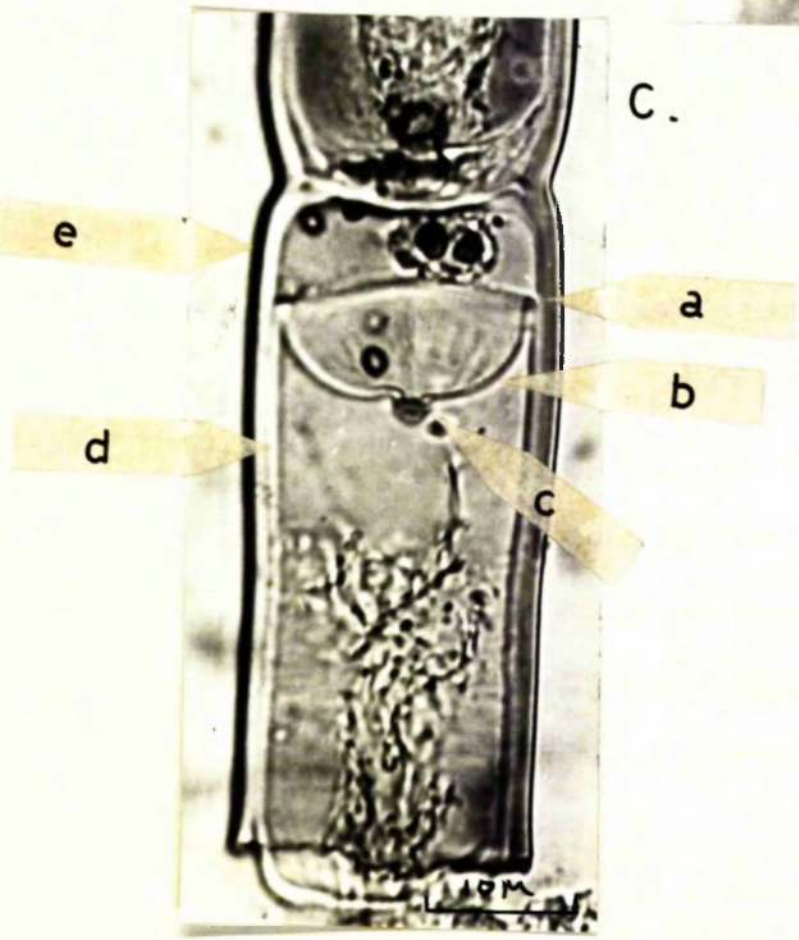


A.

B.



C.



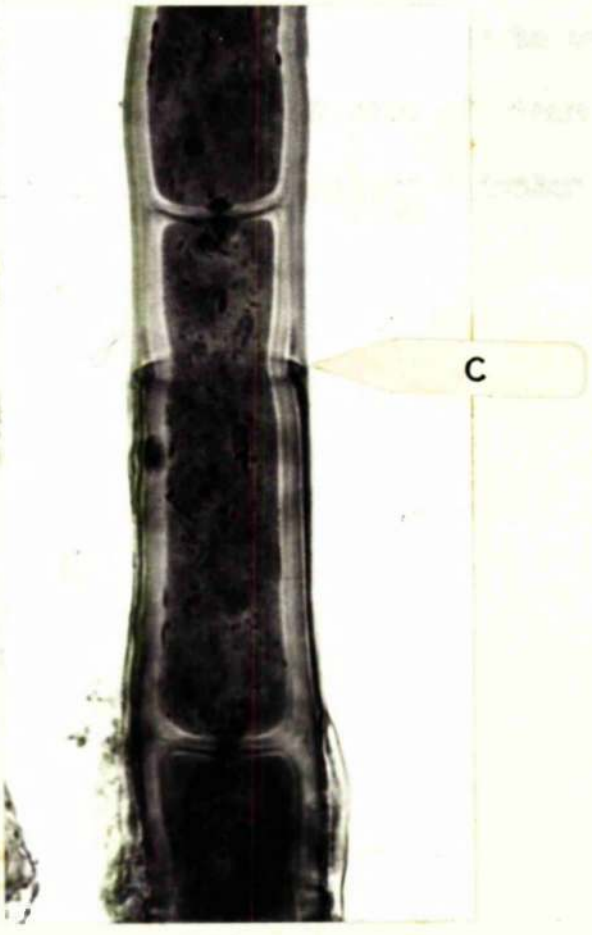
Regeneration.

The apical cells of the filaments appear to be highly susceptible to damage when the plants are growing under natural conditions. The factor or factors responsible for the damage are as yet unknown, but it is possible that the abrasive action of water-borne sand particles may be of some importance and it is perhaps significant that in culture, in the absence of moving sand, damaged apices are very rare.

While the apical cell remains healthy it dominates the filament so that there is generally no intercalary meristematic activity. However, following the death of an apical cell, or a group of cells, the first cell below the line of damage becomes meristematic and grows out through the old cell wall and assumes the function of the apical cell. Due to the fact that in the initial stages of the growth this cell is confined within the limits of the old outer wall of the filament, at first it is less broad than average (Plate 12). The narrowing is however only temporary and soon the original cell width is re-established. The region of damage is indicated by two things; (1) a region of constriction in the first-formed cell, (2) the presence of a 'collar' formed by the broken edge of the old outer wall (these features have been used to determine the rate of growth of the alga in culture as described in the following section).

Regeneration of an apical cell takes place no matter how distant from the original apical cell damage occurs, therefore every cell may be assumed to be potentially meristematic although the potentiality is in some way suppressed by the presence of a healthy apical cell. This suppressing of

Plate 12.



Photomicrograph of region originally damaged showing the collar of the old outer wall (c) and the momentary narrowing of the first-formed cell below it.

potential meristematic activity of intercalary cells by an active apical cell may be due to the production of a growth-regulating hormone by the apical cell and its subsequent diffusion along the filament in a manner analogous to the suppression of axillary bud development in higher plants by the diffusion of hormones from the apex. This theory does not, however, appear to be compatible with the observed production of downward-growing filaments from apical portions of plants possessing an undamaged apical cell.

2. The germination of the tetraspores and the morphology of the sporelings.

Germination of tetraspores

The development of released tetraspores has been followed both in culture and in fixed wild material. No differences have been observed between the two with the exception that in culture there is a high proportion of germination in situ within the sporangium. This is occasionally found in wild material and may be due to the absence of alternate desiccation and immersion which is probably necessary for the rupturing of the sporangial wall.

Observations

The mature spores are released through an apical pore in the sporangium. They are spherical, uninucleate, multipyrenoid and possess a thin cell wall. Since the sporangia are not uniform in size there is some variation in spore diameter within the region of 18-20u. Upon coming into contact with a suitable substrate the spore moulds itself closely to it. The initial attachment and moulding are probably due to the gelatinisation of the lower wall; this is followed by the secretion of a colourless substance around the base which becomes firmly attached to the substrate (Fig. 12 A,B,C). Further development is of two types:

1. The spore produces one, rarely two, germ tubes, between 5-10u in diameter, from the side, occasionally from the top. This is cut off by a cell wall and functions for a time as an apical cell, producing a short filament of up to three cells in length. The filament may be simple, more frequently primary laterals are produced from near the top of the cells, generally one, occasionally two, per cell. The primary laterals

Figure 12.

A diagramatic representation of the stages of spores germination.

A, B and C are common to both types.

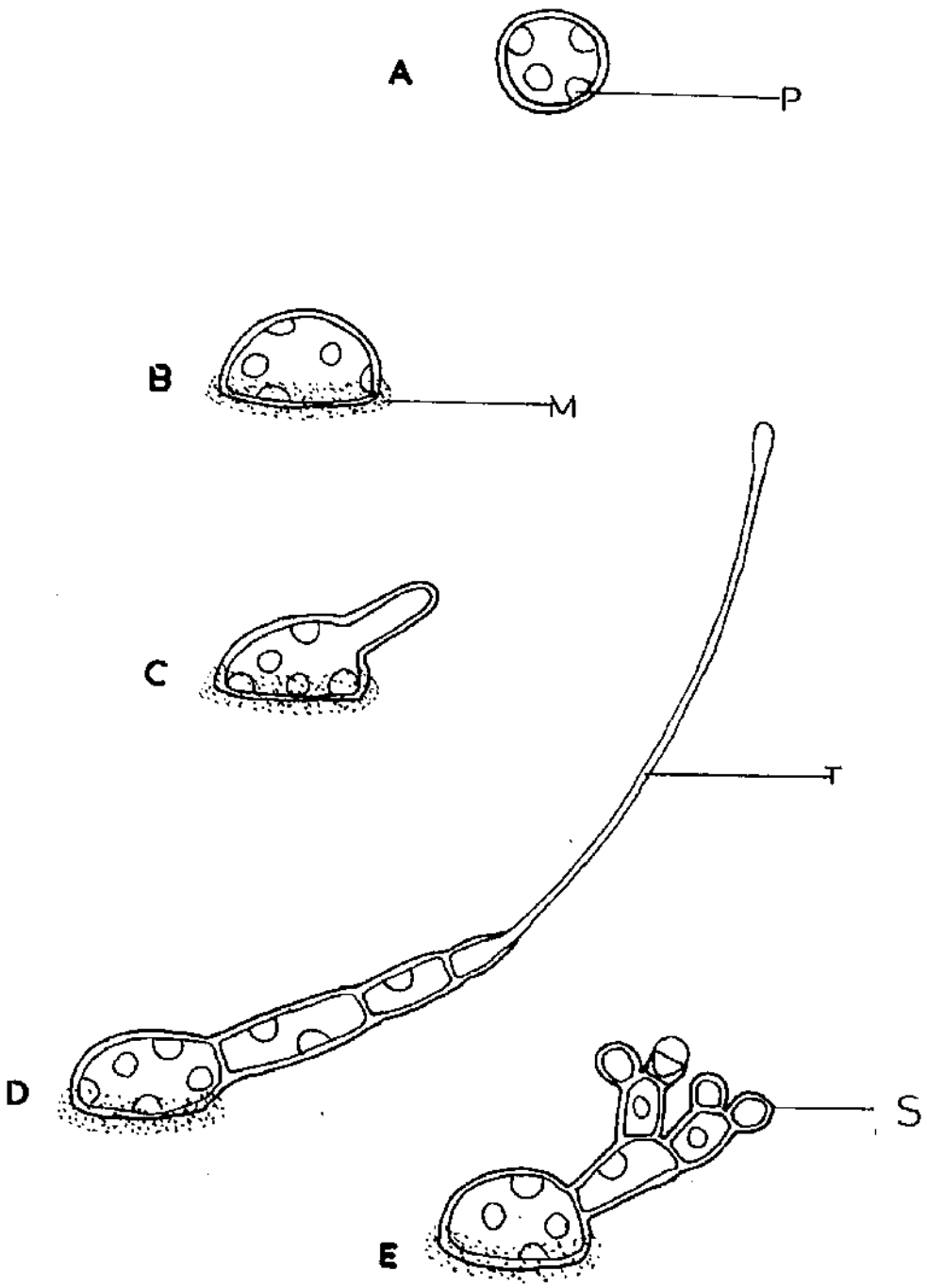
D and E are female and male plants respectively.

P = pyrenoid

M = mucilage

T = trichogyne

S = spermatangium.


A
P
B
M
C
T
D
S
E

may sometimes bear secondaries. Finally from the apical cells of the filaments there are budded off one or two globose, pear-shaped laterals, the contents of which round off and are released through an apical pore as a colourless 'sperm' between 6 and 8u in diameter (Fig. 12E).

2. In the second case the germ tube is in the region of 10-15u in diameter. It is cut off by a cross-wall from the spore, and gives rise to a filament of up to four cells in length. Both cell length and breadth diminishes towards the apex. The apical cell finally produces a long colourless protoplasmic hair with a bulbous tip (Fig. 12D). Only one instance of branching of the filament has been observed.

In both cases pyrenoids and chromatophores similar to those in the cells of the tetrasporophyte have been observed in all but the terminal cells.

Germination in situ

Many instances have been recorded of the germination in situ of the tetraspores, especially in cultured material. Instances have however been seen of germination in situ of spores in wild material.

In all cases so far observed, the products of germination are similar to those of the released spores.

There is no indication that the spores which germinate in the sporangium have failed to undergo meiosis, as is suggested by Boney (1963) for Antithamnion plumula.

Prior to germination, the spores swell considerably, stretching the

sporangial wall, but failing to rupture it (Plate 14A). The released spores probably swell in a similar manner but due to the variation in sporangial (and thus spore) size it is difficult to be certain of this.

The spores produce a germ-tube in the manner previously described, and this pierces the sporangial wall (Plate 14B).

The products of germination are of the types previously noted (Plate 14 C,D).

Although all four spores have been observed to germinate within the sporangium, no examples have been seen in which the four products were mature, with the result that it is not yet known; whether each of the pairs in the top and bottom halves of the sporangium are of the same sex, or whether the arrangement is random.

Discussion.

In view of the fact that it seems definitely established that reduction division does take place during the first nuclear division in the tetrasporangium (Cytology), and considering the form of the sporelings described above, it appears justifiable to accept the two types of sporelings as being male and female.

The type of germination described is rather different from that recorded by Chemin (1937) who observed the formation of a 'massif pluricellulaire' before the initiation of an erect filament. However, Chemin's illustrations of germination are not clear on some points; for example, he makes no mention nor gives any indication of the presence of

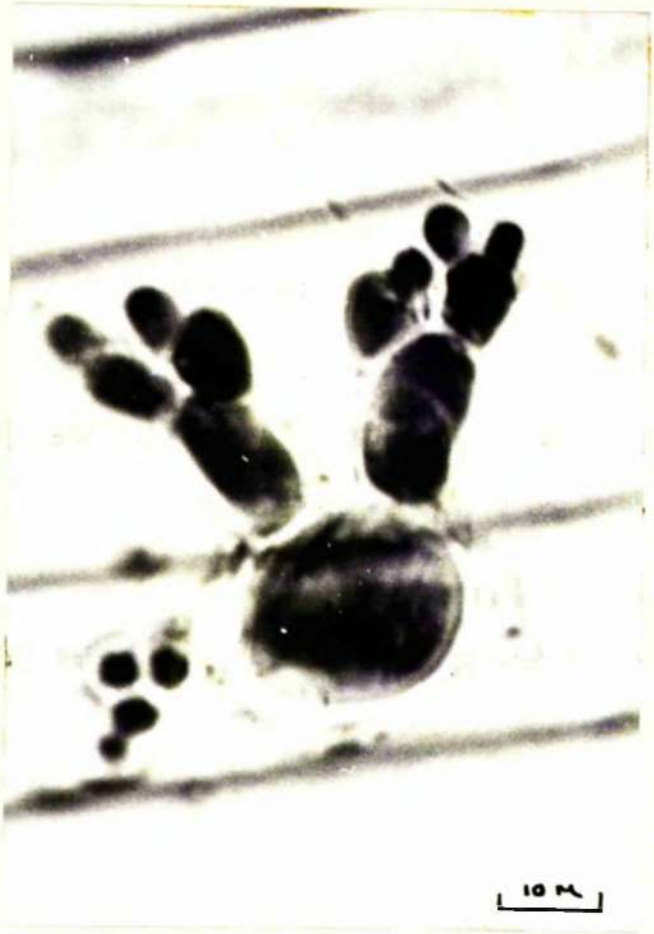
Plate 13.

A. photomicrograph of a male plantlet.

B. photomicrograph of a female plantlet taken prior to the production of the trichogyne.

Wild material fixed in FAA and stained with cotton blue in lactophenol.

A



B

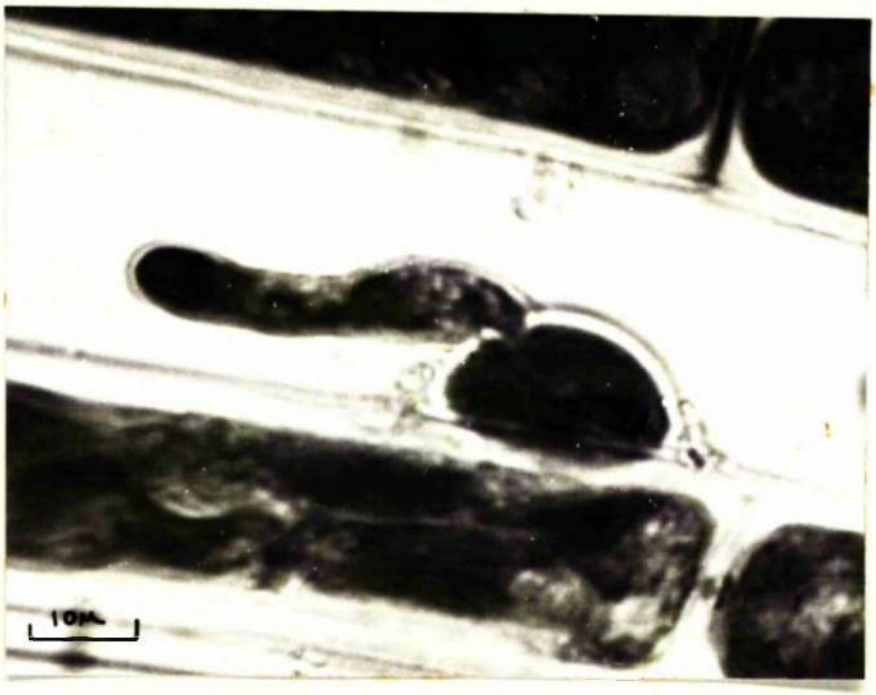


Plate 14.

Photomicrographs showing stages of the *in situ* germination of tetraspores. Material fixed in acetic alcohol stained with acetocarmine and cotton blue in lactophenol.

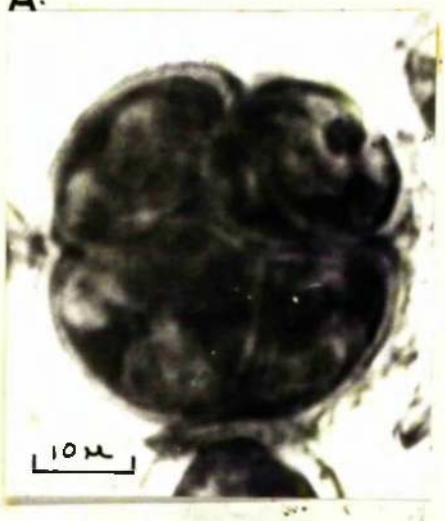
A. The first stage in germination, the spores have swollen, stretching the sporangial wall.

B. One of the spores has produced a germ-tube (t).

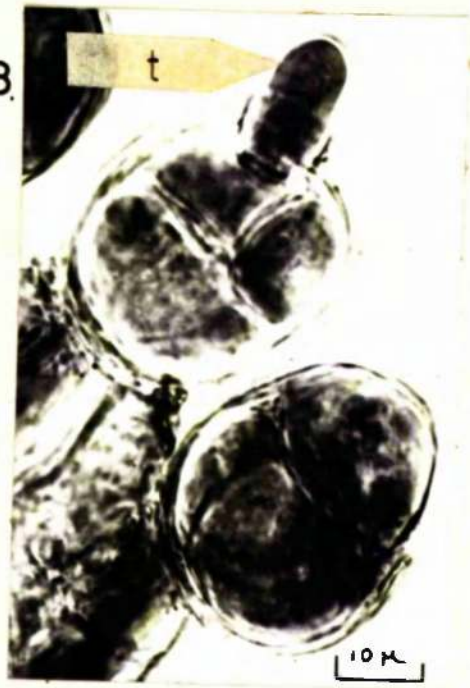
C. One of the spores has produced a young male plantlet.

D. Early stage in the development of a female plant.

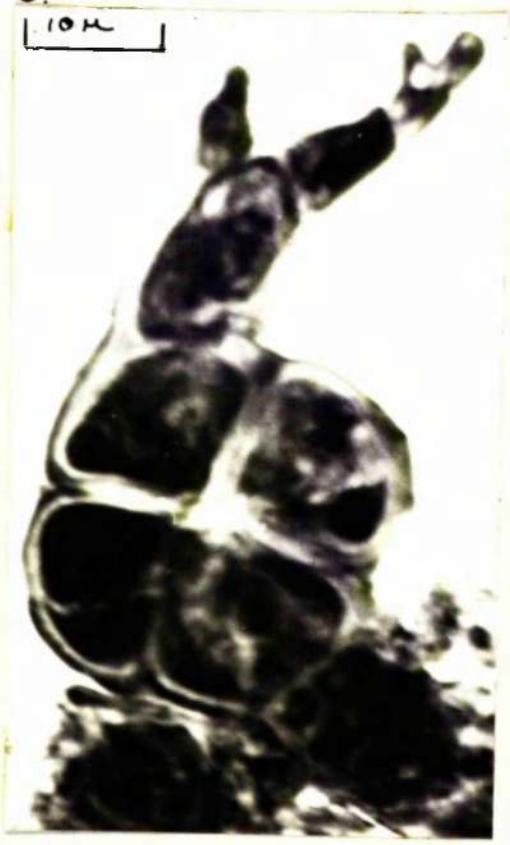
A.



B.



C.



D.

pyrenoids which in healthy material are most conspicuous; moreover, his description of the plastids suggests that they were clumped, a feature common in degenerating spores. He also mentions that under the conditions of culture which he employed, the majority of spores were completely degenerate within a fortnight, indicating that the conditions were unfavourable.

It may be however that under certain environmental conditions tetraspores will form a multicellular mass before the formation of the erect system. This remains to be seen.

On the other hand, the type of germination described is not unlike that of the species of Acrochaetium epiphytic on all samples of R. floridulum collected during the present investigation, and since Chemin describes his difficulty in following germination due to the presence of numerous small algal epiphytes, it may be that these have been a source of confusion.

So far no stages in fertilisation or post-fertilisation changes have been observed. The sporeling cultures are being maintained in an effort to discover these critical phases in the life-history.

C. Cytology of R. floridulum

Cytology
Cytology

Introduction:

The first 50 years of the present century were ones in which the foundations of Rhodophycean cytology were laid, through the investigations of workers such as Wolfe (1904), Yamanouchi (1906, 1921), Lewis (1909), Kylin (1914, 1916a, 1916b, 1917, 1924, 1940), Cleland (1919), Svedelius (1915, 1933, 1937), Drew (1934, 1935, 1939, 1943), Westbrook (1935), and Drew (1944) gives an exhaustive review of the available knowledge on the subject up to that time.

Since then investigations have been continued by various workers including Magne (1952, 1961), Prasaka Rao (1953), Krishnamurthy (1959) and Austin (1955, 1956, 1959, 1960).

Of these investigators only Drew (1935) studied a member of the genus Rhodochorton, the fresh water species R. violaceum, in which she found a tetrasporophyte and separate male and female plants similar in form to each other and to the tetrasporophyte, but she did not determine the chromosome number or the site of meiosis.

1. Cytological techniques:

(a)

The Brazalin technique (Drew 1934).

Method:

1. Fix in a solution prepared by mixing 100ml. 70% alcohol with 6 ml. 40% formaldehyde. Shake well and leave for 24 hours. Pour off the fixative

and replace with fresh. Place material in a strong light to bleach.

2. Wash in 70% alcohol.
3. Mordant for one hour in alcoholic alum prepared by mixing 23 ml. 4% iron alum with 77 ml. of 90% alcohol.
4. Wash for one hour in several changes of 70% alcohol.
5. Stain in a 0.5% solution of Brazalin in 70% alcohol for 18-48 hours.
6. Wash in 70% alcohol until no excess stain comes out.
7. Pass through a series of 5% grades of alcohol, leaving the material in each for 15-20 minutes.
8. Place in absolute alcohol for 1 hour changing liquid once.
9. Pass through a series of seven grades of absolute alcohol-xylol, leaving the material for 15-20 minutes in each.
10. Place in xylol for 1 hour.
11. Place in dilute balsam-xylol mixture and leave for several hours.
12. Pass through three higher grades of balsam-xylol mixtures and mount.

Results:

The procedure gives satisfactory staining of the chromosomes but is too lengthy for general use.

(b)

The acetocarmine technique (Belling 1926).

Prasaka Rao (1953) reported obtaining satisfactory staining of algal chromosomes using the acetocarmine method described by Belling (1926), and in a modified form it has also been employed by Austin (1956). The following modified method was used.

Method:

1. Fix in a solution of 1/3 glacial acetic acid/ absolute ethyl alcohol, for a minimum of 5-10 minutes. A few drops of a saturated aqueous solution of ferric chloride added to the fixative, or a similar amount of ferric acetate in the acetic acid of the fixative gives satisfactory mordanting.
2. Wash in several changes of distilled water.
3. Transfer material to a slide, add a drop of acetocarmine prepared as a saturated solution in 45% acetic acid and tease out the filaments with a pair of fine pointed needles.
4. Cover with a number 0 cover-glass and heat to boiling over a bunsen pilot flame.
5. Add fresh carmine and boil again.
6. Squash between several layers of blotting paper.
7. Seal the edges of the cover-glass with rubber solution.

Results:

The chromosomes stain an intense reddish-black. The cytoplasm of actively elongating or dividing cells such as apical cells or young sporangia also stain intensely and because of this it is often difficult to clearly resolve the chromosomes.

Short periods of hydrolysis (2-4 minutes) in 1 N CHl at 60°C. considerably reduce the stainability of the cytoplasm.

(c)

Aceto-orcein technique (La Cour 1941).

Method:

1. Fix in 1/3 glacial acetic acid/absolute ethyl alcohol for a minimum of 5 minutes.
2. Transfer material to a slide, add a drop of aceto-orcein (prepared as a stock solution of 2.2% in glacial acetic acid and diluted to 45% of this concentration before use), and tease out the filaments with a pair of fine pointed needles.
3. Allow the preparation to stand for several minutes to give even staining and then cover with a number 0 cover-glass.
4. Squash between several layers of blotting paper.
5. Ring edges of cover-glass with rubber solution.

Results:

Metaphase and anaphase chromosomes stain intensely.

(d)

The Feulgen technique. (The stain being prepared according to Lillie (1951) as described by Sass (1959)).

Method:

1. Fix in 45% acetic acid or 1/3 glacial acetic acid/absolute ethyl alcohol for a minimum of five minutes.
2. Hydrolise for 2-4 minutes in 1N HCl at 60°C.
3. Wash in several changes of distilled water.
4. Stain for 3-4 hours.
5. Transfer to a slide, tease out material and cover with a number 0 cover-glass.
6. Squash between several layers of blotting paper.
7. Seal edges of cover-glass with rubber solution.

Results:

The chromosomes stain black while the cytoplasm remains unstained.

(e)

Aqueous Azure A. (Flax and Pollister 1949).

Method:

1. Fix in 1/3 glacial acetic acid/absolute ethyl alcohol for a minimum of five minutes.

2. Hydrolise in 1 N HCl at 60°C. for 4-10 minutes.
3. Stain for 30 minutes in a mixture of 50 ml. 2% aqueous Azure A, 3 ml. 10% $NaHSO_4$, 3 ml. 1 N HCl.
4. Remove excess stain by washing in 25% alcohol.
5. Transfer material to a slide, tease out in a drop of distilled water and cover with a number 0 cover-glass.
6. Squash between several layers of blotting paper.
7. Seal edges of cover-glass with rubber solution.

Results:

The chromosomes stain dark blue-red while the cytoplasm stains a light blue-red.

Choice of standard stain.

The acetocarmine technique employed after hydrolysis was chosen as the standard stain because of the rapidity with which material could be prepared for cytological examination by this method.

Because of the intensity with which the cytoplasm of actively elongating and dividing cells stained in unhydrolised preparations, the acetocarmine technique can also be used to determine the general condition of culture material and so provides an easy method of roughly comparing algal activity under different conditions.

2. Culture techniques:

Methods. 1.

Apparatus.

The alga was grown in 250 ml. conical flasks, fitted with dreschel heads when aerated, otherwise lightly plugged with non-absorbent cotton wool. During screening experiments the flasks were placed inside 800 ml. beakers into which solutions of the dyes were run.

The cultures were kept in a Prestcold cold-cabinet at a temperature of 5°C., under artificial illumination from two four-foot Mazda Daylight fluorescent tubes placed 14 inches above and 8 inches to one side of the culture racks, giving a maximum light intensity of 860 lux on the top shelf, 430 lux on the second, and 215 lux on the third. The lights were controlled by a time switch to give eight hours illumination daily. In addition, the cold-cabinet contained separate units screened from the general illumination, in which lighting was provided by two-foot tubes controlled by a separate time switch so that daylength could be varied as required.

During experiments, the flasks were always arranged parallel to the tubes and at equal distance from them in order to obtain equal illumination.

Aeration was provided by Hy-flo electric pumps, the rate of bubbling being controlled by a screw-clip on the inlet lead of each flask. To remove air-borne bacteria and fungi, the air was cleaned before entering the cultures by passing it through a filtering column lightly packed

with cotton wool, a series of three washing bottles each containing 200 ml. of acidified potassium permanganate, and three containing 200 ml. of distilled water. To increase the efficiency of the system, pumice stone bubblers were fitted to the inlets of the dreschel heads.

Before use, all glassware was cleaned by immersion in chromic acid for forty eight hours, then, together with all tubing connections, thoroughly washed in boiling water, rinsed in distilled water, and autoclaved at 15 lbs. p.s.i. for 15 minutes.

Methods. 2.

Preparation of material.

Before use, the material was treated in the following manner to remove mud and sand:

1. Rinsed in several changes of sea water.
2. Transferred to an enamel dish and washed in a jet of sea water from a washing bottle.

The tufts were then placed in a petri dish containing a little sea water and examined with a binocular dissecting microscope. The presence or absence of tetrasporangia and the general condition of the material was recorded. Badly damaged material was rejected.

Media tested.

A. Schreiber (1927)

Sea water	1000 ml.
sodium nitrate	0.1 gm.
Di-sodium hydrogen phosphate	0.5 gm.

B. Consisting of A. supplemented with vitamin mixture from ASP 6 (Provasoli, McLaughlin and Droop, 1957)

Sea water	1000 ml.
Sodium nitrate	0.1 gm.
Di-sodium hydrogen phosphate	0.5 gm.
Vitamin mixture	1.0 ml.

The vitamin mixture has the following composition:

Thiamine.HCl	0.2 mg.
Nicotinic acid	0.1 mg.
Putrescine.2HCl	0.04 mg.
Ca pantothenate	0.1 mg.
Pyridoxine.2HCl	0.04 mg.
p-aminobenzoic acid	0.01 mg.
Biotin	0.5 ug.
Cholin.H_2 citrate	0.5 mg.
Thymine	0.8 mg.
Inositol	1.0 mg.
Orotic acid	0.26 mg.
Riboflavin	5.0 ug.
Pyridoxamine.2HCl	0.02 mg.
Folic acid	2.5 ug.
Vitamin B_{12}	1.0 ug.
Distilled water	1.0 ml.
pH	7.0

C. Drew-Krishnamurthy (Krishnamurthy 1959)

Sea water	666.0 ml.
Unheated soil extract in sea water	333.0 ml.
Mineral mixture	25.0 ml.

The mineral mixture has the following composition:

EDTA (disodium salt)	0.02 gm.
Sodium nitrate	0.20 gm.
Di-sodium hydrogen phosphate	0.04 gm.
0.2% solution of boric acid	1.00 ml.
0.1% solution of ferric citrate	1.25 ml.
Distilled water	50.00 ml.

D. Foyn's Erdschreiber (Foyn, 1934)

Sea water	1000.00 ml.
Sodium nitrate	0.1 gm.
Di-sodium hydrogen phosphate	0.02 gm.
Soil extract in sea water (½ v/v)	50.00 ml.

E. V 37 (Provasoli, McLaughlin and Droop, 1957, modification 2 (Stewart, Thesis))

Sodium nitrate	0.10 gm.
Sodium chloride	5.00 gm.
Magnesium chloride	0.75 gm.
Calcium chloride	24.0 mg.
Di-potassium hydrogen phosphate	2.50 gm.
Potassium sulphate	0.01 gm.
Ferric citrate	0.01 gm.
Citric acid	0.01 gm.
Distilled water	1000.00 ml.

Trace element supplement added:

The composition of the trace element supplement is as follows:

Fe (as Cl)	0.4 mg.
Mn (as Cl)	0.1 mg.
Mo (as Na salt)	0.1 mg.
B (as H_3BO_3)	0.1 mg.
Cu (as SO_4)	0.01 mg.
Zn (as SO_4)	0.01 mg.

F. ES enrichment (Provasoli, personal communication)

Sea water	1000.00 ml.
Enrichment	20.00 ml.

The composition of the enrichment is as follows:

Distilled water	100.00 ml.
Sodium nitrate	350.00 mg.
Di-sodium glycerophosphate	50.00 mg.
Fe EDTA (1:1 molar $FeCl_3$ & Na_2EDTA)	2.50 mg.
Pll metals	25.00 ml.
Vitamin B_{12}	10.00 ug.
Thiamine	0.50 ug.
Biotin	5.00 ug.
Tris buffer	500.00 mg.
pH 7.8	

Pll metal mixture:

Distilled water	100.00 ml.
Boric acid	0.114 gm.
Ferric chloride.$6H_2O$	4.90 mg.
Manganese sulphate.$4H_2O$	16.40 mg.
Zinc sulphate.$7H_2O$	2.20 mg.
Cobalt sulphate.$7H_2O$	0.48 mg.
Na_2EDTA	100.00 mg.

In all cases the sea water was filtered through No. 1 Whatman paper and aged before use (Harvey, 1941). All additives, except where specified, were autoclaved at 15 lbs. p.s.i. and allowed to cool before use.

Culture experiments to determine the most suitable medium.

Control.

Before starting the culture experiments investigations were made of the activity of the plant under natural conditions. This was done in the following manner:

Samples of material were fixed at the same time at different levels on the shore so as to include submerged plants as well as those which had been exposed for varying lengths of time.

Samples of material from a particular level on the shore were fixed at intervals during the period of exposure: (a) as the tide retreated; (b) as the tide advanced.

The material was then stained and examined, estimations being made of the activity (See P. 66) of the apical cells at different times of the day, and under different conditions of exposure. Tables 1 and 2 show the results of two of many such investigations.

Table 1

Date: 14th September 1961.

Site: Millport.

Tide: Advancing. Low tide 10 p.m.

Time fixed.	Level on shore	% number of dividing apices in sample
10.45 a.m.	Upper mid-littoral	100
10.45 a.m.	Mid-littoral	75
10.45 a.m.	Lower mid-littoral (submerged)	93

Table 2

Date: 14th July 1962.

Site: Portmahomack.

Tide: Retreating. High tide 10 a.m.

Time fixed	Level on shore	% number of dividing apices in sample
12.30 p.m.	mid-littoral (exposed)	86
2 p.m.	"	100
4 p.m.	"	100
5 p.m.	"	98
8 p.m.	submerged	100

It was found that there was no marked difference in the percentage number of active apices between plants at different levels on the shore or in the same plant at different times of the day.

The percentage number of active apices was always high, the average being 85%, the lowest examined 60%. These percentages are based on counts of undamaged apices. Most plants examined had about 20% damaged apices, but in particular samples the figure was as high as 90%. The reason for this damage is not known; in some cases the damaged apical cells contained Chytrids, but these accounted for only a small fraction of those examined. The control figure was taken to lie within the region of 60-100%.

The media were tested as follows:

Approximately equal samples of material prepared as described above were placed in three flasks containing 200 ml. of the medium, and the

flasks arranged so as to receive equal illumination. At the end of 20 days, samples from each flask were fixed and stained with acetocarmine and the percentage number of active apical cells determined. The first medium tested (A) supported apical growth in the control range; the sporangia, however, degenerated. The other media were tested in a similar manner and the results are shown in Table 3.

Table 3

Medium	Vegetative growth	Sporangial condition
A	Within control range (See p. 50)	Degenerate
B	"	"
C	"	"
D	"	"
E	"	"
F	"	"

The results of further variations of the medium on sporangial development are shown in Table 4.

Table 4

Treatment	Sporangial condition after 20 days
Dilution of medium to ¼, ½, and ¾ of standard conc.	Degenerate
Reduced salinity, ¼, ½, ¾ standard	Degenerate
pH variation between 5 & 8	Degenerate
Alternation of depleted with enriched media, from 1-10 days in each	Degenerate
Increased aeration by means of electric pumps	Degenerate
Twice daily immersion and desiccation in tidal chamber (Aleem, 1949)	Degenerate

It was concluded that the influence of the medium was in itself not responsible for the degeneration of the sporangium.

Sea water + E.S. was arbitrarily chosen as the standard medium and was used in the experiments on the influence of light.

Rate of growth in culture.

As has been previously noted, following the regeneration of a damaged filament, the position of damage is marked by the momentary narrowing of the filament and the presence of a 'collar' formed by the remains of the old outer wall. These factors have been used as a basis for determining the rate of growth of the alga under standard conditions in culture.

Method:

A healthy plant was placed in a petri dish containing sea water and the filaments severed a uniform distance behind the apex, the operation being carried out with the aid of a dissecting microscope. The material was then transferred to a 250 ml. flask containing 200 ml. of ES supplemented sea water and placedin the cold cabinet. A portion of the material was removed daily and examined microscopically.

Results:

It was found that during the first ten days following decapitation no new cells were produced. On the tenth day the first new cell began to break through the septum and from then on a new cell was produced on average every 1.4 days.

The ten day lag period and the average time required for the production of a cell were confirmed in similar experiments in which the material was examined every 20 days.

This technique provides a potentially useful method of determining the rate of growth of the alga under different cultural conditions.

The experiments described on pages 54-59 are the results of investigations similar to those of Boney (1962). These appeared to be relevant to the degeneration of the tetrasporangia which had been observed earlier in the current investigations. They are preliminary in character and the results cannot be taken as conclusive without further investigation.

EXPERIMENTS ON THE EFFECT OF LIGHT ON SPORANGIAL DEVELOPMENT

The influence of light on sporangial development.

During the experiments previously described it was observed that the rate of degeneration of the sporangia was slowest in those sporangia situated in the centre of a tuft of the cultured material. This suggested that light was in some way responsible for the death of the sporangia, either the spectral composition or the intensity being harmful.

Boney & Corner (1962) have shown that the growth of Plumaria elegans can be influenced by the composition of incident light, the light being screened by aqueous solutions of phycoerythrin or chemically related dyes. These authors found that aqueous solutions of eosin yellow, of concentrations from 0.2-0.5 mg. dye/l, had a marked stimulatory effect on growth. This dye, structurally related to fluorescein, has an absorption peak at 515mu, approximately at the centre of the range of greatest light absorption observed by Boney & Corner with phycoerythrin.

The effect of aqueous solutions of this dye on the development of the sporangia was investigated in the following manner:

Method 1:

Cultures were prepared in the manner previously described and the flasks placed inside 800 ml. flasks each containing 100 ml. of eosin yellow solutions of concentrations from 0.2-2 mg. dye/l. They were then placed in the cold-cabinet so as to receive maximum illumination (860 lux) and left for 20 days, after which time samples from each flask were fixed, stained and examined. No attempt was made to estimate any stimulus to vegetative growth, but the condition of the apices was noted.

The results are shown in the following table.

Table 5

	Dye concentration mg./1	Veg. growth	sporangia
Control	0.0	within control range (See p. 59)	degenerate
	0.2	"	"
	0.4	"	"
	0.6	"	"
	0.8	"	"
	1.0	"	"
	2.0	"	"

Conclusion.

In the concentrations employed, the effect of the eosin screens on the development of the sporangia was negative.

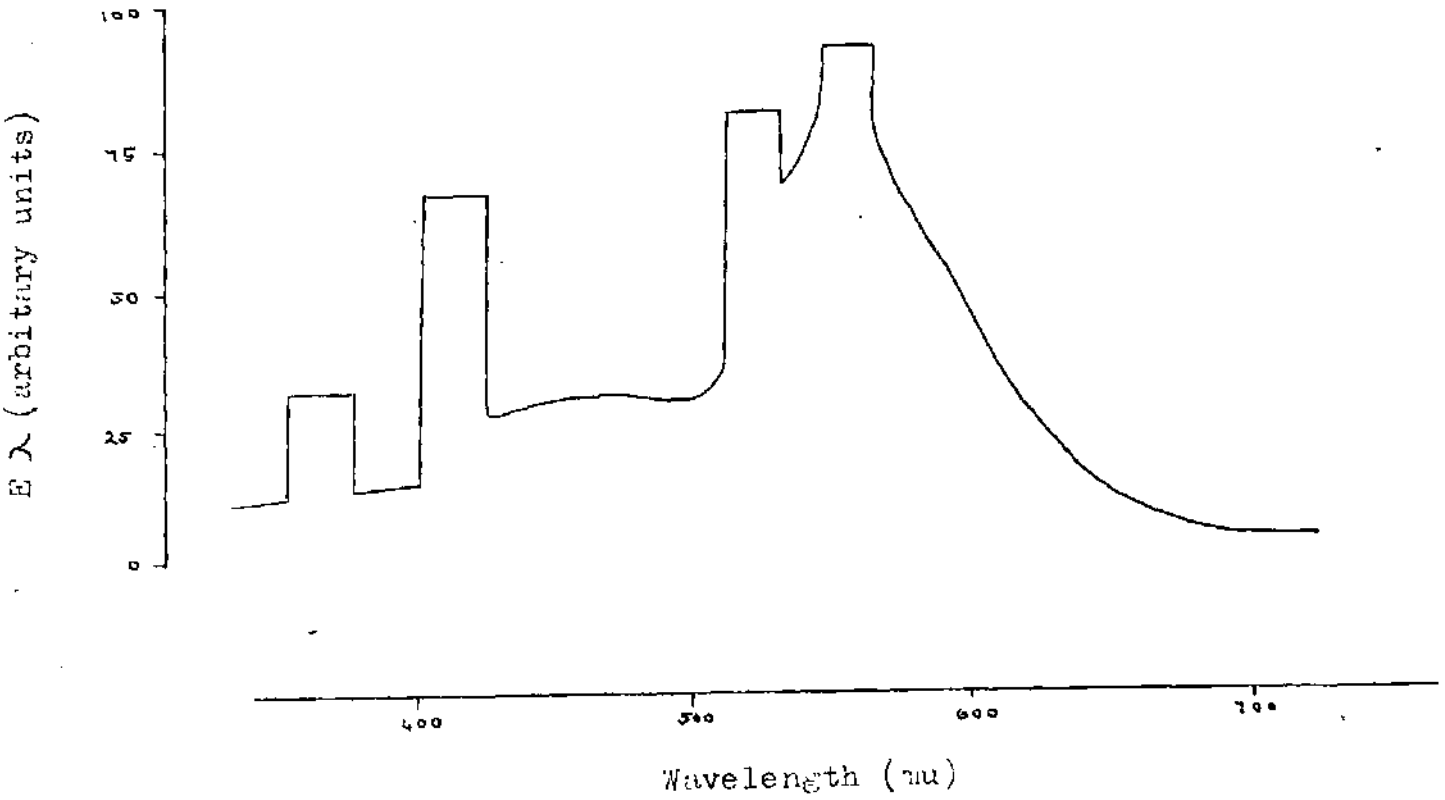
Method 2:

Cultures were prepared as previously described and screened by the following solutions:

(A) Aqueous solutions of eosin of concentrations between 0.0 and 1000 mg./1.

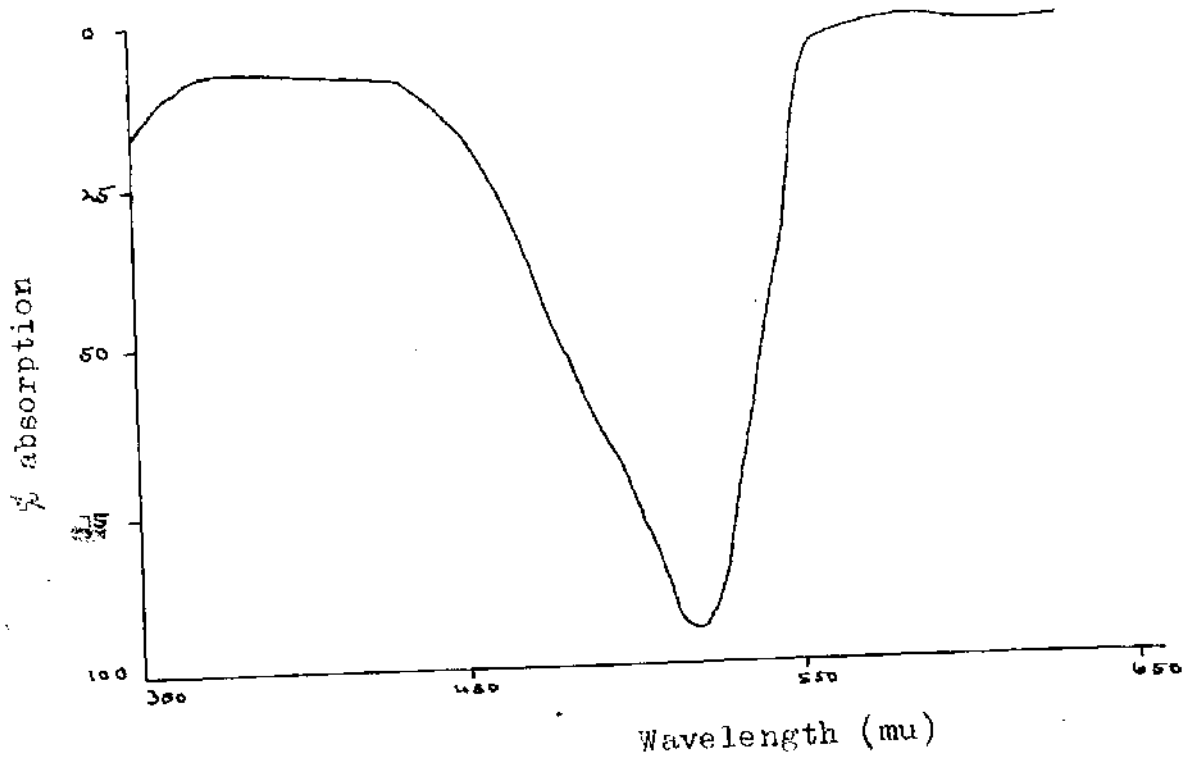
FIG. 14 Spectral energy distribution curve for Mazda Daylight fluorescent tube.

A.



B.

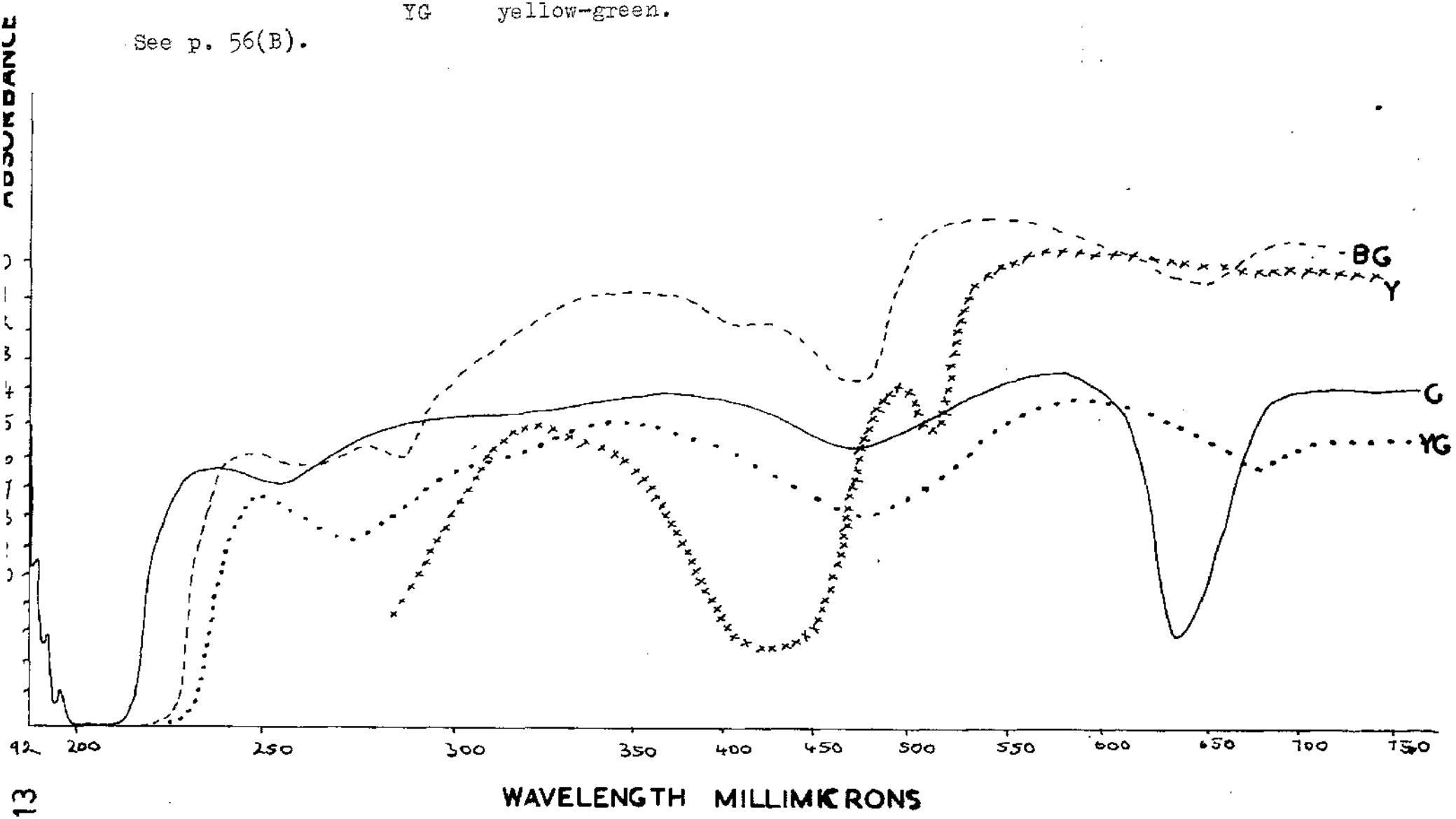
Absorption curve of an aqueous solution of Eosin yellow (10 mg./l)



The absorption spectra of Ilford gelatine filters in aqueous solution.

BG	blue-green
Y	yellow
G	green
YG	yellow-green.

See p. 56(B).



C.13

(B)

1.

Aqueous solutions of Ilford gelatine spectrum filters:

a. Blue-green (maximum transmission 4700-5200 A)

b. Green (maximum transmission 5000-5400 A)

c. Yellow-green (maximum transmission 5300-5700 A)

d. Yellow (maximum transmission 5600-6100 A)

Diluted so as to give a 40% reduction in the intensity of the incident light.

2.

The same filters diluted so as to give a 60% reduction in the intensity of the incident light.

Figure 14A shows the graph of the spectral energy distribution of the light source employed; figure 13 shows the absorption spectra of the gelatine filters; figure 14B illustrates the absorption curve of an aqueous solution of eosin yellow at a concentration of 10mg./1.

(C) Aqueous solutions of indian ink giving the following percentage reduction in the intensity of the incident light:

1. 30%

2. 40%

3. 50%

4. 60%

5. 70%.

Unscreened cultures served as a master control for the experiment.

The cultures were allowed to stand for 20 days and then samples were

fixed, stained and examined.

Results: Table 6

(A)

Dye conc.	Vegetative growth	Sporangia
0.0 mg./1	within control range (See p. 50)	degenerate
0.2 mg./1	"	"
2.0 mg./1	"	"
10.0 mg./1	"	active
0.5 gm./1	"	degenerate
1.0 gm./1	"	"

(B)

1 & 2.

It was found in all cases that although the majority of the sporangia had not degenerated, the nuclei remained in the interphase condition. The filters had therefore prevented degeneration but had failed to stimulate division.

(C)

The sporangia were found to have degenerated in 1; in 2-5 degeneration had not occurred but the nuclei were inactive.

It was later found that if the concentration of the solutions of the spectrum filters was reduced to a level giving a 30% reduction in the incident light intensity the activity of the sporangia could be stimulated.

Conclusions.

It has been observed that the degeneration of sporangia in culture can be prevented by screening the cultures with solutions of certain dyes and it seems likely that the effect is due to the removal of certain inhibitory wave-lengths from the spectrum of the incident light.

It is realised that the experiments described can be considered only as preliminary investigations, and that more detailed study is required before the effect of light on sporangial development can be understood.

The effect of day-length on sporangial development.

The effect of the duration of the period of illumination on the development of the sporangia was investigated in the following manner.

Method:

Cultures were prepared as previously described and placed for 20 days in the light chamber screened from the main body of the cold-cabinet.

In a series of experiments the time switch was adjusted to give from 3-24 hours illumination, the day-length being raised by 3 hours for each new experiment.

Results:

It was found in all cases that the sporangia had degenerated by the end of the experimental period.

CYTOLOGICAL OBSERVATIONS

Recording of observations:

All observations were recorded photographically using a Zeiss Attachment Camera with coupled photocell.

Film: Ilford Pan F, 35 mm.

Developer: Ilford ID2

Johnson 'Definol'.

Ilford ID2 was used to develop the greater part of the films and the prints taken from such negatives are marked ID2. It was later found that negatives developed in 'Definol' for 10 minutes at 20°C. had a finer grain and better contrast, and consequently 'Definol' was used in preference to ID2. The prints taken from such negatives are marked 'D'.

Throughout, an Ilford tricolour green filter was used to improve the contrast of the chromosomes against the background of cell content.

Microscope:

The greater part of the photographs were taken with the Camera attached to a Baker Patholette, using a x8 eyepiece and a x50/0.95 Fluorite oil immersion objective. The resulting prints are marked 'B'.

It was found that the Baker x100/1.30 Fluorite oil immersion objective gave negatives lacking in resolution.

Those photographs which were not taken with the Baker-Zeiss equipment were taken with a Reichert 'Zetopan' with camera attachment, using a x5 or x8 eyepiece and a x100/1.30 Fluorite oil immersion lens. The resulting photographs are of a much higher quality than the others and are marked 'R'.

CYTOLOGICAL OBSERVATIONS

1. Mitotic division.

The process of mitosis was followed in the apical cells of the erect system of material grown in culture, fixed and stained as previously described. Comparison was also made with material fixed on the shore. In general no differences were observed.

Observations:

1. The interphase nucleus.

The majority of the nuclei in any plant are in the interphase condition. The interphase nucleus is spherical with a diameter of approximately 8u. It contains a spherical nucleolus of from 2.6 - 3.6u in diameter, which, in contrast to the nucleolus of a nucleus in early prophase, stains feebly with acetocarmine. Surrounding the nucleolus is a region which contains a network of fine strands staining weakly with acetocarmine.

2. The dividing nucleus.

The nucleus of an actively elongating cell at the onset of prophase is generally spherical; in squash preparations the form may vary between spherical and pear-shaped, but this is probably due to the pressure applied during staining, since observations made with living material indicate that the nucleus is spherical. The maximum diameter of the nucleus at this stage is 13u, and apart from a non-staining region immediately within the nuclear membrane, it is entirely filled with a nucleolus which stains intensely with acetocarmine, but not with the Feulgen technique.

During the early stages of prophase there is a gradual reduction in

the volume of the nucleolus and the chromosomes appear in the zone surrounding it as greatly elongated, faintly staining strands. By late prophase the nucleolus has disappeared, and the chromosomes have shortened and thickened to dot or rod-like dimensions, and lie scattered round the periphery of the nucleus. At this stage they are sufficiently distinct to allow a count to be made, but since they tend to obscure one another it has not been possible to obtain a definite figure. The number lies between 16 and 22, with 20 being representing the average of 30 counts.

During prometaphase the chromosomes move from the periphery to the centre of the nucleus where they become arranged in an open ring around the periphery of the metaphase plate. At this stage individual chromosomes are still separately resolvable and so can be measured; in length they vary from 0.75 - 1.5u, average breadth is 0.5u.

As metaphase continues, the chromosomes move closer together until they form a compact, intensely staining mass in which individual chromosomes cease to be separately resolvable. Since the polar axis of the spindle lies parallel to the long axis of the cell only equatorial or sub-equatorial views can be obtained with the technique employed.

During anaphase the long axis of the chromosomes lies orientated parallel with the polar axis of the spindle, suggesting that the centromeric region is situated at or near the end of the chromosomes. No V-configurations have been observed, but the size of the chromosomes is such that even if they are present it is unlikely that they would be apparent.

The movement of separation at anaphase is undergone by the upper daughter chromatid group only, which may travel up to 60u from the position of the metaphase plate to a point a short distance behind the apex of the cell. Very often the separating groups assume a saucer-like appearance, with the convex side directed towards each other; sometimes one of the pair can be seen in polar view.

At telophase the chromosomes uncoil, gradually losing their affinity for the stain as they do so. At the same time the nucleolus becomes stainable and increases in size until the early prophase appearance is re-established in both the upper and lower daughter nuclei. The nucleolus in the lower nucleus, after attaining a maximum diameter of about 10u, gradually shrinks as it enters the interphase condition.

In occasional preparations twin nucleoli exist in both the upper and lower nuclei. It is not known whether or not this is a normal condition of short duration, the two nucleoli fusing to form the single one which is normally observed, or whether it is an abnormal condition. That it may be the former is suggested by the fact that two pairs of chromosomes have been observed to possess satellite chromosomes which may act as nucleolus organisers.

The behaviour of the cytoplasm during nuclear division.

The behaviour of the cytoplasm during cell division, as discussed elsewhere, can be related to phases in nuclear division.

During the early stages of prophase when the nucleolus is still conspicuous, the cell is densely and uniformly filled with intensely

staining cytoplasm. The shrinking of the nucleolus is accompanied by the appearance of a small vacuole in the cytoplasm adjacent to the cross-wall. The size of the vacuole increases progressively during prophase until by metaphase it occupies the greater part of the cell between the nucleus and the septum. During anaphase and telophase it is gradually extended beyond the lower daughter nucleus up to a point a short distance below the upper daughter nucleus. The upper region remains uniformly filled with dense cytoplasm and becomes cut off from the lower by the formation of a septum.

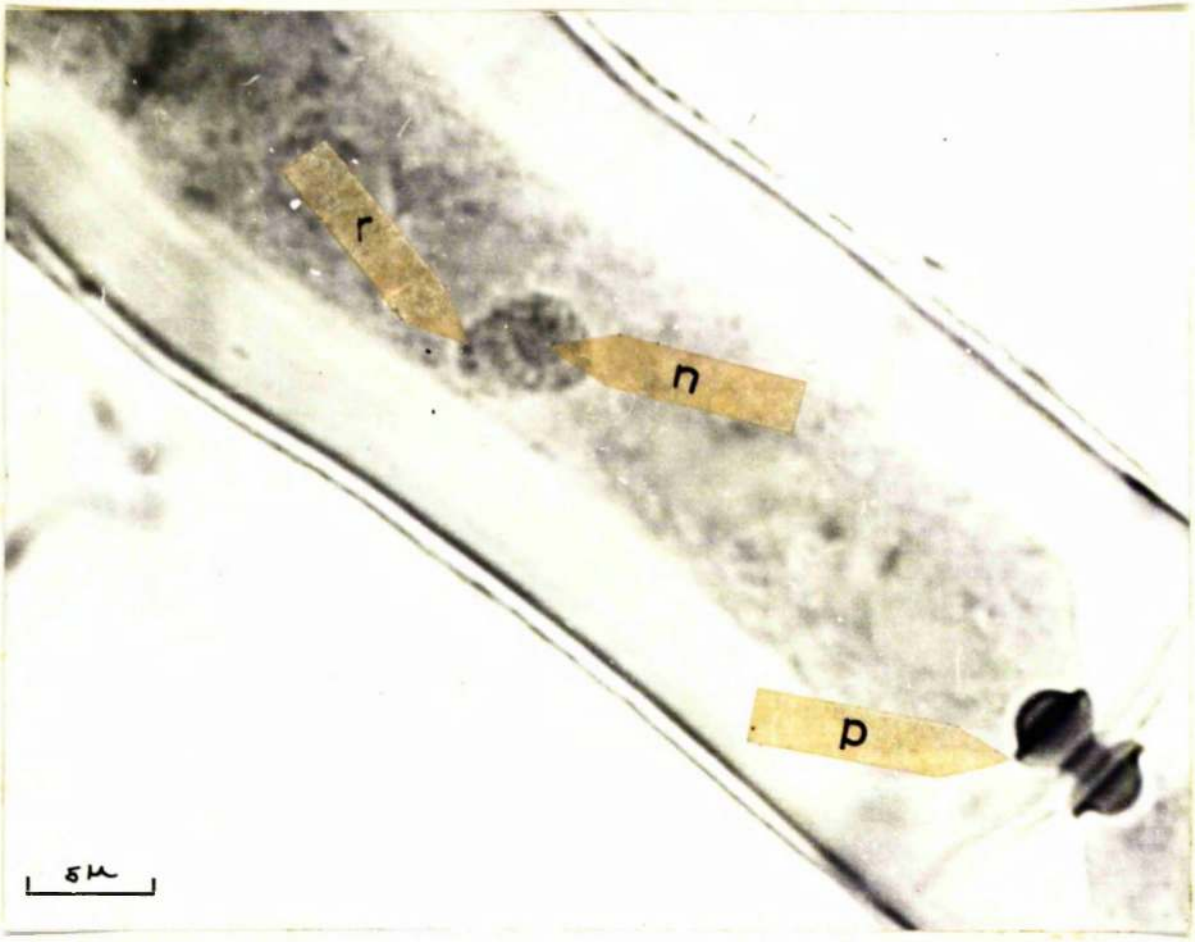
Abnormalities in mitosis

On two occasions apical cells of cultured material have been observed to contain abnormal nuclei. One cell showed circa forty chromosomes in late prophase, and it was thought that the plant to which it belonged might be a tetraploid Examination of dividing nuclei in other apical cells of the same filament showed however that with this and one other exception, all nuclei had the normal complement of c. 20 chromosomes. The same material also produced a cell in which two metaphase plates were arranged above each other, each having c. 20 chromosomes. These two results taken together suggest that under certain conditions two nuclei become included in the one cell, dividing in the same region, with the result that during prophase the two complements of chromosomes become mixed, only to separate again at metaphase.

It has not been possible to investigate this abnormality.

Photomicrographs of stages of mitosis.

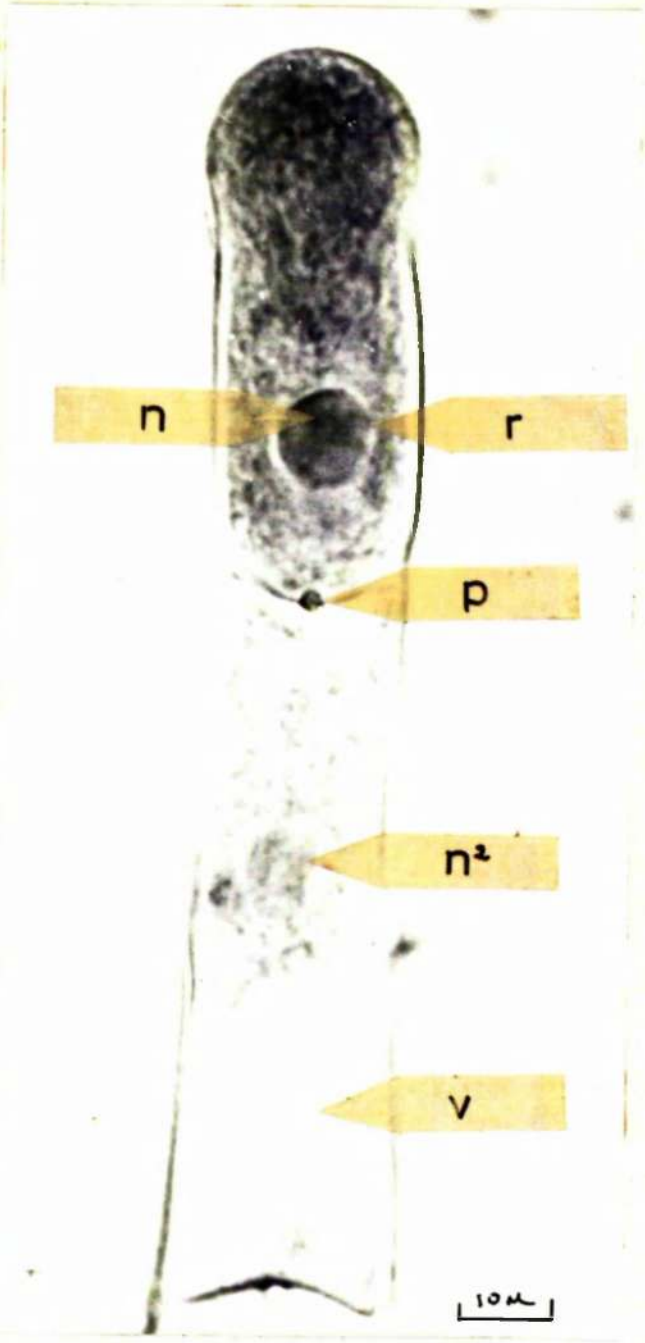
Plate 15.



Interphase nucleus in intercalary cell showing reticulum (r) and nucleolus (n), the conspicuous pore (p) connecting the cells and the attachment of the cytoplasm to the rim.

(R - D)

Plate 16.



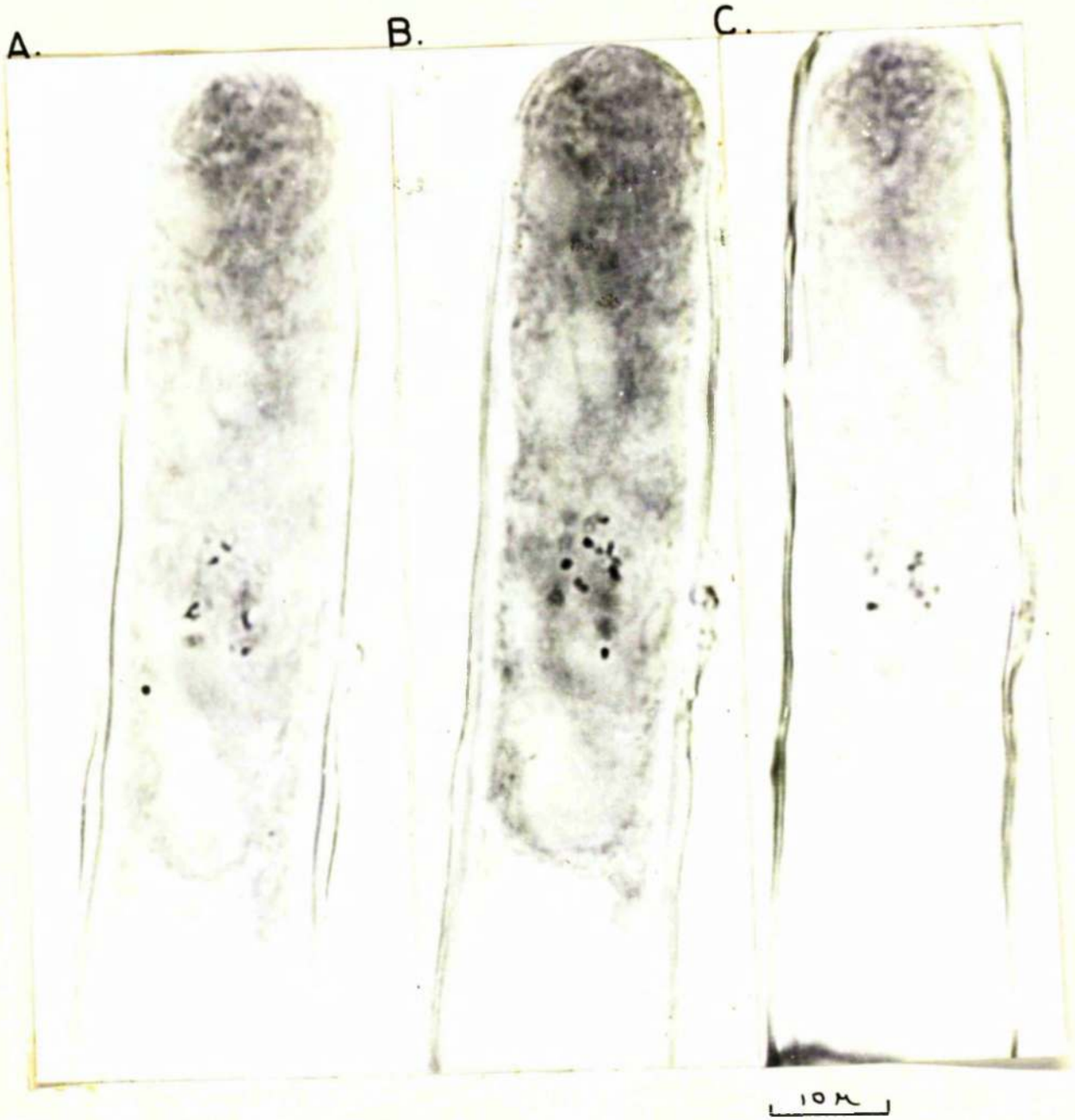
Very early prophase nucleus in an active apical cell showing the enlarged nucleolus (n) surrounded by the clear ring (r), also the intensely staining cytoplasm of the apical cell, the convex septum with the pore (p), and the feebly staining nucleus (n^2) of the daughter cell. v = vacuole.

Plate 17.

A, B and C are photomicrographs taken at three different levels in a late prophase nucleus showing the number of chromosomes to be in the region of 20-21.

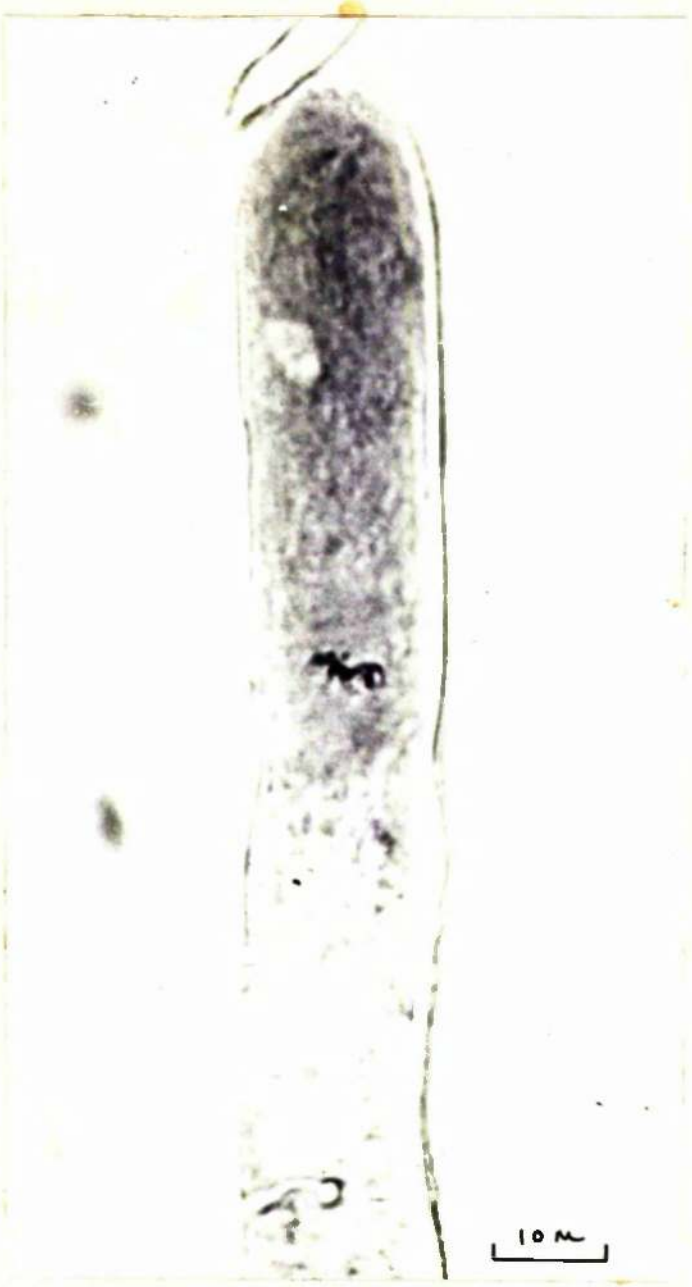
D is a diagramatic interpretation of the same nucleus.

(B - ID2)



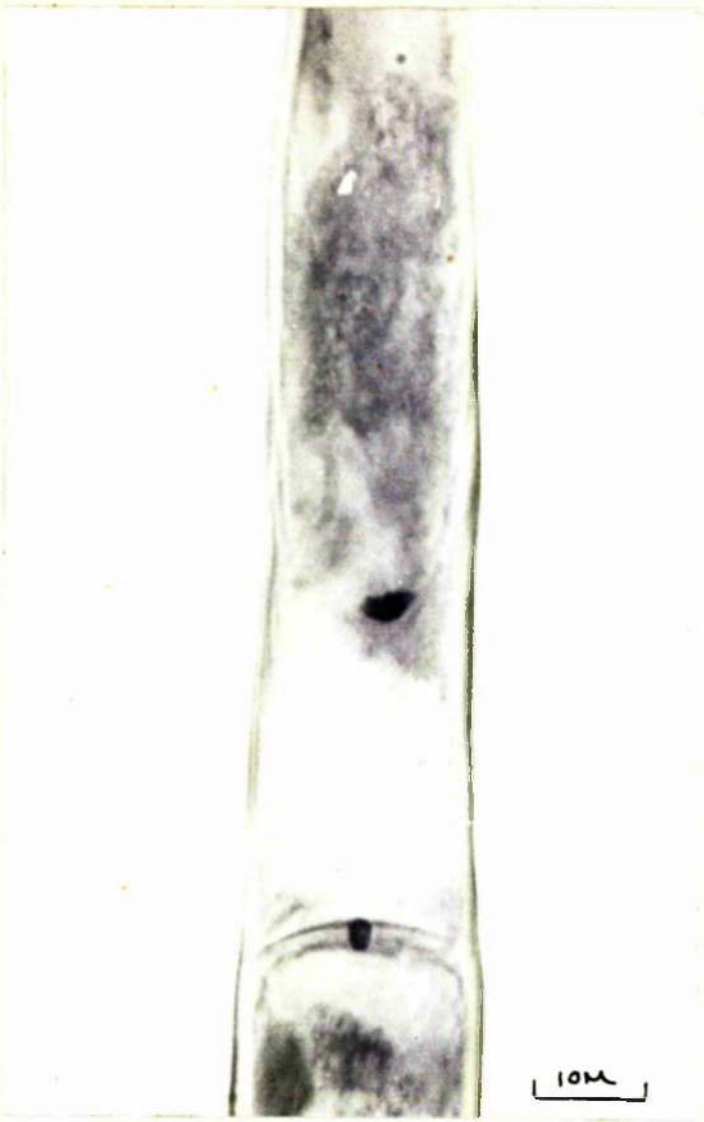
D

Plate 18.



Equatorial view of early metaphase, showing the more intensely staining cytoplasm in which the chromosomes are embedded, and the appearance of vacuoles in the lower region of the cell.

Plate 19.



Subequatorial view of late metaphase; the chromosomes are now very closely arranged so that the group is rather amorphous.

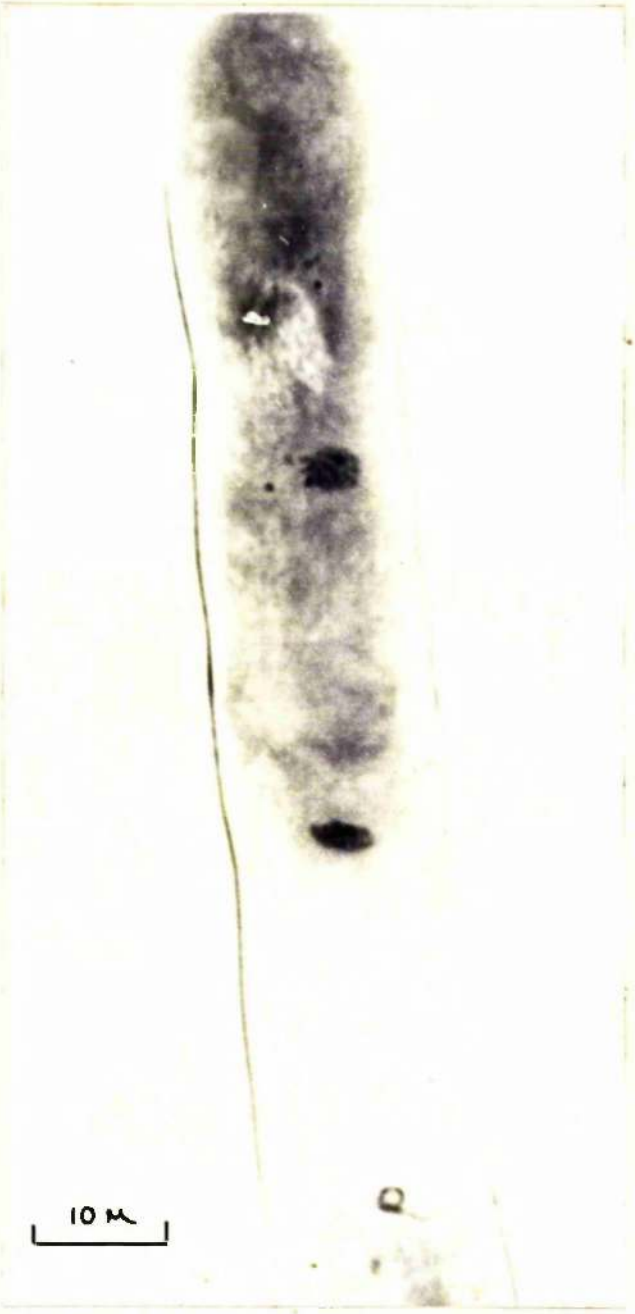
(B - ID_2)

Plate 20.

Early anaphase, equatorial view.

(B - ID_2)

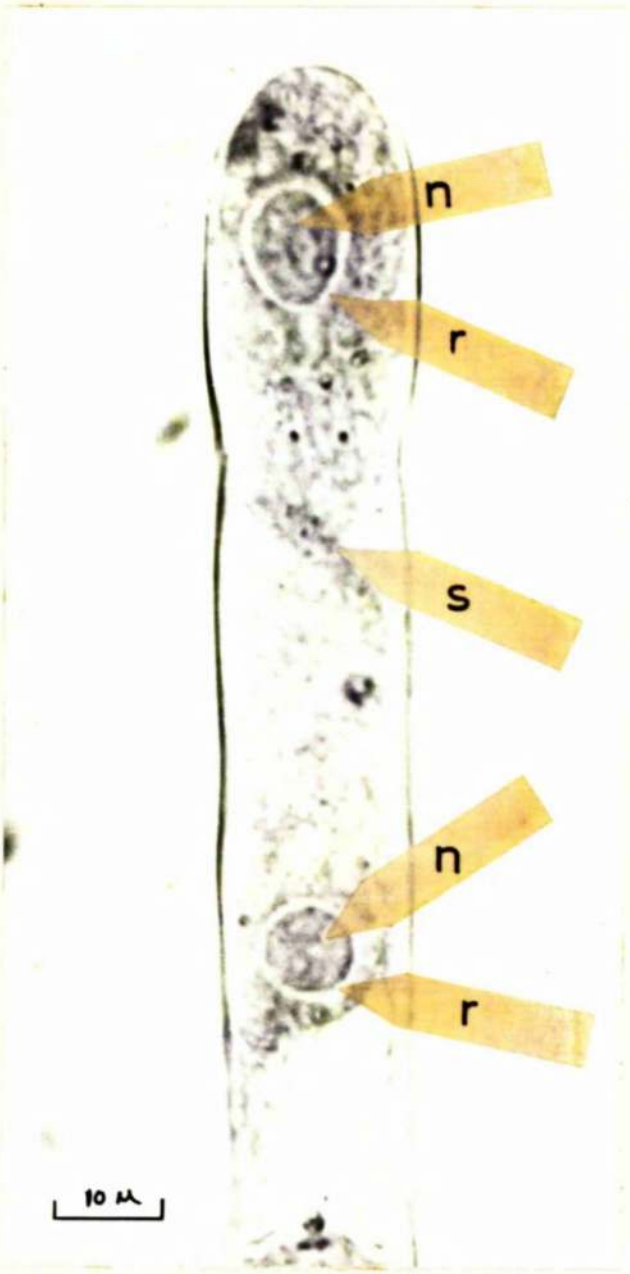
Plate 21.



Early telophase. The top daughter nucleus is seen in polar view, the lower in equatorial view.

(B - ID_2)

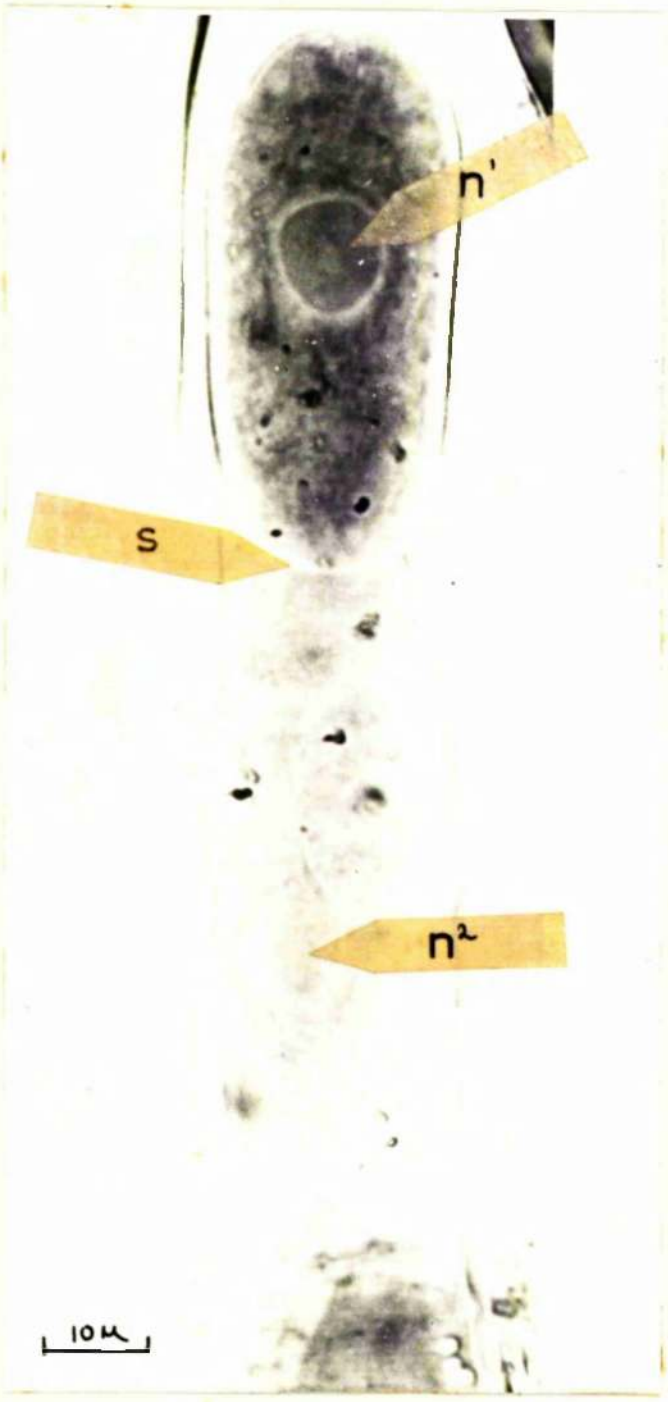
Plate 22.



Telophase. The nucleoli (n) have been reconstituted in both nuclei and the non-staining region (r) is conspicuous. s = septum.

(B - ID_2)

Plate 23.



The septum (s) is more clearly defined; the upper nucleus (n^1) is in very early prophase, the lower (n^2) is entering the interphase condition.

(B - ID_2)

The Periodicity of Mitosis.

It is known that periodic mitotic rhythms exist in certain algal species, division occurring exclusively at night in species of the genera Cladophora and Stigeclonium (Braun, 1851), Spirogyra (Braun, 1851; Tamintzin, 1867; Sachs, 1874; Strasburger, 1880), Zygnema (Kursanow, 1912), and Vaucheria, Hydrodictyon and Ulothrix (Sachs, 1874), whilst Karsten (1918) found three maxima in each 24-hour period for species of Closterium, Cosmarium and Mesotaenium.

Leedale (1959) found that mitosis was almost exclusively confined to the dark period in species of Hydrodictyon, Ulothrix, Mougeotia, Spirogyra Zygnema, Closterium, Cosmarium and Staurastrum grown in biphasic culture.

No publications dealing with periodicity of mitosis in the Rhodophyceae have been mentioned in the literature examined and it appears that no previous work has been done on the subject.

Investigation was made of the periodicity of mitosis in Rhodochorton floridulum grown in culture.

Material:

Cultures were employed which had been established for not less than three months and which had previously been shown to be growing vigorously.

Methods:

The material was divided into 24 equal portions and each portion placed in a 250 ml. conical flask containing 200 ml. of ES supplemented

sea water. The flasks were then so arranged in the cold-cabinet as to receive similar illumination.

At the end of two weeks, fixations were made at hourly intervals during the period of illumination (9 a.m. to 5 p.m.), the contents of an individual flask being used each time, and during the period of darkness in the following 24-hour period.

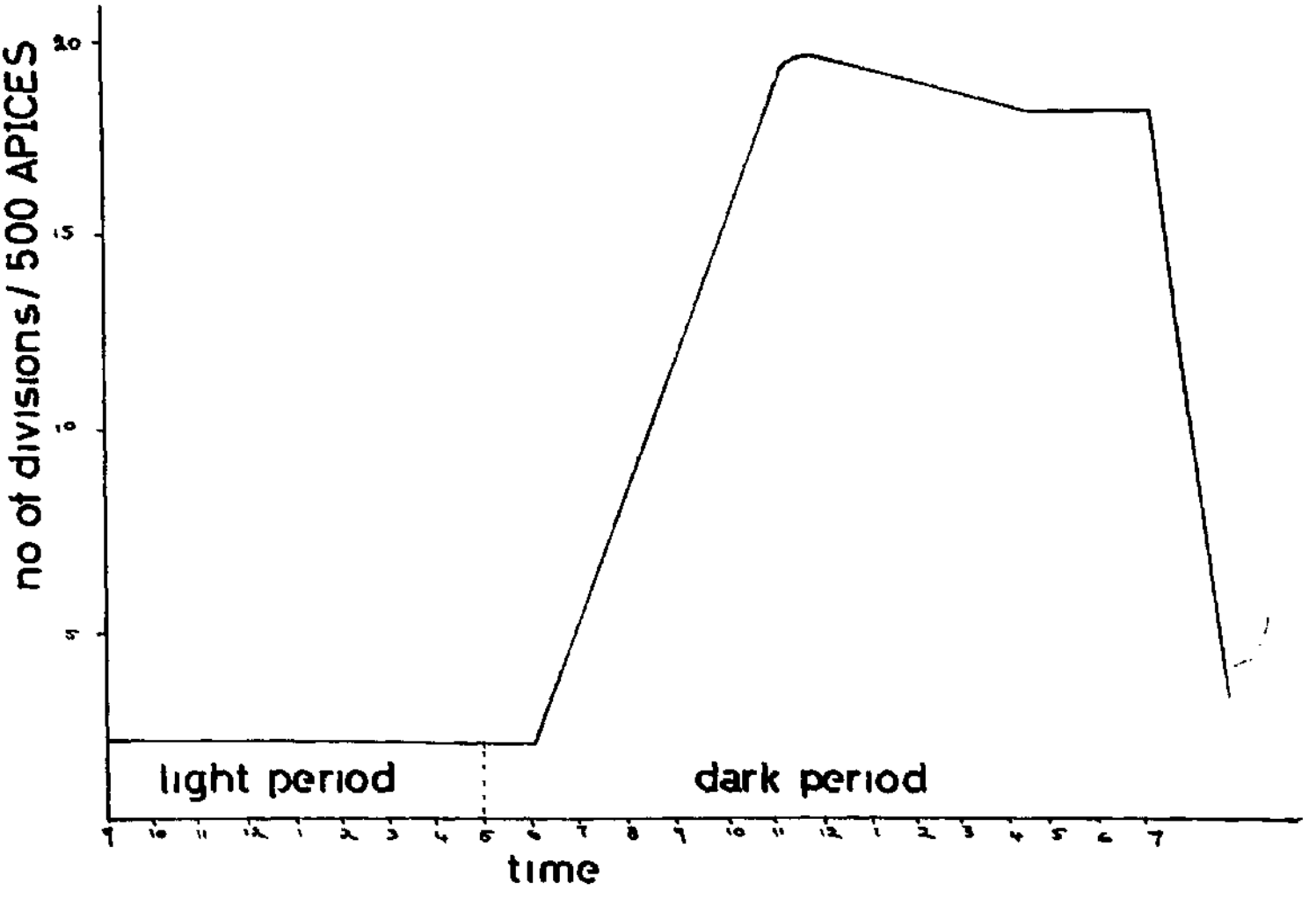
The fixed material was then stained in acetocarmine and a random selection of 500 apices from each sample examined and the ratio of dividing to predivision nuclei determined. Only nuclei in which the chromosomes were visible were taken to be dividing, nuclei containing a greatly enlarged nucleolus (as opposed to the small nucleolus of the interphase nucleus) were taken to be predivisional.* (see P 49) The number of these predivisional nuclei remains relatively constant throughout the 24-hour period in any selection of healthy material.

Results:

The results are shown graphically in figure 15. It was found that the number of dividing nuclei during the light period was small and relatively constant (3/500); during the first hour after the onset of the dark period the ratio remained at the same level, but from the second hour the ratio rose steadily to a first maximum of 38/500 at 11 p.m., falling to a minimum of 18/500 at 1 a.m., before rising to a second maximum of 34/500 between 5 and 7 a.m., after which it declined to the level of the light period.

From the results of similar experiments a like pattern emerged. Although there was some variation in the actual ratios obtained, there

FIG 15



Graph showing the increase in the number of mitotic divisions in the dark period of R. floridulum. Samples fixed hourly.

was always an increase in the number of mitotic figures during the period of darkness as opposed to that obtained during the light period.

Thus Rhodochorton floridulum grown in liquid culture under the conditions described exhibits a similar periodicity of mitosis as has been reported for other species of algae.

2. Nuclear division in the tetrasporangium.

In contrast to mitotic division, the study of nuclear division in the tetrasporangium has for the most part been confined to material fixed on the shore. Towards the end of the fruiting season of 1962-63 methods for obtaining the continued development of sporangia in culture were discovered, and it was possible to examine some of the more important stages of division in freshly fixed, clean material.

Observations.

The nucleus of a young sporangium at the onset of prophase contains a nucleolus of up to 12u in diameter which stains intensely with acetocarmine. Surrounding the nucleolus is a narrow non-staining zone of about 1u in width. The nucleolus always contains at least one conspicuous non-staining region.

During prophase there is a reduction in the volume of the nucleolus and the chromosomes become stainable in the enlarging zone around it. The earliest stages of prophase are not easily observed. This has been noted in other species, e.g. Austin (1960) in Furcellaria fastigiata (L.) Lam.,

but it can be seen that the chromosomes become more intensely stainable as the nucleolus is progressively reduced in volume. Instances of apparent pairing have been observed in some cases. Diplotene has yet to be found, but two preparations showed diakinesis. At this stage the nucleolus is no longer visible although the nuclear membrane persists. A count of c. 10 pairs was made at diakinesis, two of the pairs were observed to have satellite chromosomes.

During prometaphase 1 the bivalents become arranged around the periphery of the nucleolus before moving onto the metaphase plate where they become peripherally arranged with their long axis parallel to the polar axis of the spindle, suggesting terminal or sub-terminal centromeres.

At anaphase the daughter groups move to the opposite poles of the sporangium where they assume a saucer shape, the convex side being directed towards the pole. This has been reported by Naylor (1958) for Halidrys siliquosa Lyngb., and Austin (1960) for Furcellaria fastigiata (L.) Lam. At the poles the clumped chromosomes despiralise, becoming more elongate and faintly staining, while at the same time the nucleolus reappears and gradually enlarges until the predivision condition is re-established. By this time the sporangium has been bisected by a septum.

The length of the interphase varies in the two halves so that the onset of the second division is not always synchronous.

At the onset of prophase 11 the nucleolus shrinks as before and the chromosomes appear in the surrounding zone as elongated strands which condense into intensely staining dot-like bodies arranged around the periphery of the nucleus. These move to the centre of the nucleus and

become arranged as before around the periphery of the plate. The polar axis of the spindle during metaphase II is orientated at right angles to the long axis of the sporangium, but that of the upper is not parallel to that in the lower so that at telophase II the four nuclei lie in different p planes; that in the top right corner of the sporangium being in the same plane as that in the bottom left. The formation of a septum between each of the daughter nuclei results in the production of four spores, which later round off, and during this process of rounding off the four nuclei come to lie in the same plane.

Discussion.

The nuclear phases observed during the first division of the nucleus in the tetrasporangium are distinct from those found in mitosis and although the early stages of meiosis have not been positively recognised, several dialkinesis figures have been observed. Furthermore, counts made at metaphase give a figure of 10 as compared with 20 at mitosis.

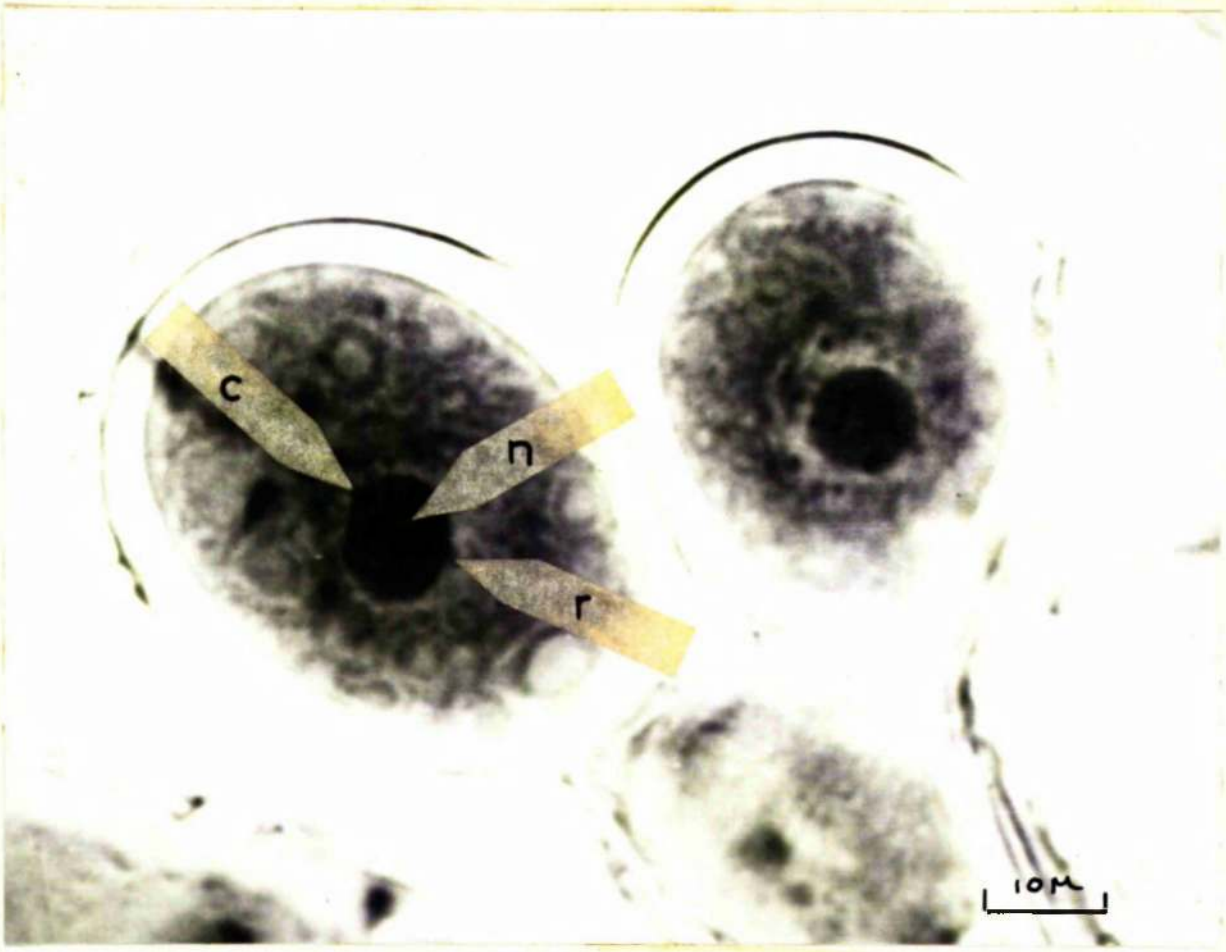
Reduction division therefore takes place in the tetrasporangium of Rhodochorton floridulum (Dillwyn) Nag. and the spores produced are haploid.

Comment on the position of reduction division.

With its incomplete life-cycle Rhodochorton floridulum (Dillwyn) Nag. has usually been classified with those Florideae which show a haplophasic nuclear cycle. If the present observations are correct then Rhodochorton floridulum must be considered a normal tetrasporophyte and the plant included among those Florideae possessing a haplo-diplophasic life-cycle.

Photomicrographs of stages of nuclear division in the sporangium.

Plate 24.

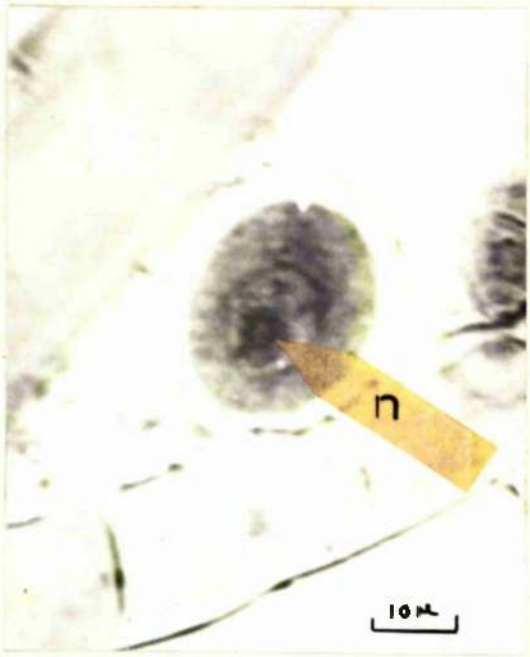


Early 1st prophase in the sporangium, showing the conspicuous nucleolus (n) and the non-staining region (r) containing dark-staining regions (c) of the chromosome stands.

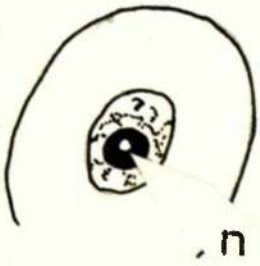
(R - D)

Plate 25.

A



B

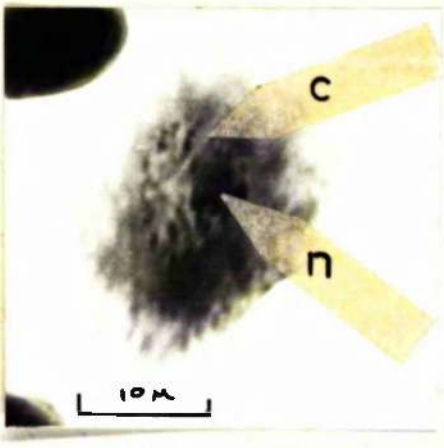


A. Later stage of 1st prophase showing the smaller nucleolus (n) and the non-staining region containing the chromosomes.

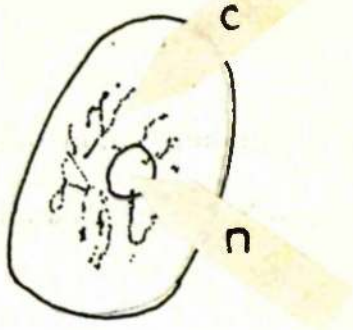
B. Diagramatic interpretation of A.

(B - ID_2)

C



D



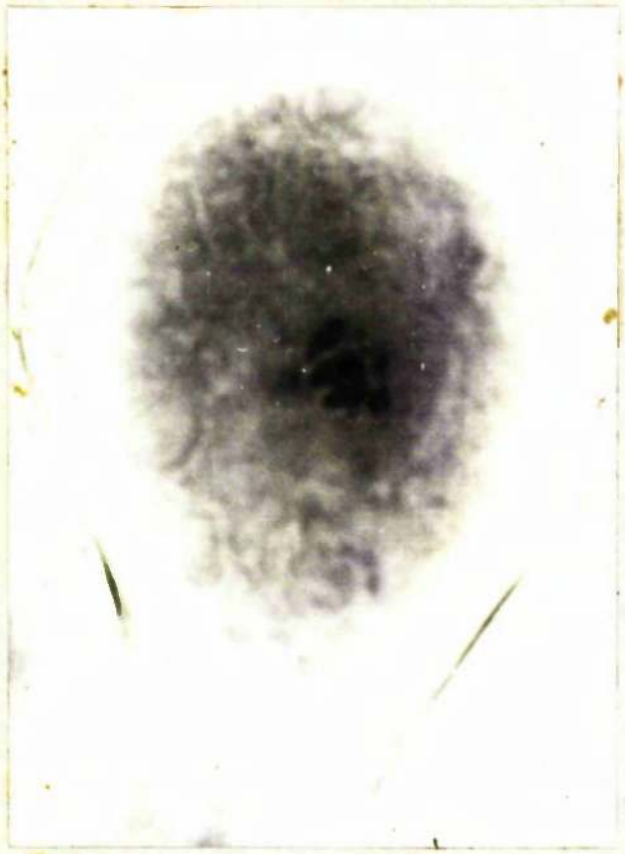
C. Contents of a sporangium pushed out of the case during squashing, showing the nucleolus (n) and the chromosomes (c).

D. Diagrammatic representation of C.

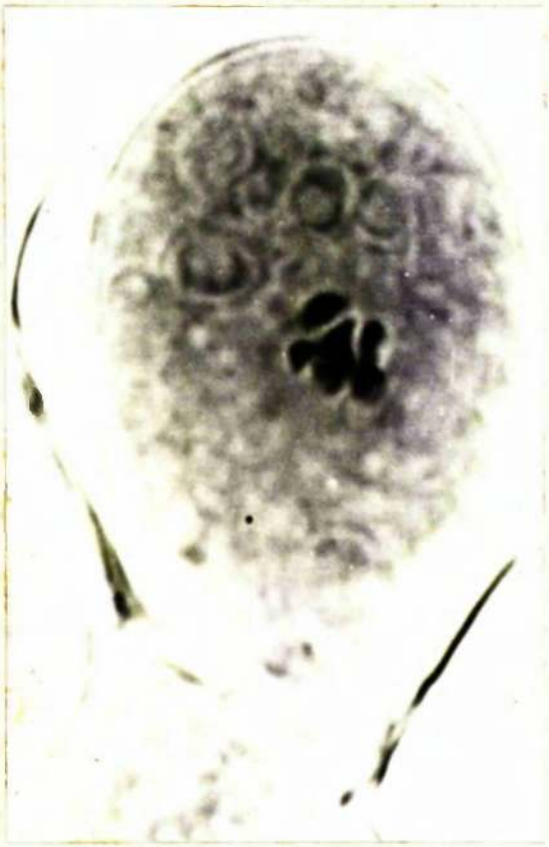
(B - ID_2)

Plate 26.

A



B



A and B are photomicrographs taken at two different levels in a diakinesis nucleus.

C is a diagrammatic interpretation of the same nucleus.

A (R - ID_2)

B (R - D)

C

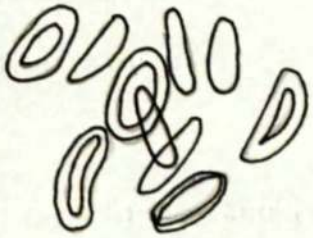
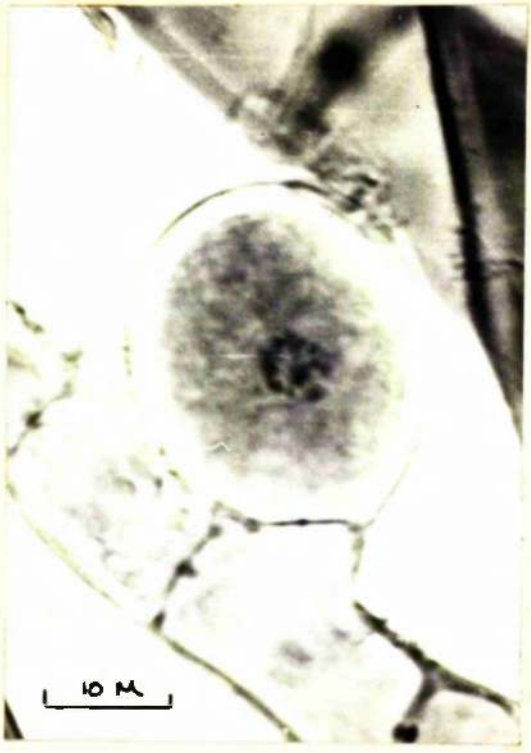
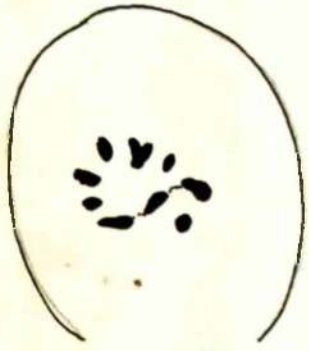


Plate 24.

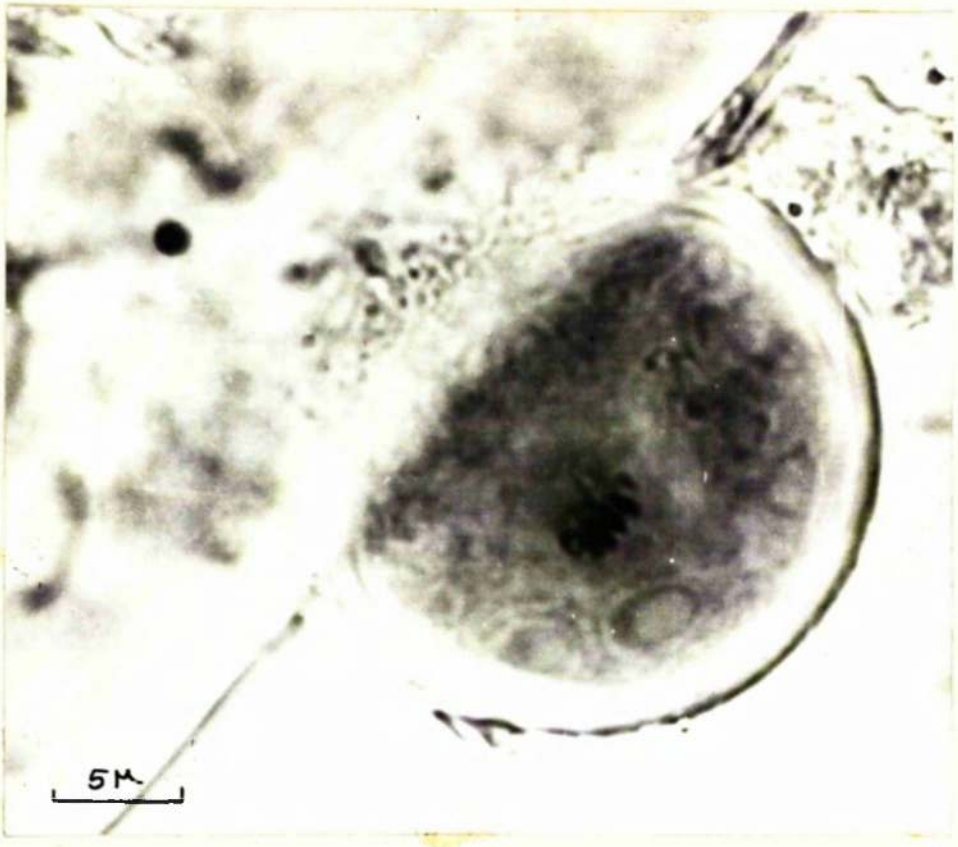
A 

B 

A. Prometaphase showing circa 10 chromosomes arranged around the periphery of the nucleus. B is a diagrammatic interpretation of A.

(B - ID_2)

Plate 28.



1st metaphase in a sporangium showing the rod-like chromosomes. The rest of the sporangium and its supporting filament lie in a higher plane and do not appear on the photograph.

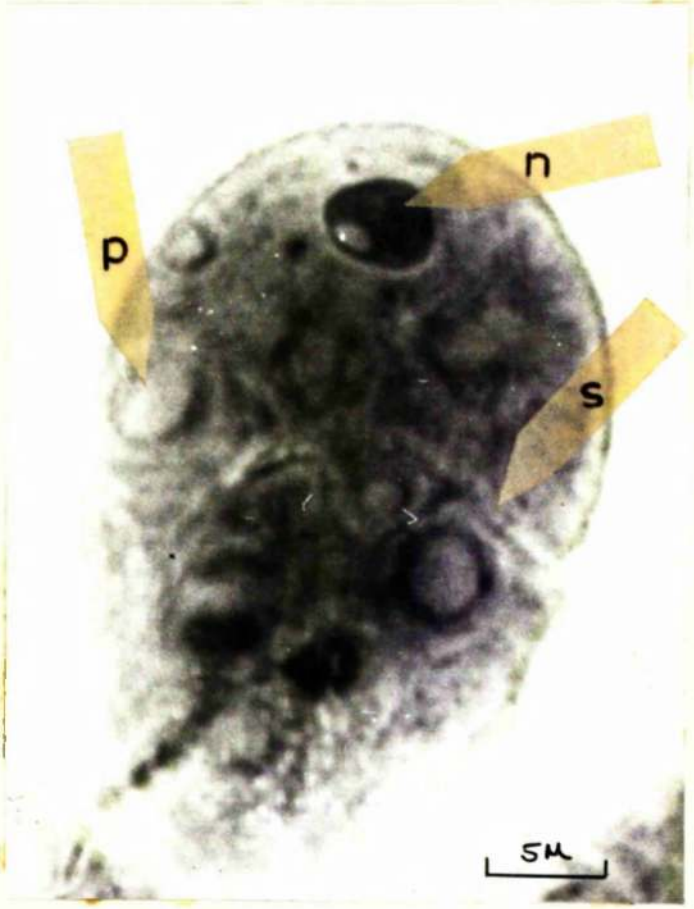
($R - ID_2$)

Plate 29.

1st anaphase in a sporangium, the contents of which have been forced out of the case during squashing.

(R - ID_2)

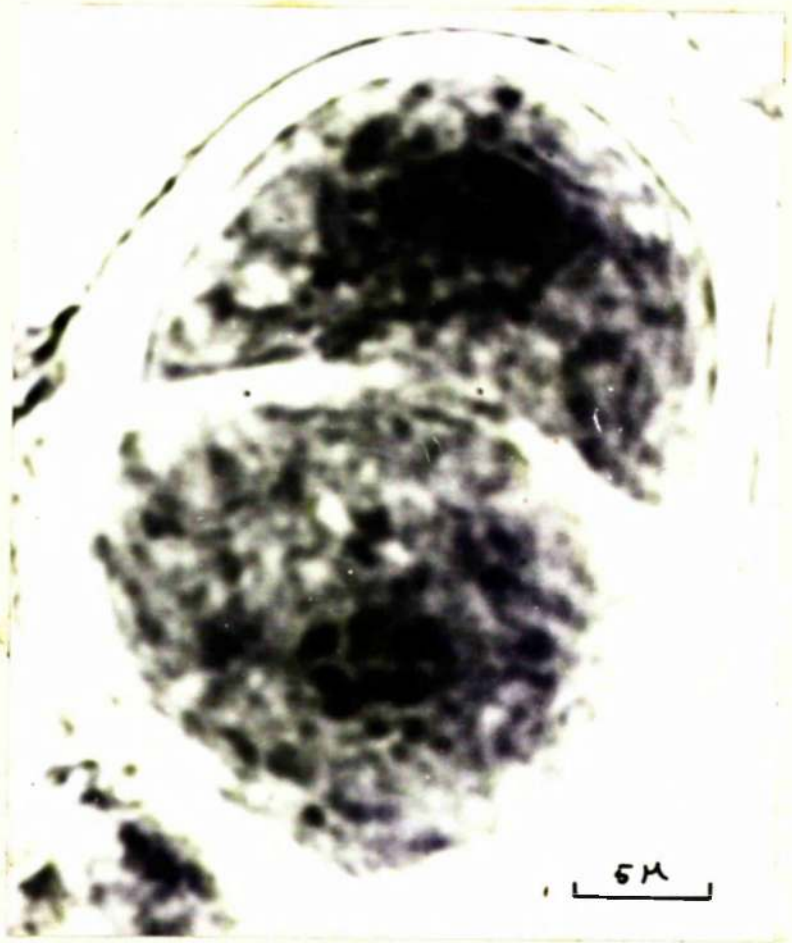
Plate 30.



Early 2nd prophase in the top portion of a sporangium showing the enlarged nucleolus (n); the septum (s) and the pyrenoids (p).

(R - D)

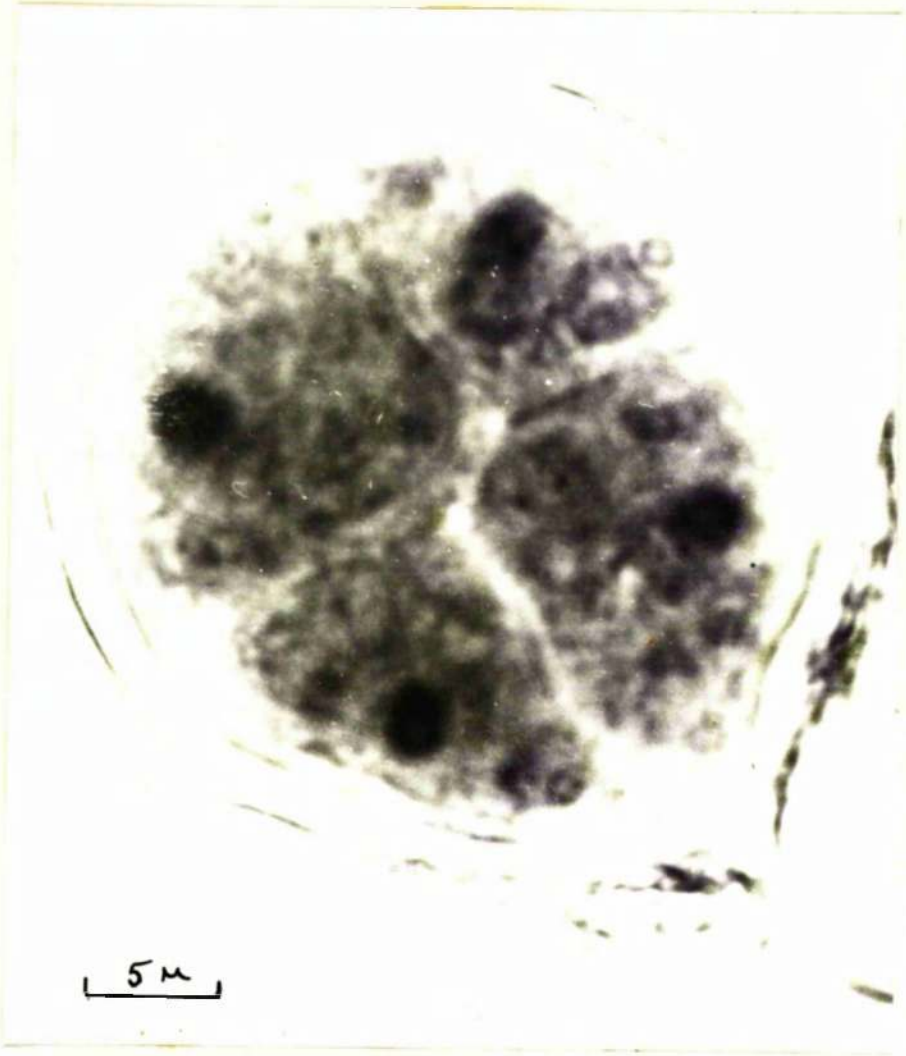
Plate 31.



2nd prometaphase showing around 9 chromosomes in the lower portion.

($R - ID_2$)

Plate 32.



Sporangium showing the four spores shortly after formation.

('R' - ID_2)

PART TWO. RHODOCHORTON PURPUREUM

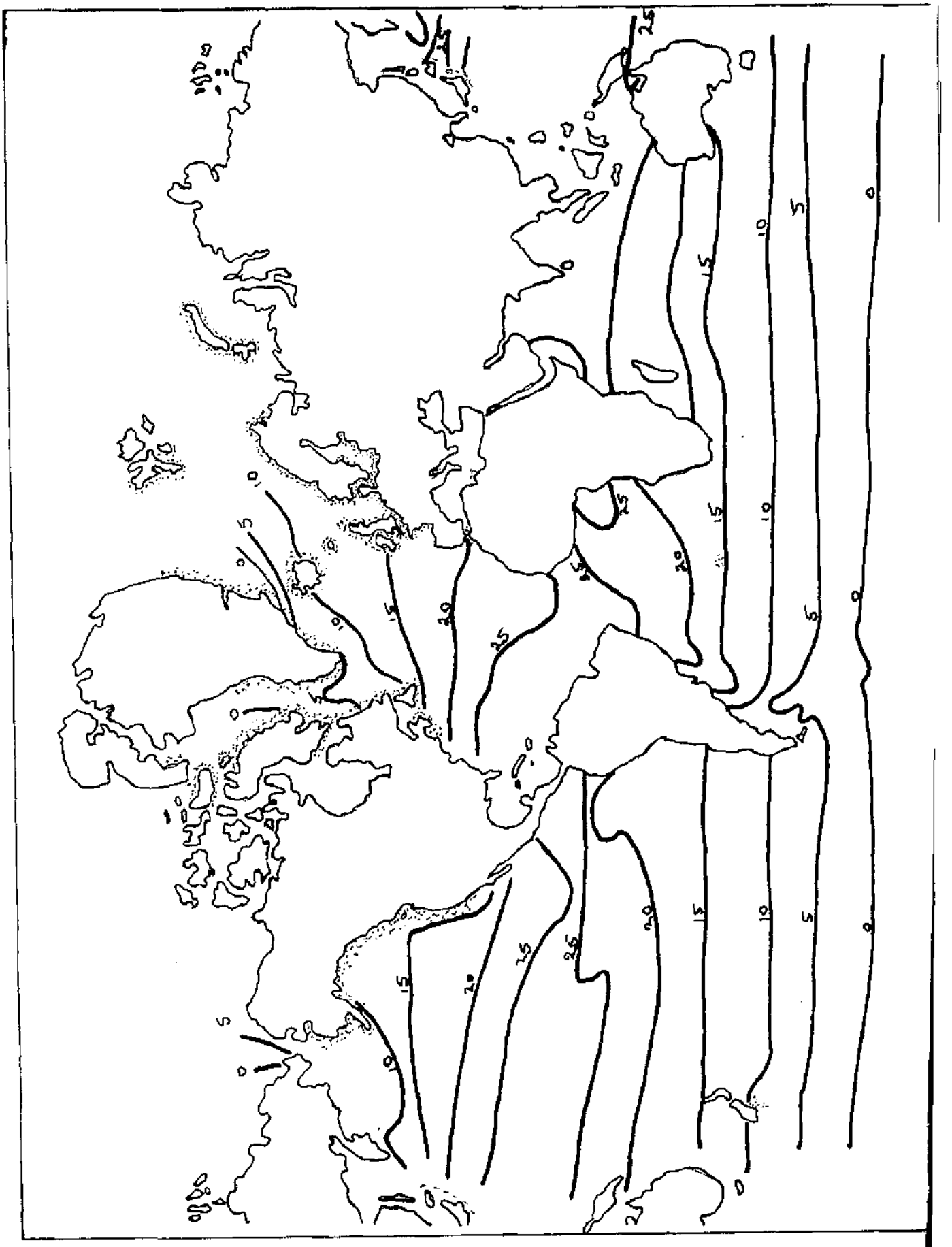
A./1

ECOLOGY AND MORPHOLOGY OF THE TETRASPOROPHYTE.

Figure 16.

Map of the world, after Larousse (1953), showing the August surface water isotherms (continuous black lines) and the distribution of R. purpureum (shown in red; dotted areas are those for which precise localities are recorded; areas delimited by a broken line are those for which precise localities are now known).

FIG.16



Ecology.

Previous observations:

(A) Geographical distribution.

The geographical distribution is shown on the accompanying map (Fig. 16), and Table in the appendix lists the authorities for the regions marked.

It will be seen that R. purpureum has been widely recorded in the northern hemisphere, where its distribution is circumpolar, whereas records of its occurrence in the southern hemisphere are extremely sparse, being two in number, one of which is doubtful (R. bisporiferum Baarsdeth). In general the southern distribution of the species is bounded by the 20°C. August surface temperature isotherm, although it has been recorded from the Adriatic where in August the surface water temperature rises to 25°C. It has been most widely recorded from waters of August surface temperature 15°C. or below, and the overall temperature range of waters in which it has been found is from below 0°C - 25°C. It may be the difference in surface water temperature which is responsible for its unequal latitudinal distribution on the east and west coasts of North America.

Since the species is intertidal, during low water it will be subjected to air temperature and isolation and the air temperature to which it is exposed may be as low as -18°C. (Novaya Zemlya) or as high as 26.7°C. (Tangier).

Rhodochorton purpureum is therefore tolerant of a wide range of temperatures.

As shown in Table 7 the species is also tolerant of a wide range of

salinities.

Table 7.

Locality	Salinity	Authority
Gulf of Finland	4.0°/oo	Waern (1952)
Pacific (Monterey)	34.0°/oo	Sverdrup (1942)
Spitsbergen	35.3°/oo	Kjellman (1883)
British Isles	34-35.0°/oo	Larousse (1953)
Mediterranean (West)	36.0°/oo	Guilcher (1957)
Adriatic	38.0°/oo	Larousse (1953)

The salinity figures listed in Table 7. refer to seas, but Rhodochorton purpureum has also been recorded from fresh water (Jonsson, 1902; Rosenvinge, 1900; Borgesen, 1902; Waern, 1936 and 1952; Gillham, 1954).

As Kjellman (1883) has pointed out, in the Arctic regions of its distribution the species must necessarily spend several months in constant darkness and so must be capable of adapting itself accordingly. R. purpureum is therefore a most adaptable species, capable of growth under a wide range of physical conditions. This adaptability is further illustrated by its distribution on the shore where it has been shown to occupy a wide vertical range; from a depth of 36m. (Rosenvine, 1923-24), through the intertidal region and upwards into the supralittoral region often occurring well above the limits of M.H.W.S. (Jonsson, 1902; Rosenvinge 1900; Borgesen, 1902). It is also accepted by many authorities as being capable of growing under purely terrestrial conditions (as R. islandicum Rosenvinge; Byssus purpurea Lightfoot).

Habitat.

In the sub-littoral the species is commonly found as an epiphyte or pseudo-endophyte on the stipes of Laminaria spp. (the endophyte was originally considered a separate species - R. parasiticum Batters, but Jonsson (1902) has presented evidence which shows that it is not), and it is also frequently found on stones and shells (Kjellman, 1883; Waern, 1952; Lund, 1959; Sundene, 1959).

In the intertidal region R. purpureum is most commonly found growing on rock under the dominant fucoids, or in shaded crevices, both in exposed (Jonsson, 1902) and sheltered localities (Sundene, 1959). It can also be found as an epiphyte on the bases of Fucus vesiculosus, Sphacelaria arctica, Cladophora rupestris, etc. as well as on Membranipora crustulenta (Waern, 1952). Frequently the tufts are intermixed with other small algae such as Sphacelaria cirrhosa and Catenella repens, and are seldom free from epiphytes, especially diatoms and blue-greens. Its characteristic habitat is the marine cave (Svedelius, 1901; Lami, 1940) and it is here that the species attains its greatest development in extent of coverage (though not in thallus size), becoming the dominant or only species present, and covering the roof and walls often in unbroken patches several yards square (Borgesen, 1902; Rosenvinge, 1900; Hamel, 1925; Rees, 1935; Anand, 1937; Waern, 1952), and up to 5 mm. high (Waern, 1952). Anand (1937) reports finding R. purpureum in small caves on the south coast of England where the light intensity is as low as 2.5% of that on the open shore.

In the supra-littoral the species normally occurs in rock crevices

or fissures, on the under sides of overhanging rocks, or in caves, mostly in places where it can be reached by spray during high water and gales, but in many places it is doubtful whether sea water reaches the plants except during storms of unusual violence (Borgesen, 1902). In such situations as these the plant may be periodically soaked with rain-water (Borgesen, 1902).

Rhodochorton purpureum has therefore a wide vertical distribution on the shore and can exist in a variety of habitats. These different habitats exert modifying influences on the development of the plant with the result that ecological forms arise, and it is to the existence of these ecological forms that much of the nomenclatural confusion can be ascribed. The major ecological forms are as follows:

(1) Rhodochorton purpureum f. purpureum (Byssus purpurea Lightfoot, 1777; Callithamnion pupurea Harvey, 1841).

As mentioned in the general introduction, the type specimen of R. purpureum (Byssys purpurea Lightfoot) is missing and for this reason it cannot be considered certain that Papenfuss (1945) and others are correct in considering Lightfoot's plant to be a form of R. rothii (Turton) Naeg. But this assumption has now been generally accepted and there is certain indirect evidence to support it.

The original specimen was collected from the Abbot McKinnon's tomb, near Iona Cathedral. The tomb is no longer extant and therefore collections cannot be made from exactly the same site; however, in 1826, Greville visited the island and made a collection of an alga which he found growing on the cathedral walls. The two descriptions which follow

are of this material; the first being after Rosenvinge (1900), the second being the results of an examination undertaken during this investigation of a specimen lodged in the algal herbarium of the Department of Botany, the University, Glasgow and labelled:

'Trentepohlia purpureum Ag.,
Iona (Cathedral)
Dr. Greville.'

(A)

The thallus formed an incrustation, up to one millimeter thick, on rock.

Cell size:

(a) The prostrate system.

The cells of the prostrate filaments are short and swollen or long and cylindrical. In the latter case they are from 8-12u broad, in the former they are much broader (no figures given).

(b) The erect system.

The cells of the erect filaments are 8-12u broad and 2-4 times as long as broad.

Branching:

(a) The prostrate system.

The prostrate filaments are much branched, the branches being often fused into a pseudoparenchymatous mass.

(b) The erect system.

The erect filaments are simple or sparingly branched.

Fructification:

The material examined by Rosenvinge was sterile.

(B)

The Glasgow specimen in parts was similar to that described by Rosenvinge, but in addition the following features were noted:

The erect filaments as well as being simple or sparingly branched, were also frequently branched throughout, in some instances up to three consecutive cells bearing laterals in a secund manner.

The cells of the erect filaments were generally cylindrical, but occasionally were short and inflated. Cell dimensions varied between 6.5-14.5u long, by 6.5-10.5u broad.

Tetrasporangia were absent, but several of the apical cells of the primary filaments were swollen in a manner similar to that found in the early stages of tetrasporangial formation in material of R. purpureum collected from the supra-littoral at Esha Ness in Shetland.

There is nothing about Greville's material which suggests that the plant is not a specimen of a high level form of R. rothii (Turton) Naeg. similar to those found in the supra-littoral and in caves, and since the nature of the site is very similar to that from which Lightfoot collected Byssus purpurea, as well as being in close proximity to it, the assumption has been made (Rosenvinge, 1900) that the two collections are of the same plant, in which case Papenfuss (1945) is correct in applying the name 'purpureum' to the plant originally called 'rothii'.

(2) Rhodochorton purpureum f. typica, R. rothii (Turton) Naeg.;

Borgesen, 1902; Rosenvinge, 1923-24; Hamel, 1925; Drew, 1928.

Morphology:

The plants form dense flat expanses, up to 1.5 cms. in height on rock and wood.

Cell size:

(a) The prostrate system.

The cells of the prostrate system are variable in size and shape, being short and inflated or else long and cylindrical; normally between 15-30u in breadth, but occasionally greater, and from 1.5-3 times longer than broad.

(b) The erect system.

The cells of the erect filaments are variable in size and shape, often barrel-shaped, frequently long and cylindrical; breadth from 6-17u in Danish waters (Rosenvinge 1923-24), from 10-29u on the Faeroes coast (Borgesen 1902), and from 10-20u on the Pacific coast of North America (Drew 1928). Cell length varies from 1.5-3 times as long as broad.

Branching:

(a) The prostrate system.

The filaments of the prostrate system are sparingly to frequently branched, the branches often densely interwoven.

(b) The erect system.

The erect filaments are generally unbranched at the base and sparingly branched above, the laterals typically arising from within a limited length of the main axis (Borgesen 1902), secondary laterals infrequent.

Fructification:

Tetrasporangia, borne terminally and laterally on much branched laterals arranged alternately or oppositely in the sub-apical regions of the erect filaments; ovoid to sub-spherical in shape; variable in size: from 25-28u long by 14-19u broad (Rosenvinge 1923-24); 26-32u long by 17-21u broad (Drew 1928).

Habitat:

On rock and wood in the littoral region, growing under Fucus spp. or in shaded crevices and in caves on the walls covered by the tide (above this level forma purpureum is often found).

It has been recorded from the sub-littoral, growing to a depth of 36m. on stones and shells. The sub-littoral material is generally smaller than that from the littoral both in overall height (up to 1mm.) and cell size (Breadth from 7-13u, length 2-4 times breadth).

(3) Rhodochorton purpureum f. globosa: Kjellman 1883; Borgesen 1902; Jonsson 1912; Printz 1926.

Morphology:

The plants form small semi-globular, solid masses about 2mm. in diameter, often growing together at the edges so as to form irregular crusts.

Cell size:

(a) The prostrate system.

The cells of the prostrate filaments may be barrel-shaped or cylindrical; dimensions are variable, similar to forma typica.

(b) The erect system.

The cells of the erect filaments are variable in form from barrel-shaped, averaging 14u in diameter, to cylindrical, 11-17u broad and from 2-3 times as long as broad.

Branching:

(a) The prostrate system.

The prostrate system is composed of densely interwoven branched filaments, sparingly to frequently branched.

(b) The erect system.

The erect filaments are rarely simple, more often repeatedly branched in a fastigiate manner; the laterals are often 1-4 times branched and are densely interwoven. The majority of the laterals arise from near the base and equal the main axis in length, but unlike it, taper towards the apex where the cells are half as broad as at the base.

Fructification:

Tetrasporangia borne in the same manner as in forma typica.

Habitat:

This form is typical of rock surfaces exposed to heavy surge and wave splash, from the upper mid-littoral to above high water mark.

4. Rhodochorton purpureum f. intermedium: Kjellman 1875; Rosenvinge 1923-24; Jonsson 1902; Lund 1959.

Morphology:

Larger plants than the other forms, from 1-3 cms. in height, forming loose mats often of considerable extent.

Cell size:

(a) The prostrate system.

The filaments of the prostrate system are composed of shorter or longer cells of varying shape, sometimes differing little from those of the erect filaments, more often irregular and then as figured by Borgesen (1902, fig. 63), and Rosenvinge (1923-24, fig. 388).

(b) The erect system.

The cells of the erect filaments are between 12 and 16u broad and up to six times as long as broad, generally cylindrical.

Branching:

(a) The prostrate system.

The basal filaments are branched in a manner similar to the other forms.

(b) The erect system.

Branching may be irregular throughout the length of the main axis, or it may be confined to the upper regions; successive laterals may be secund or alternate; laterals arising from near the base are generally equal to the main axis and similar to it in cell size and mode of branching.

Fructification:

Tetrasporangia borne laterally and terminally on much branched laterals which may be clustered (Kjellman 1875) or scattered (Lund 1959).

Habitat:

On rock in the littoral region, also reported from fresh water (Borgesen 1902).

Previous workers - e.g. Borgesen (1902) - have reported the occurrence of morphological intermediates between the various forms that exist on any one shore, and this suggests that the forms arise as a result of varying environmental factors. In the following section it will be shown that under the constant and similar conditions of culture there is a tendency for the differences between the forms to become less marked and this observation would seem to support this suggestion.

The ecology and morphology of those forms cultivated for cytological examination, with observations on the morphological changes induced by the cultural conditions.

1. Rhodochorton purpureum f. purpureum.

Habitat:

(a) On the top and the sides, down to 14 feet below soil level, of a well in the Broch court at Jarlshof, Shetland.

(b) On rocks and in crevices on the cliff tops about 50 feet above H.T.S., Esha Ness, Shetland.

Morphology:

The plants formed extensive incrustations up to 0.24 mm. high on rock and soil.

Cell size:

(a) Prostrate system.

The cells are irregular in size and shape, varying between 10.5 and 19.5u in length, and between 10.5 and 16.0u in breadth.

(b) Erect system.

The cells are either barrel-shaped or cylindrical, between 10 and 19.5u in length, and 7.5 and 10.5u in breadth.

Branching:

(a) The prostrate system.

The prostrate system is irregularly, sparingly to frequently branched, the branches being interwoven into a pseudo-parenchymatous mass.

(b) The erect system.

The upright filaments are simple or occasionally branched from near the base, the branches being as broad as or slightly less broad than the main axis.

Fructification:

The specimens from both sites were sterile when collected.

Behaviour in culture:

The following observations were made after the material had been in culture for three months:

(a) There was an increase in cell length up to a maximum of 30u, all cells being longer than the longest cell previously produced.

(b) The cells produced in culture were uniformly broad in any filament, the average breadth being 9.0u.

(c) All cells were cylindrical.

(d) The production of laterals was frequent in the apical regions of the filaments (Fig.17c).

(e) Tetrasporangia, 15.0-18.0u long, by 12.0u broad, had formed singly and terminally on the main filaments, or laterally and terminally on short sub-apical laterals (Fig.17a).

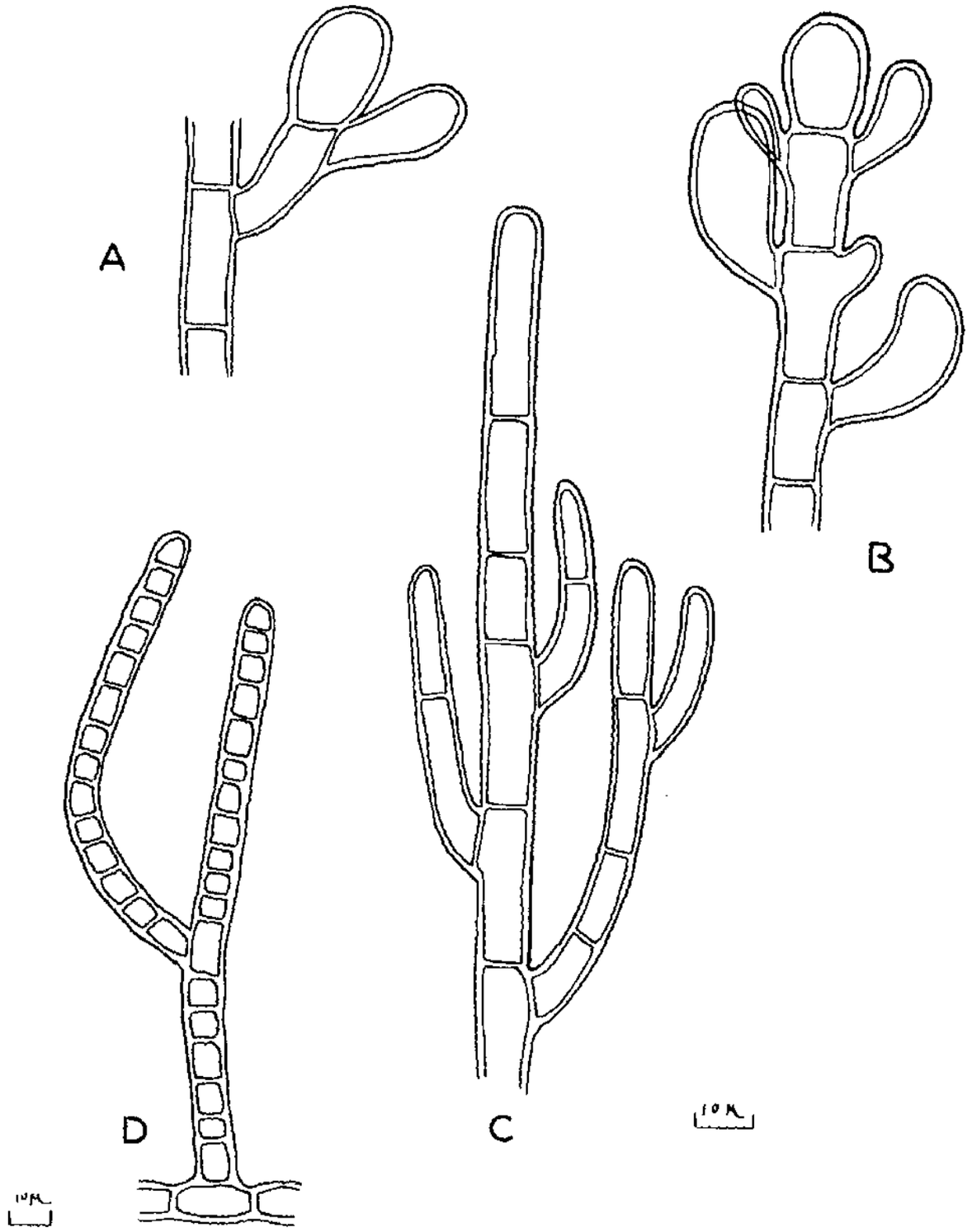
Figure 17.

A. Drawing of a young tetrasporic lateral of R. purpureum f. purpureum (from culture).

B. Drawing of the apical region of a primary erect filament of R. purpureum f. purpureum showing the production of tetrasporangia directly from the cells of the filament (from culture).

C. Drawing of the apical region of a vegetative filament of R. purpureum f. purpureum showing the formation of primary and secondary laterals (from culture).

D. Drawing of a portion of the specimen of R. purpureum f. purpureum collected by Greville and now in the Herbarium, Glasgow, showing the marked variation in cell dimensions.


A
B
C
D
10 µ
10 µ
10 µ

2. Rhodochorton purpureum f. globosa.

Habitat:

(a) On the undersides of isolated boulders and on the shaded sides of rock ribs from the upper mid-littoral to the lower mid-littoral at Farland Head (Ardneil Bay).

(b) On the undersides of boulders and overhanging rock masses, and in crevices in the rocks on both wings of Portmahomack Bay.

Morphology:

The plants form hemispherical, solid bodies up to 4 mm. in height and 6 mm. in diameter, often uniting at the edges to form an irregular crust.

Cell size:

(a) The prostrate system (Fig. 18B).

The cells are variable in size and shape, barrel-shaped or cylindrical, 15-30u in length by 10-20u in breadth, except in the rhizoidal filaments where they may be over 60u long by 5-8u broad.

(b) The erect system.

The cells of the erect system are in the range of 18-27u in length by 15-18u in breadth, and may be barrel-shaped or cylindrical.

Branching:

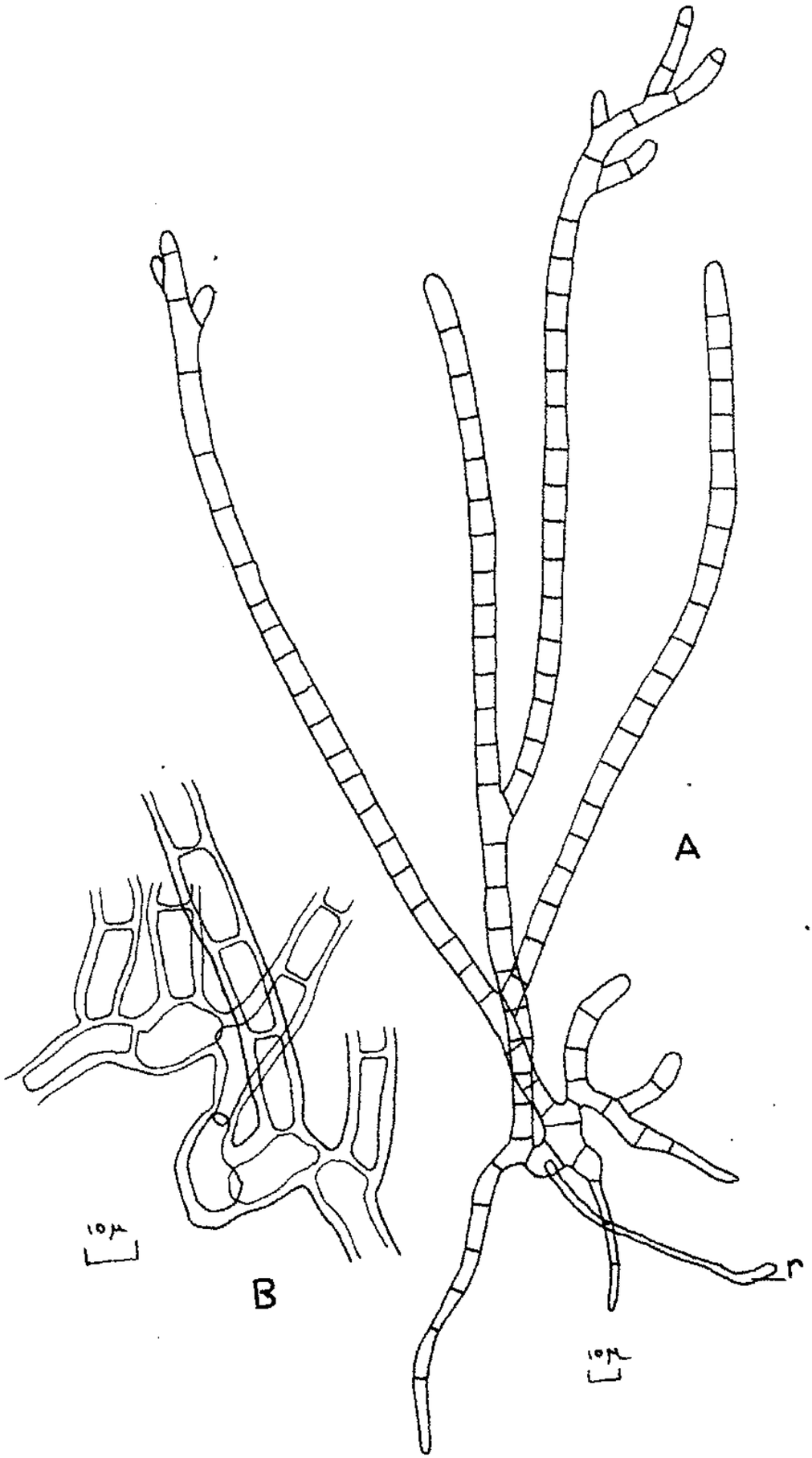
(a) The prostrate system.

The prostrate filaments are irregularly, sparingly to

Figure 18.

A. Drawing of a portion of a plant of R. purpureum f. globosa showing the system of branching of the erect filament and the production of rhizoidal filaments (r) from the prostrate system.

B. Drawing of a portion of the prostrate system of f. globosa showing the shape of the cells and the production of erect filaments.


A
B
۱۵μ
۱۵μ

frequently branched, the branches becoming interwoven to form a pseudo-parenchymatous mat. The majority of the laterals are equal in breadth to the main axis; sometimes, however, slender rhizoidal branches are formed.

(b) The erect system (Fig. 18A).

The upright filaments are sparingly to frequently branched throughout, or else branching may be confined to the basal region. Occasionally abundant branching takes place in the sub-apical regions of the filaments resulting in the formation of fastigiate tufts.

Branching is irregular on the whole, although occasionally it may be alternate or secund for a considerable length of a filament.

The laterals may be equal to, or shorter than the parent axis, generally they are equal.

The breadth of a lateral may be equal to or less than the parent filament at the point of initiation, but the breadth generally decreases towards the apex.

The majority of laterals are erect, or erecto-adpressed; there exist, however several types of rather specialised function:

(a) Spreading.

(b) Recurved - descending.

(c) Downward-growing - descending.

(d) H-shaped.

These branches are similar to those found in the typical form of R. floridulum and serve the same function, that is, they bind together the individual erect filaments into the tightly packed globose masses typical of the form.

The initiation of the erect laterals is from a point near the top of the parent cell, the angle of initiation being normally 45° or less. If it is greater, the lateral undergoes pronounced growth-curvature until the plane of growth is parallel to that of the parent axis, except in the case of spreading laterals. Recurved laterals are initiated in a similar manner, the direction of growth is however reversed after the production of several cells in the plane of initiation so that subsequent growth is in a downward direction. In contrast, downward-growing laterals arise from a point near the bottom of the parent cell and growth is in a downward direction from the first. Both recurved and downward-growing laterals can bear erect and downward-growing secondary branches.

The H-shaped laterals arise in the same manner as in R. floridulum, and each arm may bear secondary laterals of any of the types described.

These non-erect laterals and the branch systems which they bear, become entangled with the branches of neighbouring filaments as do the similar laterals of R. floridulum, and this form, unlike the others examined, is therefore a sand-binder.

Rhizoidal branches are commonly found, similar to those described by Rosenvinge (1900). These can be either erect or descending and generally arise from an intercalary cell of the filament.

Fructification:

The tetrasporangia are borne terminally and laterally on short, densely branched laterals arranged alternately or oppositely below the apices of the primary erect filaments and their branches. They are rather variable in size, measuring between 25-36u long and 14-20u broad.

Following the release of the spores a second sporangium may be formed within the first, and if this does not take place, the vegetative growth of the filament is generally continued by the assumption of meristematic activity of the cell immediately below the sporangium, a feature which was also observed by Borgesen (1902).

Vegetative reproduction:

Reproduction by fragmentation is the most commonly occurring form of vegetative reproduction and is of two types:

(a) Fragmentation resulting from mechanical damage to, and the death of, one or more cells of the erect filament. The apical portion of the filament, above the line of damage, produces a rhizoidal process from the first entire cell above the damaged region and this grows downwards until the apical cell comes into contact with a solid body (frequently another filament) whereupon it flattens so as to mould itself into intimate contact with it

Figure 19.

Drawing of a primary lateral (A) of f. globosa showing:

(a) a spreading lateral with two recurved secondary branches.

(b) a downward-growing secondary lateral bearing both upward and downward-growing tertiary laterals.

(c) a portion of the main filament.

(d) a normal erect lateral.

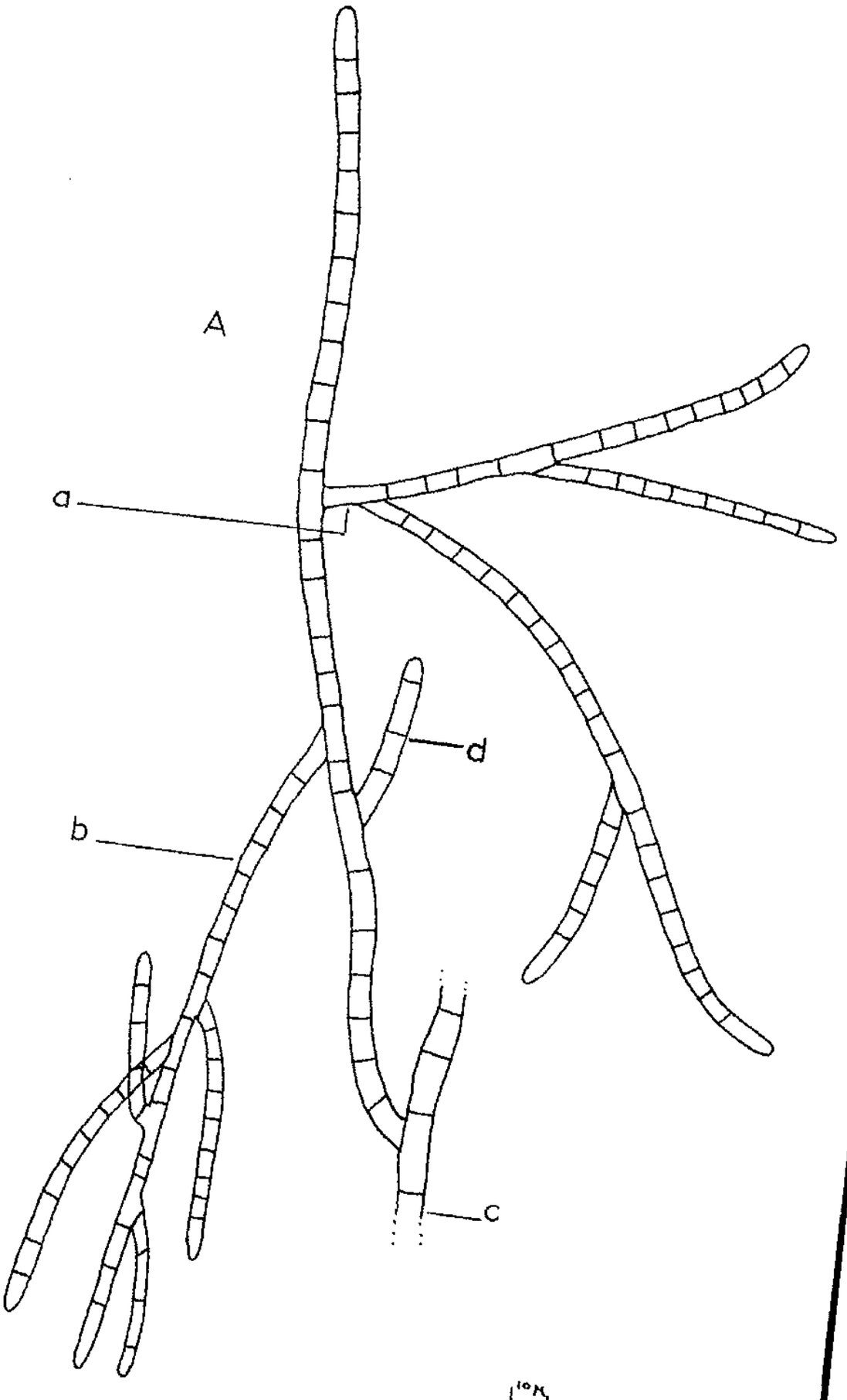

A
a
b
c
d

Figure 20.

Regeneration by fragmentation.

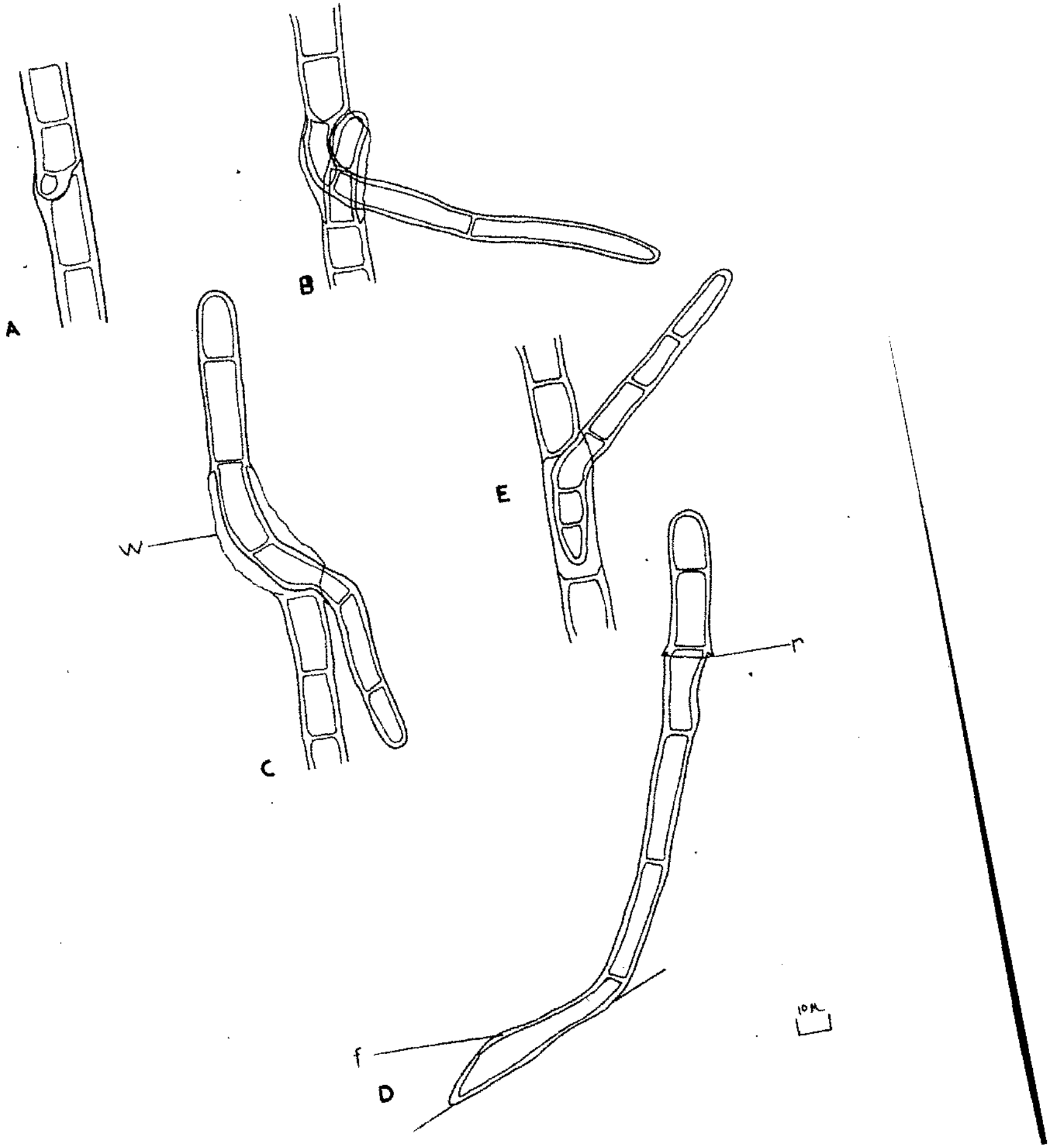
A, C, stages in fragmentation by the production of a downward-growing process.

B. Early stage of fragmentation by the production of both upward and downward-growing processes.

D. Attached fragment showing the attaching cell (f), and the 'collar' (r) of the old outer wall of the filament.

E. Fragmentation by the internal production of a filament from the contents of a single cell.

w = the outer wall.


A
B
C
W
E
r
f
D
10 µ

and attaches itself by the secretion of a colourless mucilage. Up to this point it seems that the apical cell of the fragment remains dormant, but after the rhizoidal process has become attached, meristematic activity is resumed and a new plantlet produced. A similar type of fragmentation is recorded by Rosenvinge (1923-24) but he did not observe the stages in attachment.

(b) Fragmentation as the result of intercalary meristematic activity (Fig. 20 A,B,C,D).

This type is similar to that described above with the exception that there is no obvious damage to the filament prior to the initiation of meristematic activity, and the rhizoidal process grows into what appears to be a healthy cell disrupting its content before piercing the outer wall. Eventually the wall of the pierced cell fractures, freeing the apical portion which becomes fixed to a solid body in the same manner as has been previously described. The cell below the pierced cell may produce an upward growing filament at the same time as the downward-growing rhizoidal process is being formed, or this may be delayed until separation has taken place. The upward-growing filament may be of normal width or it may be rhizoidal. The early stages in the formation of the downward-growing filament have been recorded by Rosenvinge (1923-24).

The factors responsible for the initiation of meristematic activity in intercalary cells of an undamaged filament are obscure, but it may be that there is a physiological change in the cell

Figure 21.

Vegetative reproduction.

a,b,c. Stages in the formation and germination of units formed by the rounding-off of the protoplasm of single cells.

d. Plantlet formed by fragmentation showing the production of a downward-growing lateral and the rhizoidal nature of the regenerated filament. Branching appears to take place if the apical cell of the regenerated filament does not come into contact with a suitable substrate.

a.1. The rounded-off contents of apical cell.

2. Dead sub-apical cell.

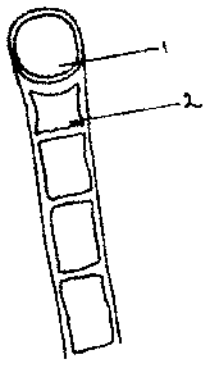
b.1. Filament produced by the rounded-off contents of the apical cell.

c.1. The rounded-off contents of an apical cell.

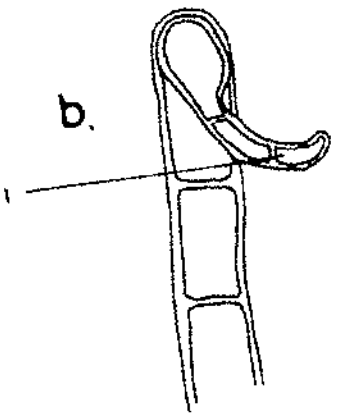
2. Germination tube.

d.r. The collar of the old outer cell wall.

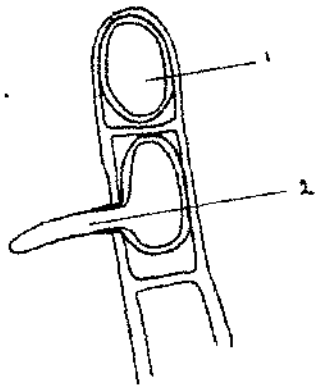
a.



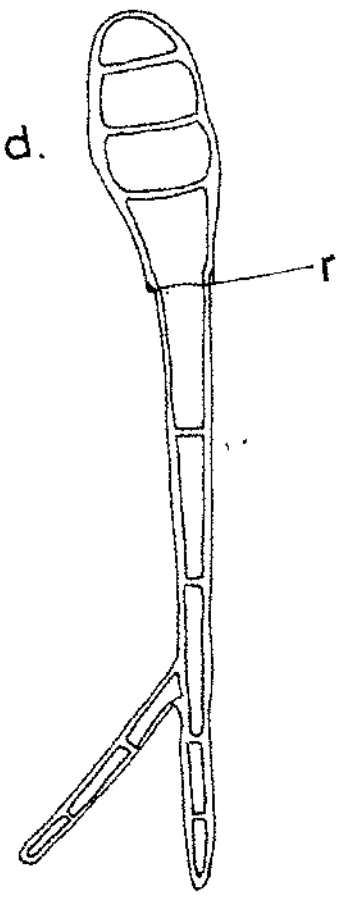
b.



c.



d.



below the one which becomes meristematic resulting in a physiological situation similar to that produced by damage. Unlike type (a), (b) is commonly found in culture where the chances of mechanical damage are slight.

It seems likely that both regeneration following mechanical damage and fragmentation of the type just described are under hormonal control and since little is known of hormonal systems in the Rhodophyceae (Brian, 1963) further investigation of the processes involved in fragmentation is likely to be rewarding.

Although fragmentation is the most commonly occurring type of vegetative reproduction two other types have been found:

(1) On one occasion a specimen was collected which showed a single instance of an unusual type of vegetative reproduction; it seems that the protoplasm of a single cell had given rise to a short filament which had then grown through the wall of the parent filament as shown in Figure 20E.

(2) Material collected at Spiggie on Shetland showed a type of reproduction which has not been found in material from any other site:

The protoplasm of cells, particularly cells in the apical regions of the filament, rounds off and secretes a cell wall. The unit thus formed later produces a germ tube which pierces the wall of the filament (Fig. 21 a,b,c). The fate of the units is not known.

Behaviour in culture:

After six months in culture it was found that there was an increase in the length of the cells produced with an accompanying reduction in breadth; length 36-45u, breadth 13-15u. The dimensions of the cells in any one filament were more uniform, and all cells were cylindrical.

The newly formed portions of the filaments were not bound together into the globose form, but remained separate.

There was an increase in the number of laterals produced in the apical regions of the filaments, all laterals being erect or adpressed.

3. Rhodochorton purpureum f. intermedium.

Habitat:

In the mouth of a supra-littoral cave at Queen's Bay, Lerwick, Isle of Shetland, growing on rocks and soil in association with Cratoneuron filicinum (Hedw.) Roth. The growth was most luxuriant on rock slabs under a jet of fresh water.

This site was several feet above that from which f. globosa was collected.

Morphology:

The plants formed a continuous expanse up to 1 cm. in height over an area of 1 sq. yard.

Cell size:

(a) The prostrate system.

The cells of the prostrate system are irregular, isodiametrical or cylindrical, between 21-35u long by 12-30u broad.

(b) The erect system.

The cells of the erect system are cylindrical, between 25.5-48.0u long by 13.5-16.5ubroad.

Branching:

(a) The prostrate system.

The prostrate filaments are irregularly, sparingly to frequently branched, the branches becoming densely interwoven to form a pseudo-parenchymatous mat.

(b) The erect system.

The upright filaments are irregularly branched throughout; the branches erect or erect-adpressed or spreading, 1-3 times branched. The basal laterals generally equal the main axis in length.

Binding laterals of the types found in forma globosa are absent, the filaments are therefore free and the plant lax.

Fructification:

The plants were sterile when collected; there were, however, short densely branched laterals, similar to the fruiting branches of forma globosa, arranged alternately in the sub-apical regions of the filaments.

Figure 22.

Drawing of a portion of f. intermedium showing the arrangement of branching of the erect filament, and the comparative shape and size of the cells of the erect and prostrate filaments.

The dotted lines indicate that portions of the filament have been omitted.

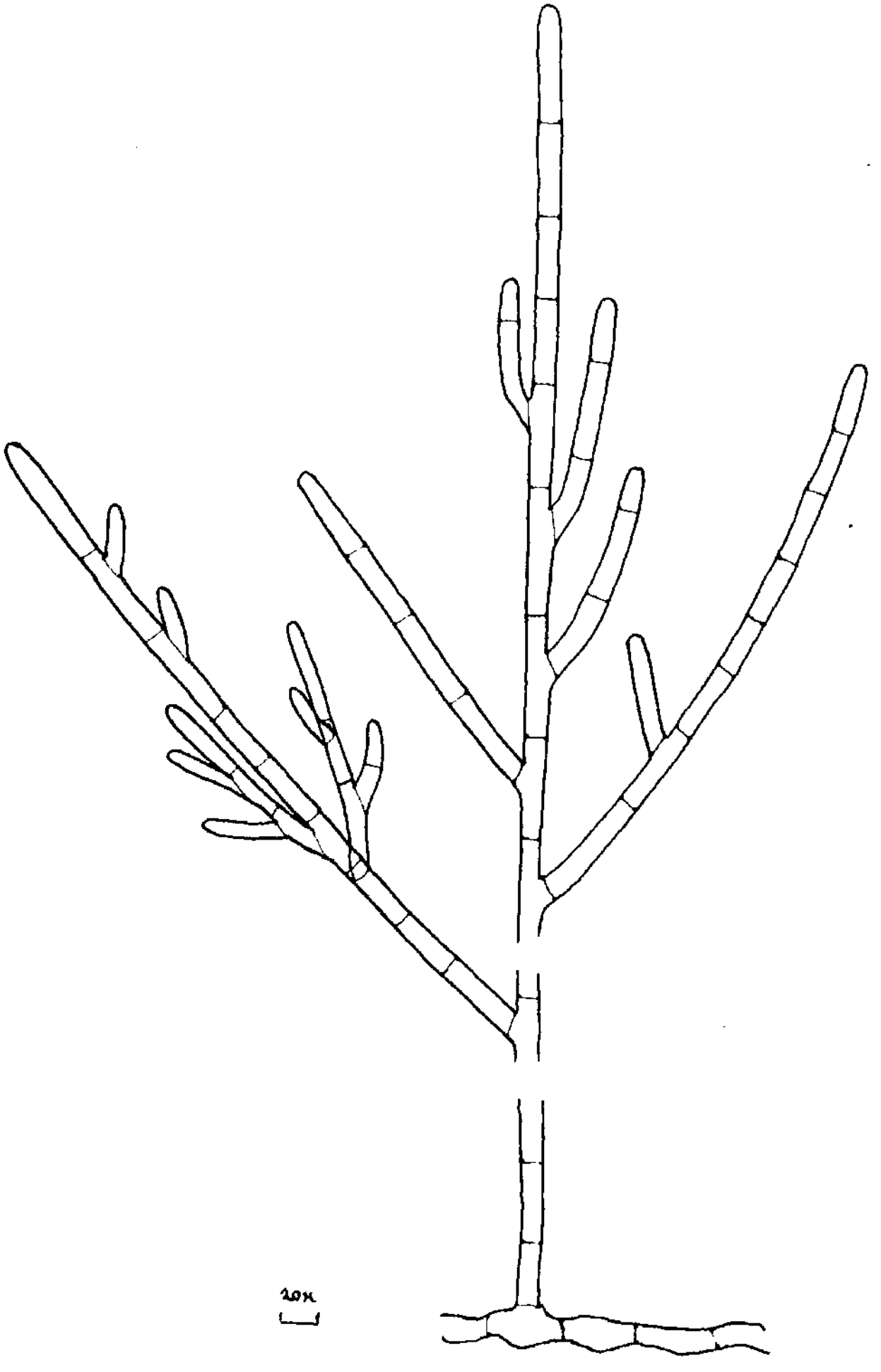
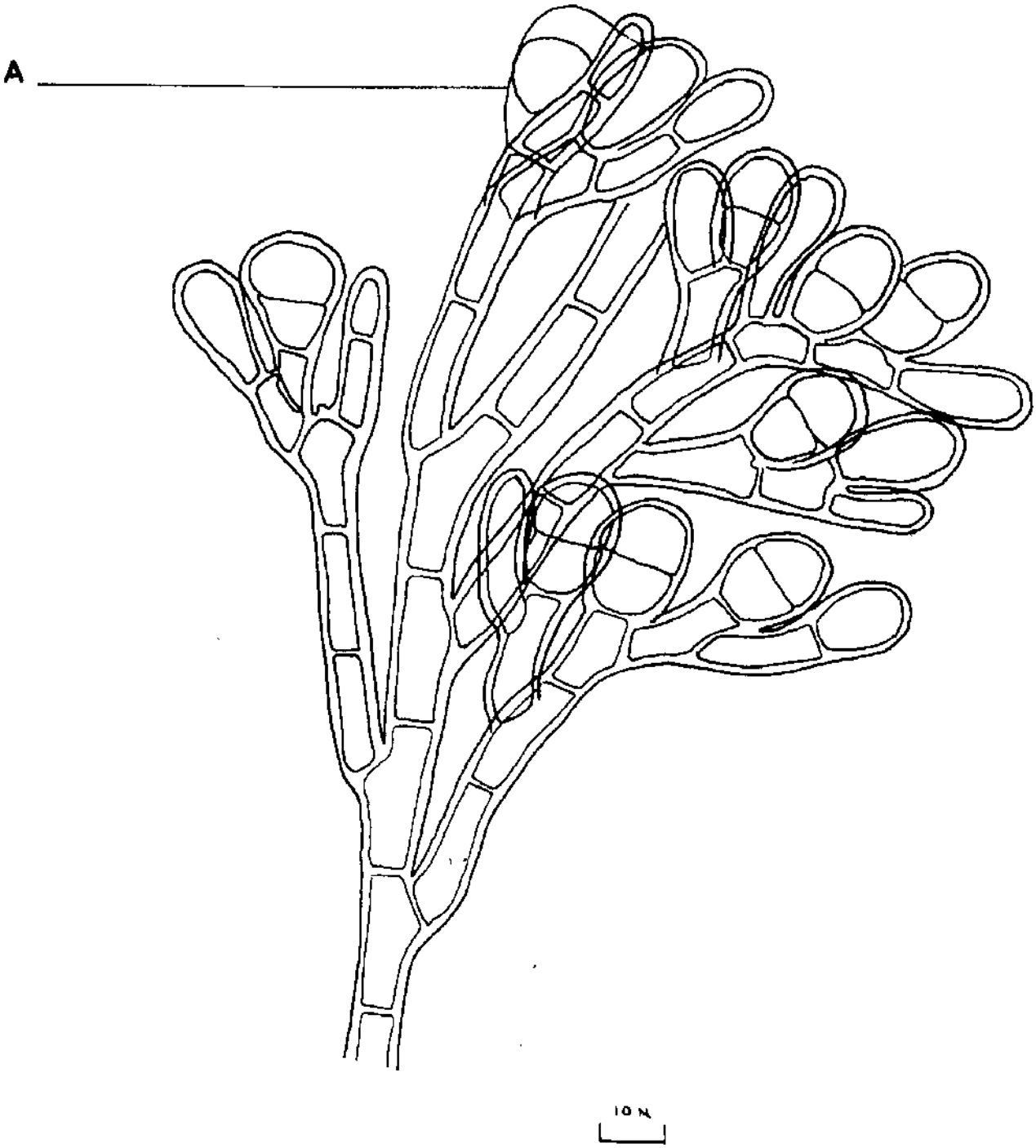

10x

Figure 23.

Portion of a tetrasporic lateral of f. intermedium showing the pattern of branching and the arrangement of the sporangia.

A. is a spore caught between the sporangial wall and the filament which has grown up through the sporangium.


A
10 N

Vegetative reproduction:

Vegetative reproduction by fragmentation is common.

Behaviour in culture:

It was found that the cells produced in culture were longer and narrower than those previously formed. The maximum length recorded was 63.0u, while the cells averaged 12u in breadth.

After three months in culture tetrasporangia were produced both terminally and laterally on the adaxial surface of the densely branched laterals originally present and on similar laterals formed in culture (Fig. 23).

On average the sporangia were slightly smaller than those of forma globosa, being 22u long and 14u broad.

Following the shedding of the spores, the cell beneath the sporangium grew out into a short filament on the end of which another sporangium was borne. Occasionally this occurred before the spores were shed, so that they became lodged between the sporangial wall and the newly formed filament (Plate 33).

Many instances were observed of bi-spore formation similar to that recorded by Klavestad (1957) in material collected from cracks in rocks above sea-level on the Baltic coast, and Baarsdeth (1941) on Rhodochorton bisporiferum Baarsdeth, collected on Tristan da Cunha.

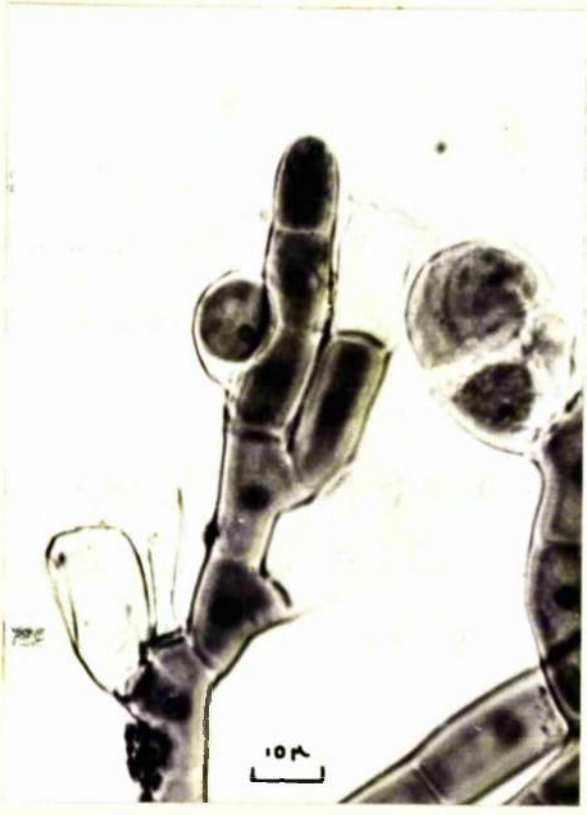
These bi-spores are formed as the result of the failure of

Plate 33.

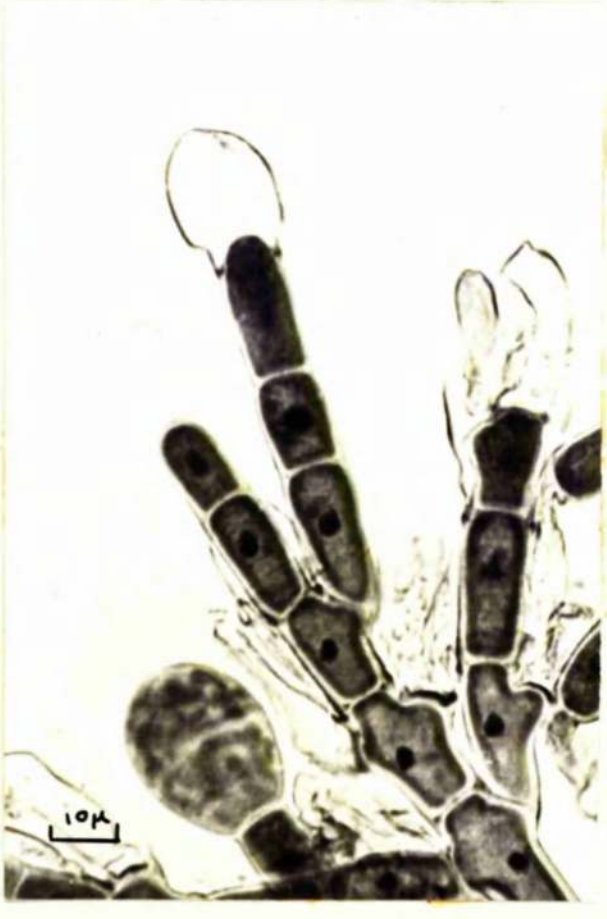
A. Photomicrograph of a portion of f. intermedium showing a spore caught between the sporangial wall and the filament which has grown up through the sporangium.

B. Photomicrograph of a portion of f. intermedium showing the initiation of vegetative growth by the cell immediately beneath an empty sporangium.

A.



B.



septum formation following the second nuclear division in the sporangium. Each spore therefore contains two nuclei.

A similar case of bi-spore formation was found in material of forma typica collected from a cave in Spiggie, Isle of Shetland.

Since Baarsdeth (1941) admitted that the presence of bi-spores was the sole criterion for separating R. bisporiferum from the then R. rothii it seems justified, in the absence of information concerning the number of nuclei in each spore of the Tristan material, to consider the two as synonomous.

Occasionally monospores are formed as the result of the complete failure of septum formation following nuclear division. These spores which consist of the contents of a single sporangium, contain four nuclei.

The germination of these spores will be considered in a later section.

It is clear from the observations made of the changes induced in the different forms under the constant culture conditions that such features as cell size and shape, mode of branching, and presence or absence of tetrasporangia are strongly influenced by environmental factors, and that under equivalent conditions the differences between the forms undergo a marked reduction.

This supports the suggestion (Borgesen, 1902) that the various forms of R. purpureum which are known to exist are purely environmental

Cell content:

The contents of the cells of all the forms examined are similar:

Each cell has a peripheral layer of cytoplasm in which is embedded a much lobed and dissected chromatophore, or many small disc or rod-like chromatophores.

The single nucleus lies in the centre of the large vacuole below the chromatophores.

At either end of the cell, near the pore in the septum, a conspicuous, spherical translucent body is often, but not always to be seen, particularly in the older cells of the filaments. The chemical nature of this inclusion has yet to be determined and its function is unknown.

That the reserve food product is starch can readily be determined by staining fresh material with iodine solution. It is evenly distributed throughout the cell as in R. floridulum.

Cell division and cross-wall formation.

The process of cell division was followed in the apical cells of the erect system and was found to be similar to that in R. floridulum.

The cross-wall is laid down in the same way as described in R. floridulum and contains a pore which is similar to but smaller than the pore in that species.

A/2.

THE MORPHOLOGY OF THE SPORELINGS.

Germination of the spores of Rhodochorton purpureum.

1. Tetraspores.

The early stages of germination of the tetraspores were described by Harvey Gibson (1891) who reported the production of sparingly branched filaments. In the present investigation the germination of the tetraspores has been followed in cultured material of forma intermedium.

The diameter of the released spores varies between 9.0 and 12.0u; they are spherical, possess a cell wall and contain a variable number of parietal chromatophores and a single nucleus. Unlike the spores of R. floridulum they do not attahh themselves to the substrate before germination. Germination is normally monopolar, but inffequent instances of bi-polar germination have been recorded (Figure 24B/a,b,c). The germ-tube produces a simple filament of about 4u in width and several cells in length (up to 8 cells have so far been recorded). As in R. floridulum, the spore remains protoplasmic after the formation of the germ-tube. The cells of the filament are similar to those of the tetrasporophyte in that they have a single central nucleus and a peripheral system of discoid chromatophores or one much dissected chromatophore.

The number of chromosomes present in the nucleus of a tetraspore is in the region of 10 (Plate 34A).

2. Bi-spores (Plates 35 & 36A).

Bi-spores are double the volume of tetraspores since they represent two such undivided spores. Each contains two nuclei, both of which divide during germination. Normally only one germ-tube is produced and into this

passes one of the four nuclei from the spore. The resulting filament is therefore monokaryotic while the spore retains three nuclei (Figure C). The number of chromosomes present in the nucleus of a bi-spore germ-tube is in the region of 10.

3. Monospores (Plate 36).

Monospores are formed as the result of the failure of septum formation after nuclear division in a sporangium, each therefore is four times the volume of a tetraspore and contains four nuclei.

In those examples so far examined only one nucleus divides during germination, one of the daughter nuclei passing into the germ-tube, so that the resulting filament is monokaryotic while the spore remains quadrinucleate.

The development of the spores in culture is slow compared with the growth of the tetrasporophyte and so far only the filamentous sporelings have been found. The cultures are being maintained in order that the further development may be followed.

Germination in situ

In situ germination of spores in the Rhodophyceae has been occasionally reported (Dixon, 1960; Boney, 1963), and has already been described for R. floridulum. It has been frequently observed in cultured material of forma intermedium, both in the case of tetraspores and bispores, and in addition in situ germination of tetraspores has been found in wild material of forma globosa. In all cases the spores produced simple filaments similar to those produced by liberated spores. Normally the germ-tube is produced from the region of the spore distal to the base of the sporangium;

occasionally, however, the tube is produced from the proximal end (Plate 34c).

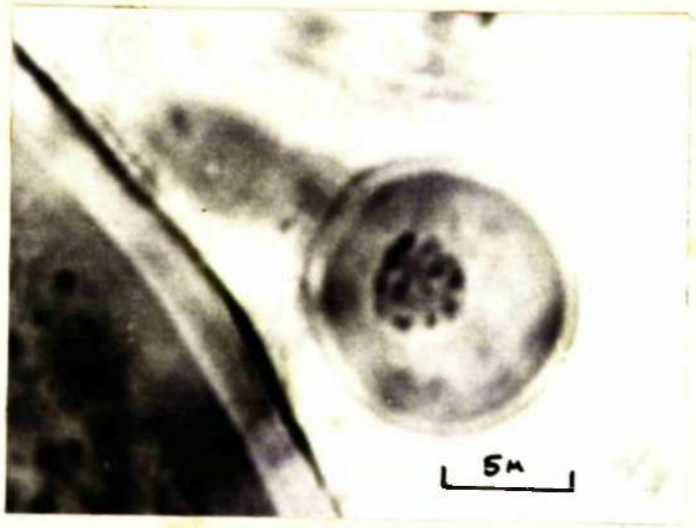
Plate 34.

A. Late prophase nucleus in a germinating tetraspore, showing circa 10 chromosomes.

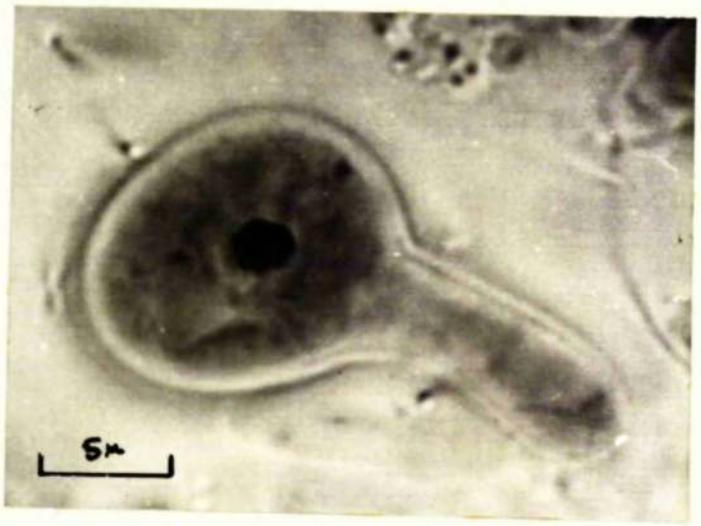
B. Sub-equatorial view of late metaphase in a germinating tetraspore.

C. Late anaphase in a germinating tetraspore showing the production of the germ tube (t) from a point proximal to the base of the sporangium (b) where the stalk cell has become meristematic, producing a cone-shaped protuberance (p).

A.



B.



C.

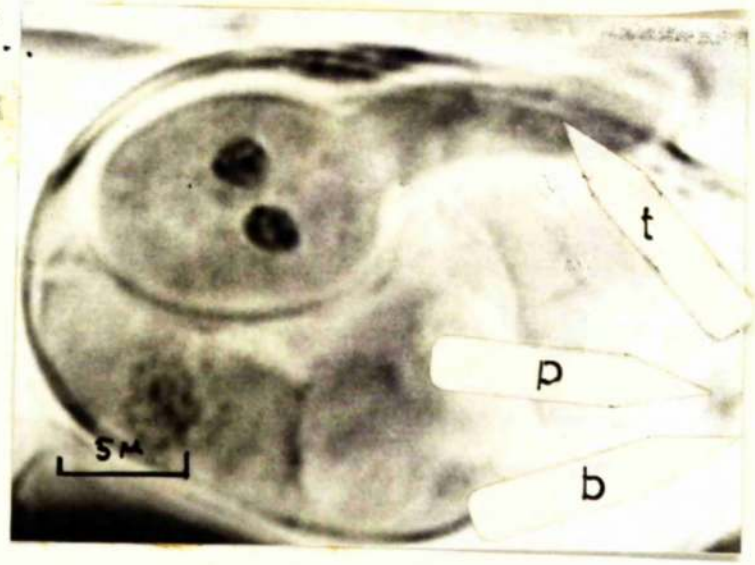


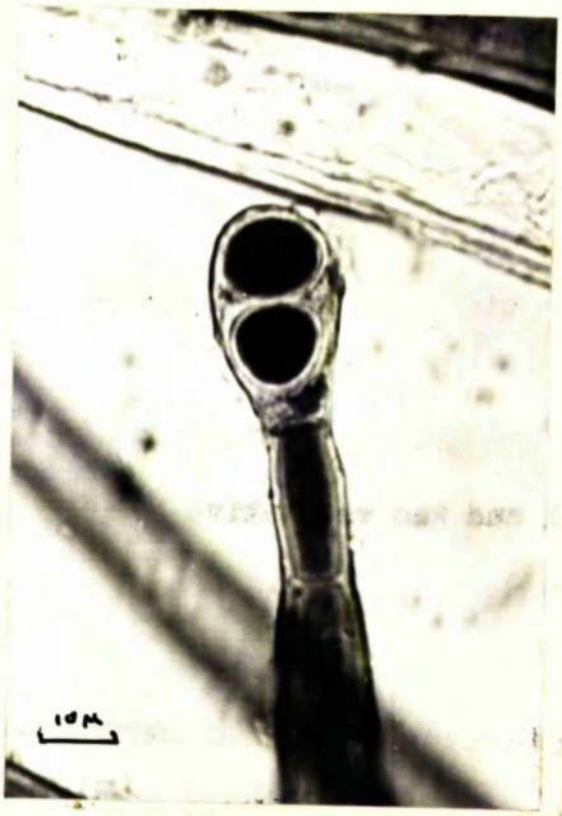
Plate 35.

A. Photomicrograph of bispores in <u>forma globosa</u>.

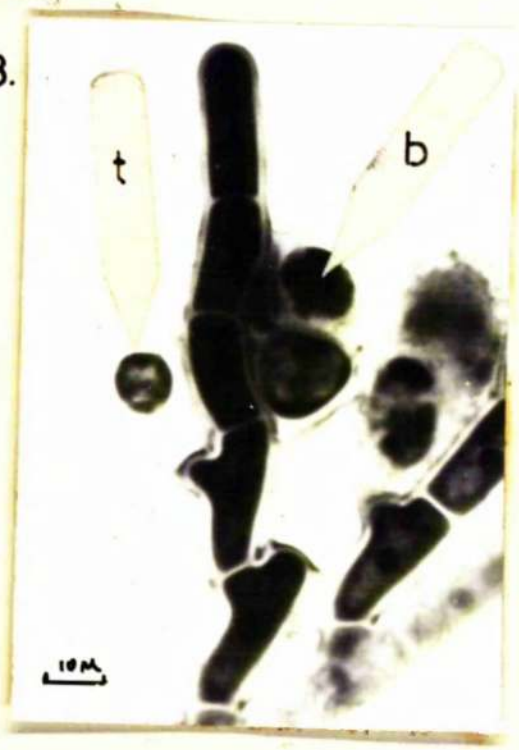
B. Photomicrograph of bispores (b) lodged between the sporangial wall and the vegetative filament. (t) is a tetraspore.

C. Photomicrograph of bispores (b): the lower shows two nuclei (n), the upper has produced a lateral germ-tube (t) in which the discoid chromatophores can be seen.

A.



B.



C.

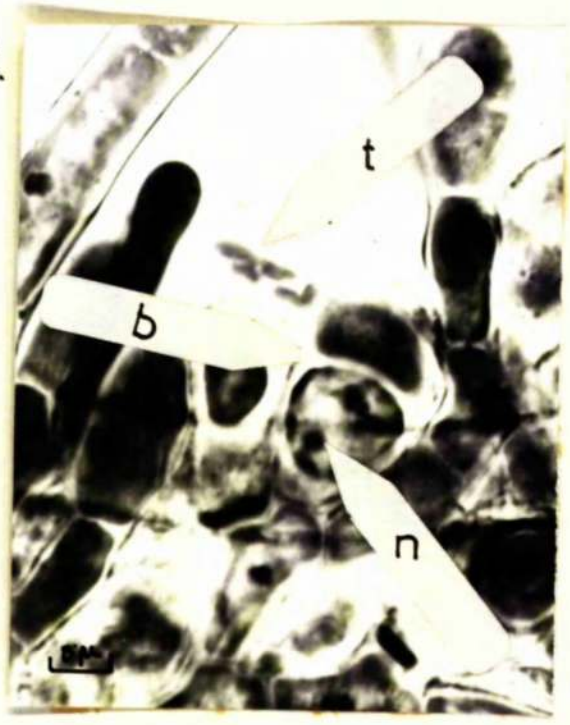


Plate 36.

A. Photomicrograph of a sporangium containing a monospore. Three nuclei (n) are visible.

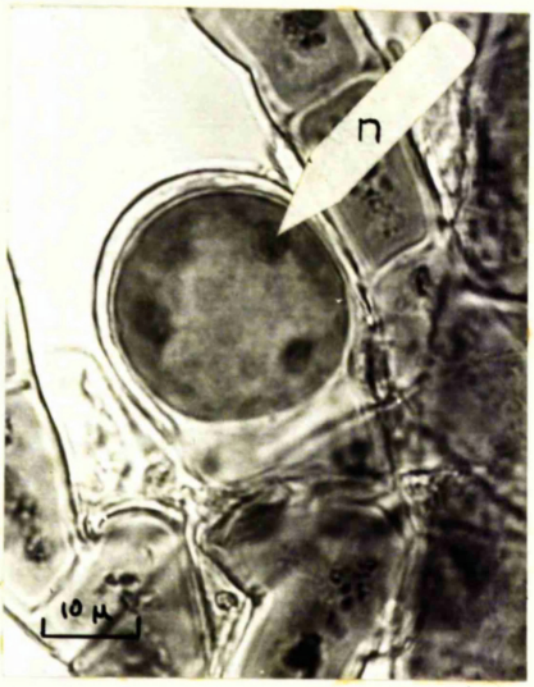

n
10 μ

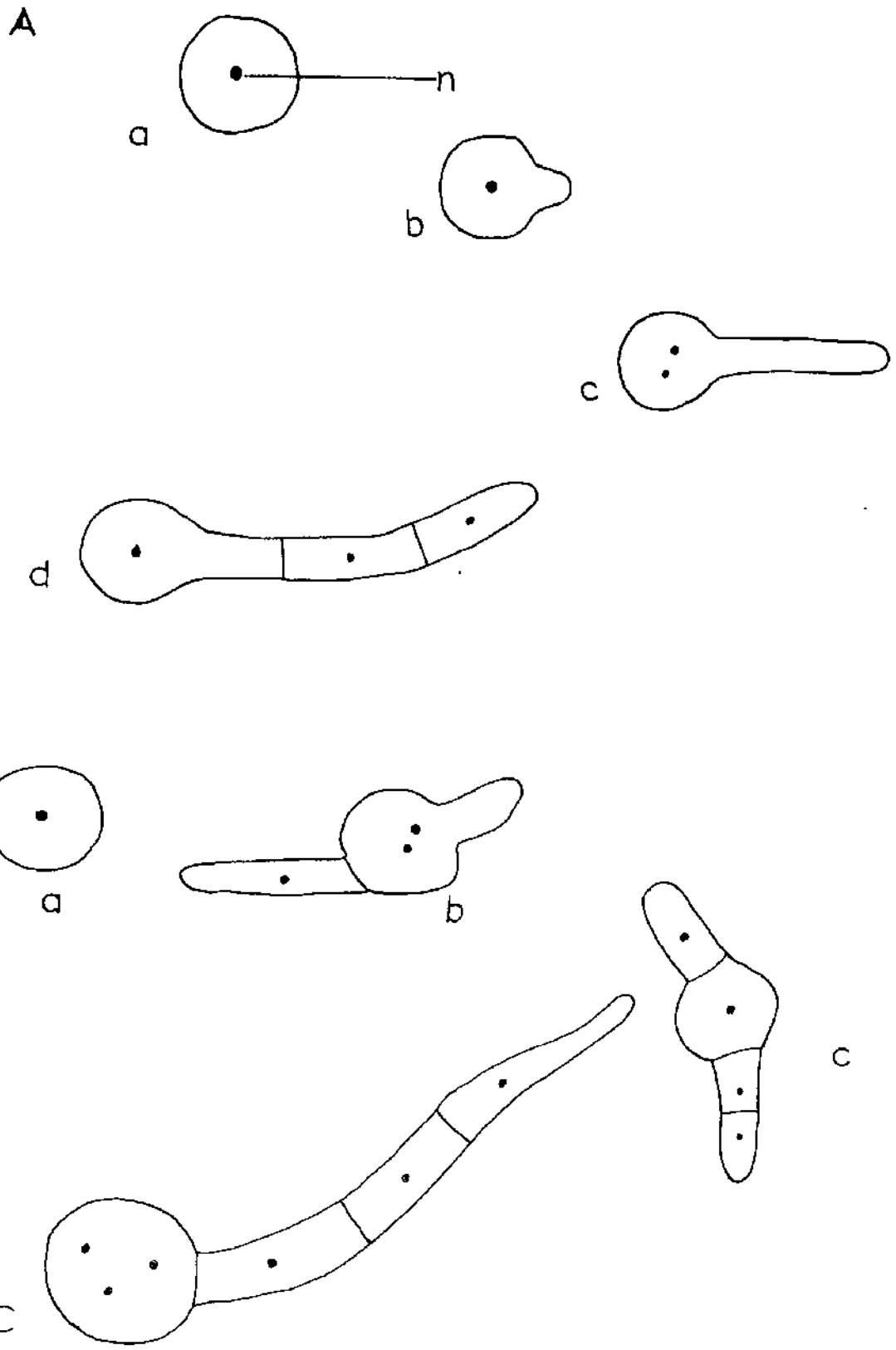
Figure 24.

A. a,b,c,d. Stages in the germination of a tetraspore showing normal monopolar germination.

n nucleus.

B. a,b,c. Stages in bi-polar germination of a tetraspore.

C. Early stage in the germination of a bi-spore.


A
n
a
b
c
d
B
a
b
c
C

B.

CYTOLOGY.

Cytology.

1. Staining techniques.

The techniques employed in the investigation of the nuclear cytology of R. purpureum were similar to the standard techniques used in the investigations on the cytology of R. floridulum, i.e. the standard fixative employed was 1:3 glacial acetic acid: absolute ethyl alcohol, the standard dye acetocarmine.

It was found that the pigment tended to aggregate in globules after fixation and these globules frequently interfered with observation of the nucleus. These globules could be removed if, before staining, the material was heated in water.

The plant material is very much smaller than that of R. floridulum and difficulty was found in separating filaments and sporangia by the normal method of teasing with a pair of needles. This difficulty could be overcome to a certain extent if a micro-spatula with a flattened end was substituted for one of the needles, or if after transferring to a slide the filaments were repeatedly severed with a scalpel before being teased apart.

2. Cultural techniques.

The same techniques as had proved satisfactory for the in vitro cultivation of R. floridulum were employed for the cultivation of the forms described in the previous section.

The standard medium was ES supplemented sea water and proved

satisfactory even for forma intermedium which had been collected from rocks covered with fresh water.

It was discovered that in order to prevent the death of the apical cells of the plants all cultures had to be screened with aqueous solutions of eosin dye of concentration between 0.01 gm. and 2.0 gms./litre.

Mitotic Division in Rhodochorton purpureum

The process of mitosis was followed in the apical cells of culture material of Rhodochorton purpureum f. intermedium. This form was chosen because of its larger size and the absence of sand between the filaments. Wherever possible, comparison was made with the other forms, both from culture and from the shore. There appears to be no difference in the number of the chromosomes in the various forms and in the behaviour of the nucleus during division.

Observations:

The interphase nucleus is spherical with a diameter of 3u. It possesses a central nucleolus between 1.5 and 2u in diameter surrounding which is a coarse reticulum staining deeply with acetocarmine. During prophase the reticulum disappears, while the nucleolus enlarges to about 3u in diameter filling the nucleus except for a narrow, non-staining region of varying width around the inside of the nuclear membrane. At this stage the nucleus resembles the early prophase nucleus of Rhodochorton floridulum. As prophase continues, the nucleolus gradually loses its affinity for acetocarmine and the chromosomes become visible as faintly staining strands in the nuclear area surrounding it. The stands shorten and thicken,

becoming more stainable as they do, until, by the end of prophase they have become dot or rod-like and can be counted. The average count is 20, so that the number is of the same order as in Rhodochorton floridulum.

At metaphase the polar axis of the spindle lies across the length of the cell so that at early anaphase the daughter chromatids move across the cell;as they do so, however, the groups rotate gradually so that the path of separation is S-shaped, by mid-anaphase therefore movement is along the length of the cell. During the later stages of separation the daughter nuclei often assume a saucer-shape with the convex side directed towards each other. Before this happens the groups are ring-shaped as in R. floridulum suggesting a peripheral arrangement on the metaphase plate as is found in that species.

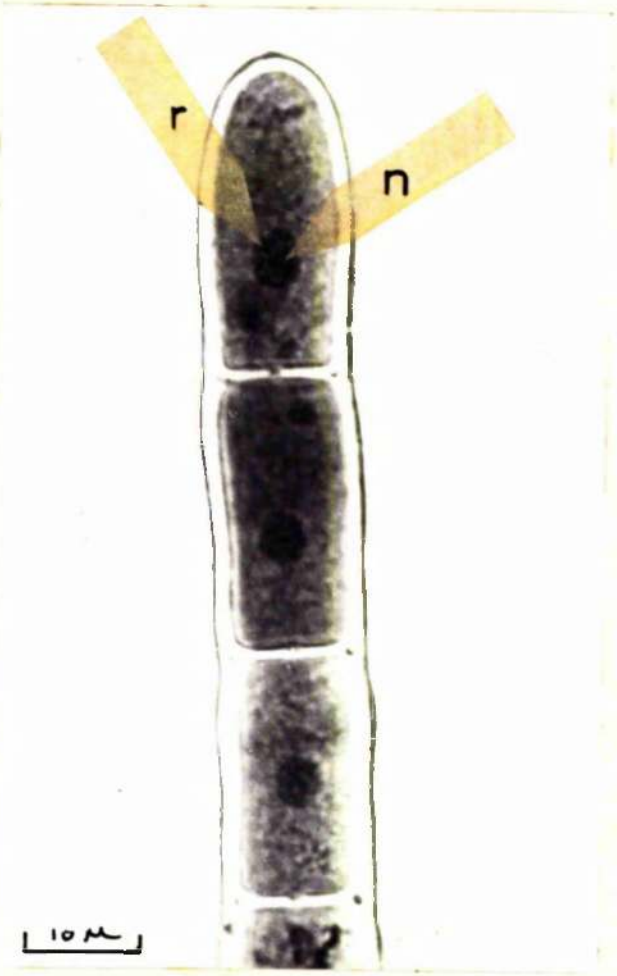
During telophase the chromosomes uncoil and lose their stainability while the nucleolus reappears. The lower daughter nucleus assumes the interphase appearance; the upper that of early prophase.

Cross-wall formation following nuclear division is similar to that described for R. floridulum.

The size of the longest chromosome at metaphase is 1u x 0.5u.

Photomicrographs of mitosis.

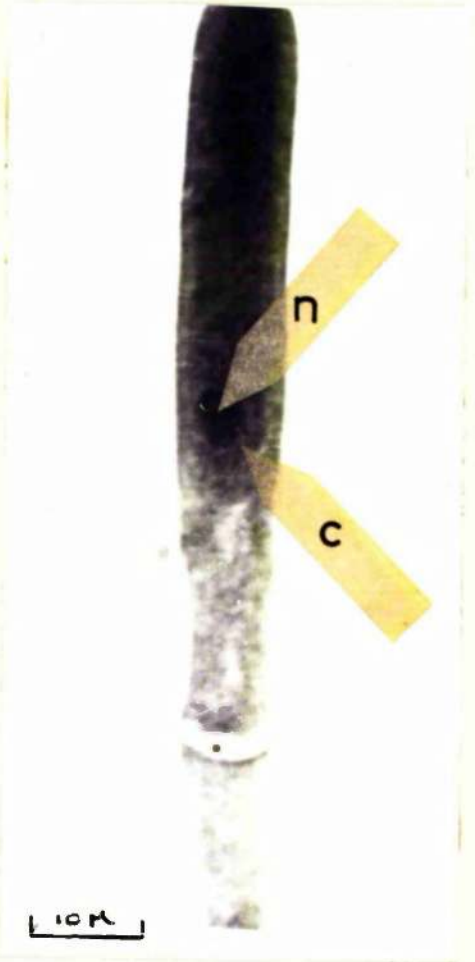
Plate 37.



Interphase nucleus in a resting apical cell, showing the small nucleolus (n) and the surrounding reticulum (r).

(B - ID_2)

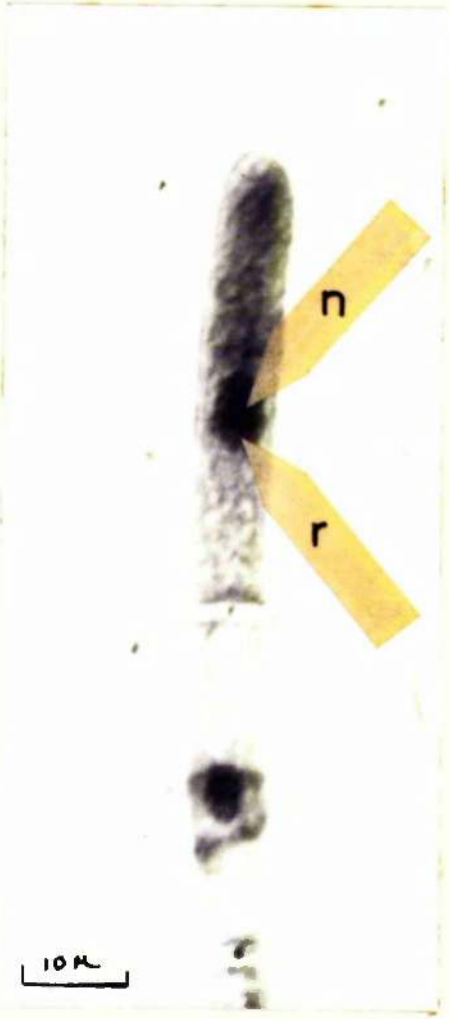
Plate 38.



Early prophase, showing the enlarged nucleolus (n) and the non-staining region (c).

(B - ID_2)

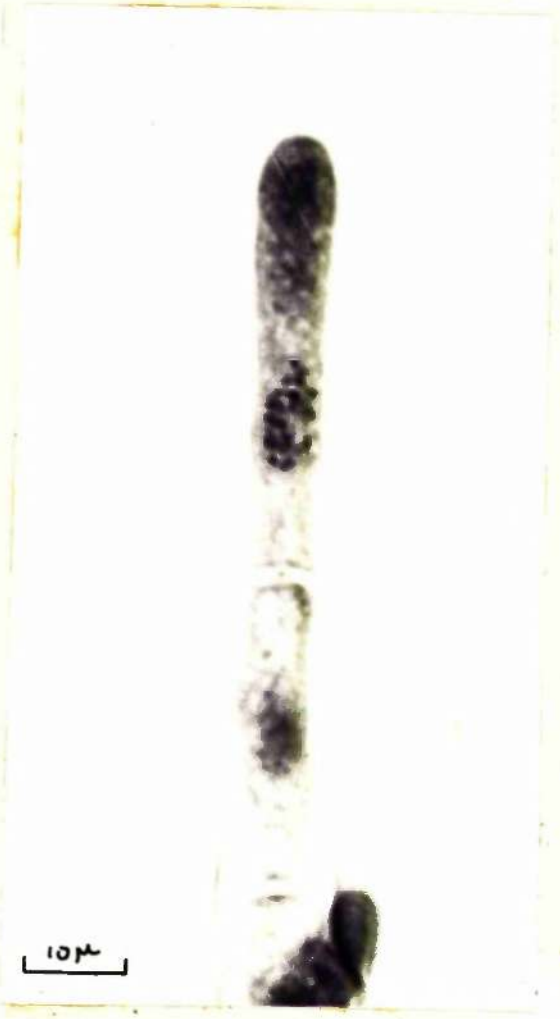
Plate 39.



Prophase, showing the nucleolus (n) and the reticulum (r).

($B - ID_2$)

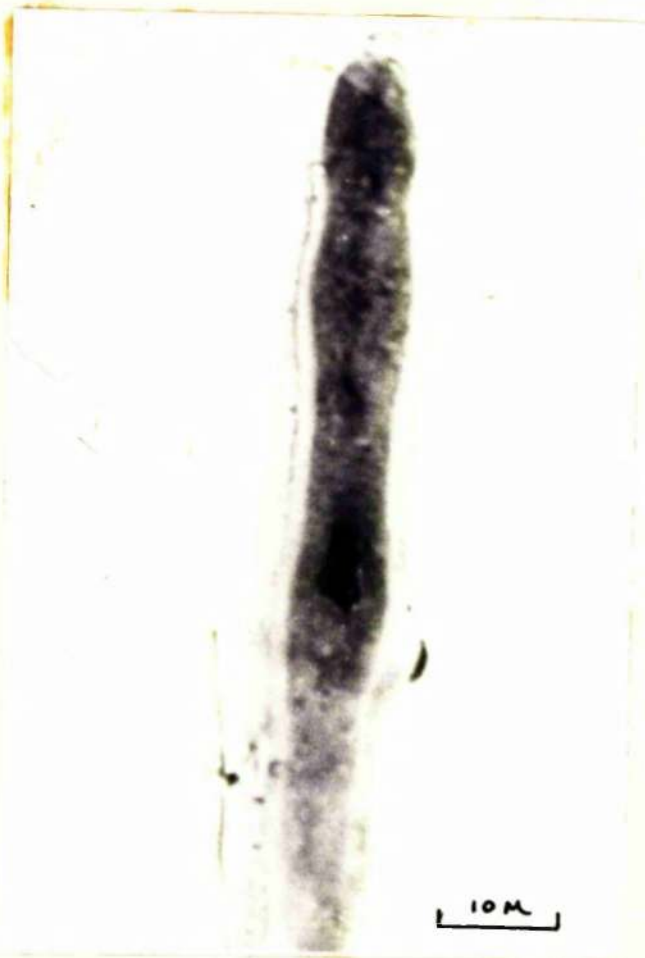
Plate 40.



Late Prophase, showing c. 20 chromosomes.

(B - ID_2)

Plate 41.



Metaphase, showing the orientation of the plate. In most cases the plate is of a greater diameter than the filament.

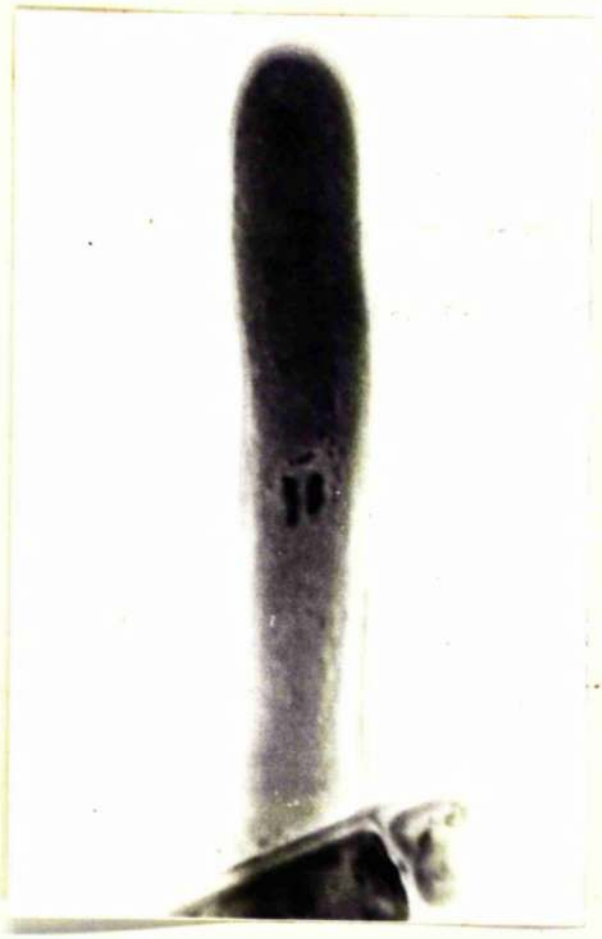
$(B - ID_2)$

Plate 42.

A. Photomicrograph of early anaphase, showing the separation of the groups across the cell.

B. Photomicrograph of a later stage of anaphase showing the rotation of the daughter nuclei.

A.



B.

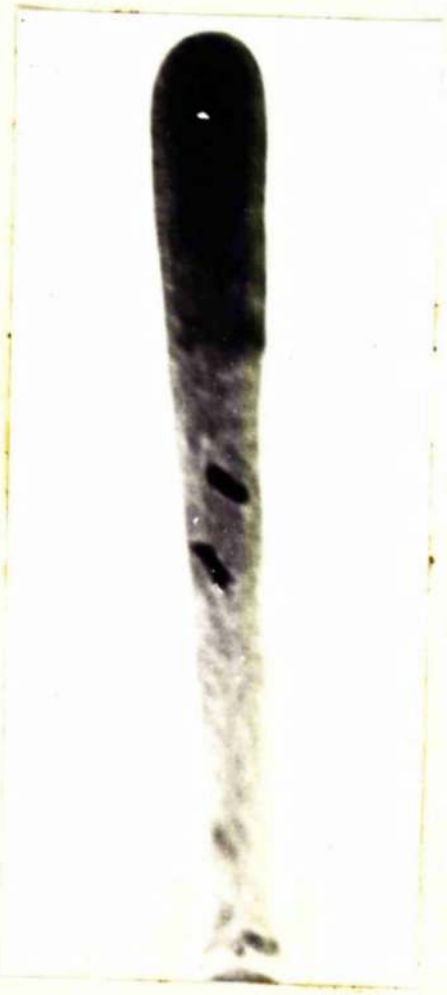
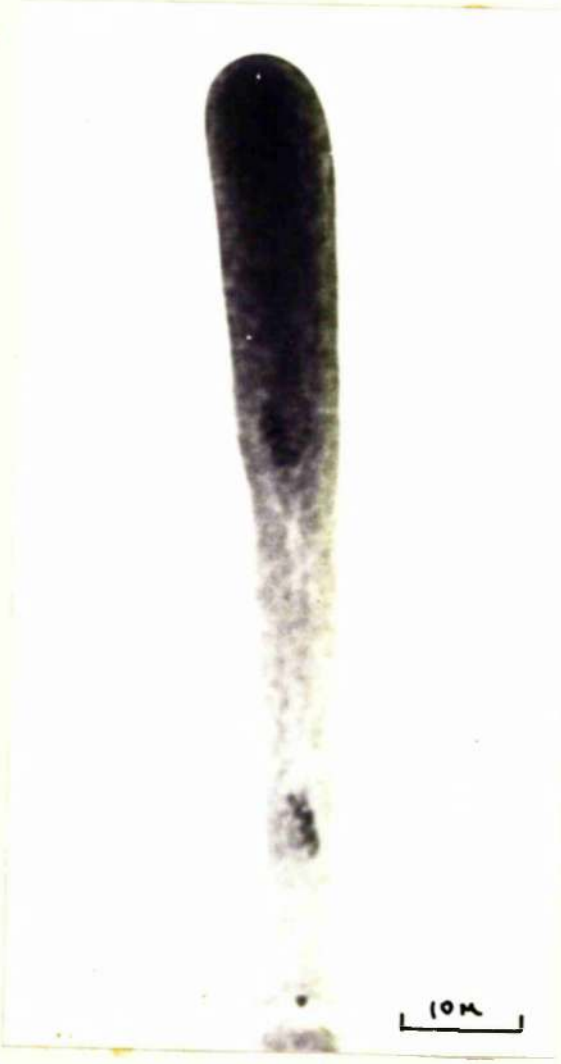


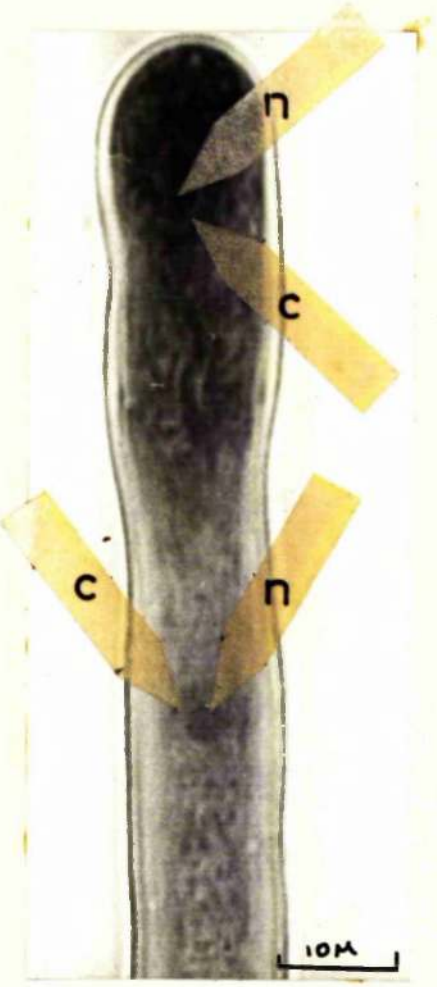
Plate 43.



Early telophase, showing the beginning of the despiralisation of the chromosomes. The nucleoli are not yet visible.

(B - ID_2)

Plate 44.



Late telophase: the nucleoli (n) and the non-staining regions (c) have been reconstituted.

($B - ID_2$)

Nuclear division in the sporangium.

The process of nuclear division in the sporangium was followed in cultured material of R. purpureum f. intermedium.

Observations:

The nucleus of a young sporangium in very early prophase contains a nucleolus of about 4.5u in diameter surrounding which is a narrow non-staining region of width up to 1u.

As prophase continues there is a continual reduction in the volume of the nucleolus and the chromosomes become stainable in the widening surrounding region.

As in R. floridulum the early stages of prophase are difficult to interpret; no characteristic meiotic phases such as diplotene or diakinesis have so far been recognised, but at prometaphase 1 the chromosomes assume an arrangement around the periphery of the nucleus which appears to be characteristic of nuclear division in the sporangium. A similar arrangement has already been observed in the sporangium of R. floridulum. At this stage the chromosomes are separately resolvable and can be counted; the number is constantly in the region of 10, i.e. half the number present in the apical cells of the filaments.

At metaphase 1, the chromosomes lie close together arranged in a ring around the periphery of the plate with their long axes parallel to the spindle. At this stage one or two chromosomes have been observed to bear satellites.

During anaphase 1 the products of division move apart along the long axis of the sporangium often assuming a saucer-shape as do the daughter

nuclei in R. floridulum.

At the poles the chromosomes despiralise and the early prophase appearance is re-established. During this process a septum is laid down across the sporangium.

As the result of a further nuclear division in the course of which the characteristic prometaphase 1 chromosome arrangement has not yet been seen, four nuclei are formed.

The onset of the second division may or may not be simultaneous in the two halves of the sporangium, but eventually four uninucleate spores are formed.

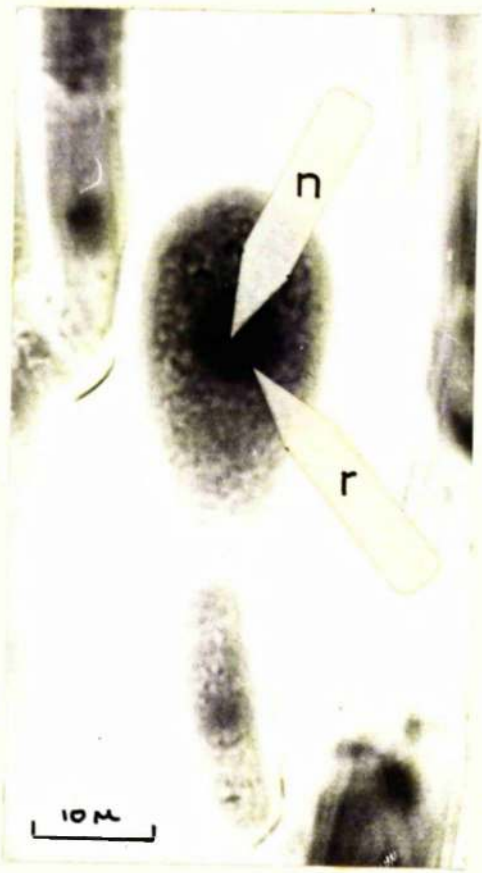
Discussion.

The chromosome number of the sporangial nucleus at metaphase 1 is in the region of 10. The chromosome number of both tetraspores and monospores is also in the region of 10. The number of chromosomes in a nucleus of a cell of the filament is in the region of 20; it would therefore appear on the basis of these counts that reduction division takes place in the tetrasporangium so that the spores are haploid.

In contrast to R. floridulum it has not yet been possible to demonstrate such phases of meiosis as diakinesis in the sporangium of R. purpureum, but it has been shown that the arrangement of the chromosomes during prometaphase 1 is similar in both cases (Plates 26 & 47) and that this arrangement is unlike any other, either in the somatic nucleus or in the second division in the tetrasporangium.

Photomicrographs of nuclear division in the tetrasporangium of R. purpureum.

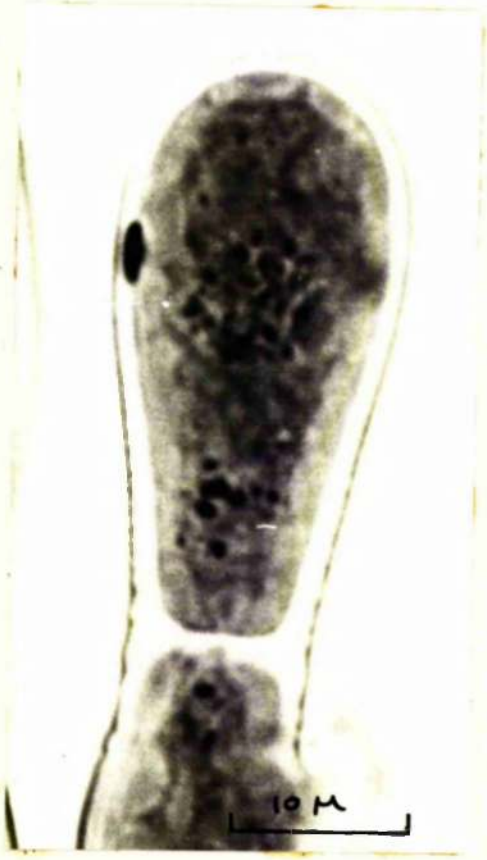
Plate 45.



Early 1st prophase showing the nucleolus (n) and the surrounding reticulum (r).

(R - D)

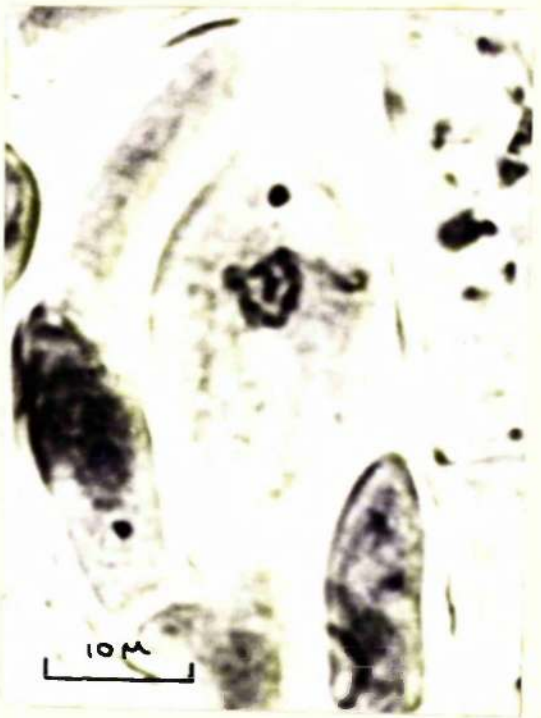
Plate 46.



1st prophase showing the thickened chromosomes.

(R - D)

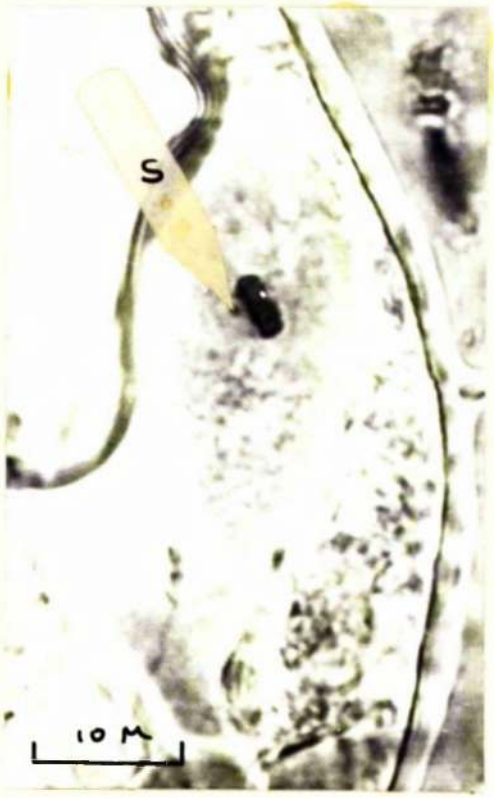
Plate 47.



1st prometaphase showing the peripheral arrangement of the chromosomes.

(B - ID_2)

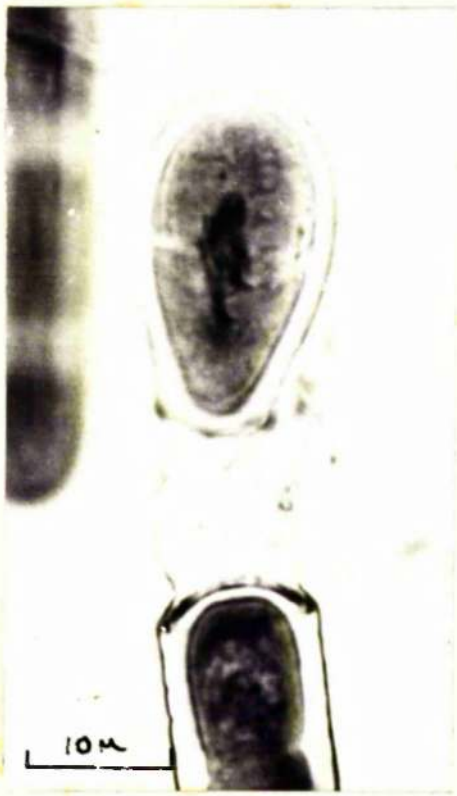
Plate 48.



1st metaphase showing a satellite chromosome (s).

(R - D)

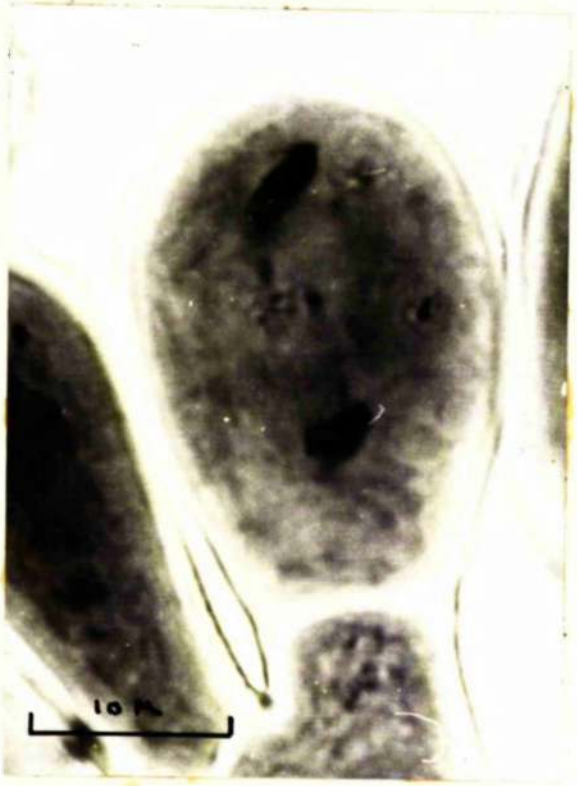
Plate 49.



1st anaphase, showing the partially formed septum.

(R - D)

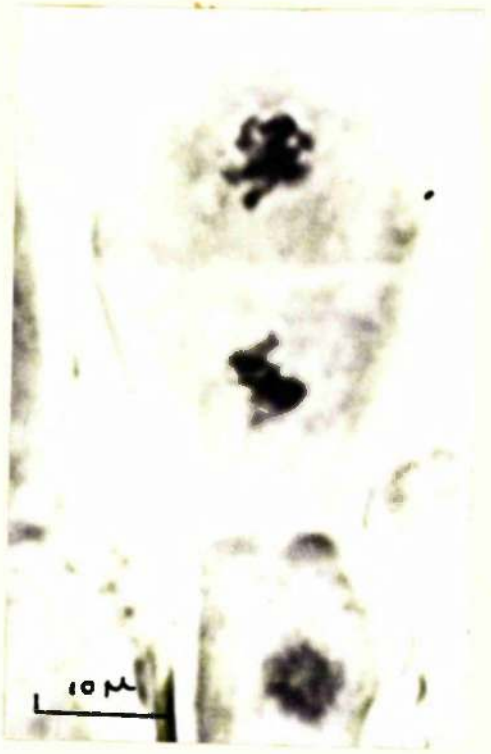
Plate 50.



Late 1st anaphase, showing the ring-shape of the chromosome groups.

(R - D)

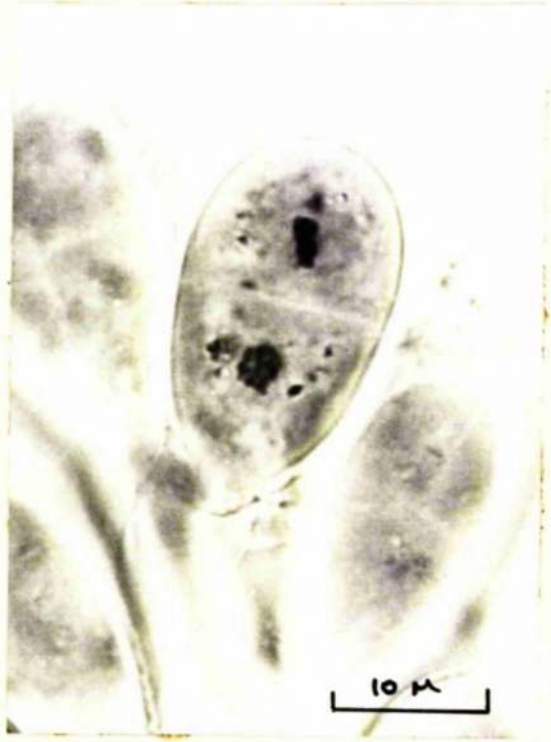
Plate 51.



Late 2nd prophase.

(R - D)

Plate 52.



Late 2nd metaphase in the top cell;

late 2nd prophase in the bottom.

(R - D)

APPENDIX

Appendix.

The ecology of R. purpureum.

Geographical distribution:

Europe:

The Mediterranean region:	Tangiers (Hamel 1925).
	Tripoli (Spigai, in De Toni, vol. 4).
	Adriatic (Zanardini, in De Toni 1889-1907, 4/3).
France:	The Channel and Atlantic coasts (Hamel 1925).
Belgium:	Kutzing (1849).
Germany:	Kutzing (1849).
Denmark:	North Sea and Baltic coasts (Rosenvinge 1923-24).
Sweden:	Baltic coast, at least as far north as Oregrund (Waern 1952).
Poland:	Gulf of Danzig (Reinke, in De Toni 1889-1907).
Finland:	Tvarminne & Helsinki (Purasjoki 1950).
Russia:	Gulf of Finland between Oranienbaum and Krasnaja Gorka (Gobi 1878).
	Arctic Coast, Sinova (1912 & 1929); Flerov & Karsakoff (1932).
Sweden:	West Coast (Kylin 1944).
Norway:	West coast (Kleen, 1874; Printz, 1926; Hygene and Jorde, 1934; Sundene, 1953; Breivik, 1957; Jorde & Klavestad, 1963).
Spitsbergen:	Kjellman 1875, 1877, 1883; Svendsen 1959.
	As far north as 79° 49' (Kjellman 1883).

Novaya Zemlya:	Kjellman 1883.
British Isles:	Common on all coasts (Holmes & Batters 1892; Traill 1890; Cotton 1912; Harvey-Gibson, 1913; Rees 1935; Anand 1937; Dunn 1939; Blackler 1951; Gillham 1954; Drew 1956, 1957; Dixon 1959; Moss, 1959.
The Faeroes:	Lyngbye 1819; Borgesen 1902.
Iceland:	Jonsson 1902.
Greenland:	Common on all coasts, recorded as far north as Koldeway Island (Rosenvinge 1893-1897).
North America:	Baffin Bay (Rosenvinge 1893).
	The west coast from Elmington Island and St. Michael, Alaska to San Diego, California (Drew 1928).
	The east coast, Long Island to Nova Scotia.
	Hudson strait, Baffin Island, Devon Island and Ellesmere Island (Taylor 1957).
Japan:	Yendo, in De Toni 1889-1907, 6.
New Zealand:	Dunedin District (Dellow, 1955).
Tristan da Cunha:	Possibly present (As R. bisporiferum Baarsdeth, Baarsdeth 1941).

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(2) The tetrasporophyte and reduction division in the tetrasporangium. Ibid., pp. 296-310.

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