Cytological aspects of growth and development in gametophytes of Dryopteris borreri

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SUMMARY

The literature on fern gametophytes suggested that they would provide a system suitable for studies of the control of cell division and expansion and unusually promising for investigation in the orientation of these processes. Work on gametophyte morphogenesis has already established that development can be altered by varying environmental factors particularly light wavelength. In this investigation, a systematic analysis has been made of the responses to light and temperature of the gametophyte and its individual cells, from germination to the onset of prothallial growth.

Drynaria borreri was the fern species chosen because each plant produces large quantities of genetically identical spores, easily stored and cultured. After a standard germination procedure the cultural conditions involved various combinations of five temperatures at $2^\circ C$ intervals from $17^\circ C - 27^\circ C$, three intensities of white fluorescent light 25, 100 and 400 lumens/sq. ft., and red and blue light of energies approximating to the red and blue components of the standard white light. A few exploratory experiments were also carried out at temperatures outside this range, and at very low light intensities.

It was confirmed that the induction of transition from filamentous to prothallial growth is dependent upon short wavelength (blue) light and demonstrated that filamentous growth and transition pattern, is also sensitive to environmental conditions, moreover, transition cannot be considered as an "all or none" re-orientation of a single division.

The growth variants encountered, included two main patterns of transition and filaments that were sometimes simple and unbranched, sometimes forked at the base and sometimes branched from interstitial cells. Considerable variations in cell number and shape during the filamentous phase were found as
well as differences in rhizoid number and the frequency of interstitial divisions along the filament. Some characteristics of filamentous growth could be related to the type of subsequent transition. Both are sensitive to light wavelength after the 3-cell stage.

In white light, low intensities and high temperatures were found to give a growth pattern resembling red-grown gametophytes i.e. highly polarised expansion and a prolonged filamentous phase, while high intensities and low temperatures gave "blue-type" growth with isodiametric cell expansion and early transition to prothallial growth.

However, in blue and red light, gametophytes were less sensitive to variations in intensity and temperature than white-grown ones. Even at every low intensities, blue-grown cells showed no tendency to become attenuated.

The data suggest three photosystems whose interactions are modified by temperature. The photosystems which are:

(a) a blue-sensitive system
(b) a longer wavelength (possible red) sensitive system
and (c) photosynthesis.

have been discussed in relation to other pigment systems in plants.

It is now possible, using selected environmental conditions, to obtain the specific predictable growth patterns necessary for further investigation of cell polarity. This investigation has also contributed detailed data on the early development of this species of fern.
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Introduction

Fundamental to morphogenesis in any multicellular organism are the twin processes of cell division and expansion. The ultimate pattern of morphogenesis depends upon the distribution and orientation of these two processes. The mechanism behind the control of development, and particularly the control of the polarity of division and expansion in individual cells within an organised multicellular system, are problems of great importance not only within the context of a specific system but also as basic problems of general significance.

Despite the universal nature of the processes, systems that lend themselves to a study of cell polarity are extremely hard to find. It was this search for a plant system suitable for a study of the orientation of cell division and expansion, that led to this investigation. An ideal system for this purpose should possess several important characteristics:

(i) It should possess a pronounced, natural polarity and a means of relating the polarity of individual cells to the polarity of the whole system.

(ii) The distribution and rate of mitosis should be easily and accurately assessable.

(iii) Similarly, the rate and pattern of expansion of each cell should be directly measureable.

(iv) Development should be so consistent that for any set of conditions it is possible not only to trace the history of individual cells but also to predict their future behaviour.

(v) The system should be amenable to culture under well defined conditions.

(vi) Its polarity should be alterable in similar ways by two or more different experimental treatments.
(vii) It should be possible to control the orientation of the system with respect to some external factor.

(viii) It should, as all good experimental material, be available for culturing throughout the year and have a convenient rate of growth and development, compatible with fairly short term experiments.

The great majority of plant systems already used in studies of other aspects of cell growth and differentiation, fall a long way short of these conditions. For example, cultures of single cells such as *Chlorella* are totally unsuited to any study of intercellular control of development. In a similar way, callus tissue, although multicellular, is devoid of normal intercellular organisation. Tissues such as root tips are rather too large in terms of cell number, for easy analysis. Radioactive tracers and other labelling techniques can provide clues to the lineage of certain cells but it is not possible to predict the future of any specific cell.

By contrast, observations on the early development of fern gametophytes, made by a variety of authors over the past hundred years suggested that gametophytes fulfil the requirements far more closely than any of these other systems.

Spores of most fern species germinate to give filamentous protonema from which the broad, flat prothalli are formed. During the filamentous stage of certain species, e.g. species of *Dryopteris*, only the apical cell divides to give new protonemal cells. This means that the rate of increase in the number of cells in the filament is a direct measure of the mitotic rate in this apical cell. It also means that the cells lie along an age gradient and the history
of each cell is evident from its position in the filament. Thus the necessity for labelling cells to determine cell lineage is avoided. The probable future of each cell can also be inferred from its position.

The transition from protonema to prothallus involves a sharp change in the pattern of development. This is marked by the re-orientation, and frequently re-distribution, of cell division. Mohr (1956) has considered the transition as representing a change from polar to apolar growth, although Albaum (1936) and others (Reuter 1953; Igura 1965) have found evidence for the existence of definite gradients in the prothallus which would imply that transition involved a change rather than a total loss, of polarity. In either case the gametophyte system certainly fulfils the requirements for a definite polarity; a polarity that is altered sharply at a well defined point in development.

With the exception of a very few ferns e.g. Osmunda which do not normally have a filamentous phase, this pattern of protonema, transition, prothallus, is general among the ferns (Orth 1936). This suggests a control mechanism with a fairly wide significance.

In addition, fern gametophytes form convenient experimental material. The spores can be kept viable in a refrigerator until required for use and then germinated and grown on a simple, mineral medium (Laage 1907; Mohr 1956a; Sussman 1965). Development from spore to young prothallus can be completed within a few weeks which allows a fairly rapid succession of experiments to be undertaken.
Dispersed amongst the considerable volume of work done on the morphology and taxonomy of Pteridophytes during the late 19th and early 20th centuries, are a few references to experimental modifications to morphology. These give indications that the pattern of development could be altered quite fundamentally under the influence of environmental factors. PRANTL for example (1881) showed that the development of fern gametophytes could be arrested at an early prothallus stage by growing them in a nitrogen deficient medium. BUCHTJEN (quoted by WILLIAMS 1938) described how light intensity could modify the morphology of Equisetum gametophytes.

KLEBS (1916-1917) found that temperature, in addition to light quality, could affect the rate and pattern of gametophyte development in species of PTERIS. An inverse relationship was shown to exist between the rates of cell division and expansion. Minimum expansion and maximum division rates occurred in white and blue grown cultures at 20°C. Maximum expansion and minimum division rates in cultures under red light at 25°C. Furthermore, KLEBS demonstrated that it was possible to alter the number of filamentous cells formed. In high intensity white or blue light, the filamentous stage was very short and the first re-orientated division occurred at the 4-celled stage. Decreasing the intensity of light, resulted in filaments of up to 10 cells. Culturing the gametophytes under red or yellow light prolonged the filamentous phase indefinitely.

These light wavelength effects were confirmed by CHARLTON (1938) who contrasted the elongated, red-grown filaments of PTERIS LONGIFOLIA and OSMUNDA CLAYTONIA with the plates of cells that these species form in the presence of blue light. These differences were found to be reversible. The observations
on *OSMUNDA* are especially interesting as this is a genus in which a filamentous stage is generally considered to be lacking. Thus, environmental conditions can not only modify the timing of a shift in polarity; they can also impose a polarity otherwise absent.

Later *MOHR* (1956b) carried out extensive and systematic experiments into the relationship between specific wavelengths and gametophyte development and drew up an "action spectrum" for transition. From measurements of the relative lengths and widths of the gametophytes under different wavelengths he demonstrated that beyond a critical point (450nm), transition to protallial growth would not occur.

Since these pioneering experiments, much of the work carried out by *MOHR* and other investigators has been concerned with the photoreceptive pigments involved and, to a lesser extent, the mechanism of the response, which has been inferred from the effects of specific light conditions.

None of the work has been primarily concerned with cell polarity or any other response of individual cells to light stimuli. However, re-orientation of division is the most conspicuous feature of transition (*REUTER* 1953; *MOHR* and *HOLL* 1965; *DAVIS* 1968a) and transition has generally formed the focal point of these photomorphogenetic studies. Therefore, since the re-orientation of division is an abrupt change, the effect of light wavelength has been assumed to be abrupt and attempts have been made to detect equally abrupt accompanying changes in biochemical parameters such as DNA (DRUM and *MOHR* 1967), RNA (HOTTA, *OSAWA* and *SAKAKI* 1959; RAGHAVAN 1965; DRUM and *MOHR* 1967; RAGHAVAN and HENG FONG TUNG 1967) and protein (HOTTA and *OSAWA* 1958; OHLENROTH and *MOHR* 1963; KASEMIR and...
Form the results of these biochemical studies, DRUMM and MOHR (1967a) have postulated that short wavelength (blue) light leads to the differential activation of genes. These genes then control:

(i) the formation of specific enzymes in the cytoplasm, and thence the characteristic morphogenetic responses (transition).

(ii) an increase in unspecific protein synthesis in the chloroplasts.

This concept of photomorphogenesis, with its assumption that the basic unit in the response is the gametophyte as a whole, is not fully justified by the published observations on gametophyte development. Differences in cellular growth during the filamentous phase, have been described, although their significance has rarely been discussed. For example, in experiments in which gametophytes were transferred from red to blue light DRUMM and MOHR (1967a) showed that within a few hours the increase in lateral expansion at the apex could be measured. The authors referred to this observation solely as evidence that the blue-light induced morphological changes were visible before and therefore not dependent on, changes in protein levels. It also, however shows that light wavelength not only initiates prothallial growth but also affects the polarity of expansion prior to transition.

There is also evidence that light wavelength can alter nuclear (BERGFIELD 1963b) and chloroplast (MOHR 1965b; BERGFIELD 1963a) volume in both filaments and prothalli, and that such changes are reversible. The variations in size have been correlated with changes in the rate of protein synthesis and have been used primarily as evidence for the role of protein in the
morphogenetic response (BERGFIELD 1963a), rather than as evidence for possibly autonomous responses at the cellular or sub-cellular level.

The literature also contains isolated references to differential responses by specific cells (e.g. KATO 1964; MILLER and MILLER 1964); such references suggest that in order to investigate the control of morphogenesis fully, it would be necessary to study the responses to environmental treatments, of individual cells as well as whole gametophytes. This present research therefore set out to characterise responses to environmental factors such as light and temperature (little studied previously), at the level of both the whole gametophyte and the individual cell, from germination to the onset of prothallial growth. Such information would, it was hoped, make it possible to then obtain specific, predictable growth patterns by selected environmental conditions this being vital to further experiments on the orientation and other aspects of cell division and expansion.

The investigation would also be likely to yield data on the relationship between the characteristics of filamentous growth and transition and might lead, incidentally, to new conclusions on the role of pigments and the mechanism of the light control over the development of at least one species.
DRYOPTERIS BORRELI was the species used in this investigation for several reasons which included:

(1) Fairly widespread and easily obtained.

(2) Gave a high yield of viable spores.

(3) Is an apogamous species which undergoes a sort of restitution division prior to a pseudomeiosis in the formation of the spores. In this way all the spores from one plant are genetically similar. Therefore any variation in the growth of the gametophytes can be attributed to environmental variations.

Fronds of DRYOPTERIS BORRELI bearing ripe sporangia were collected from Balerno in Midlothian in mid-September. Collections from individual plants were kept separate and labelled according to their origin. In the first year of the collections, each of the source plants was mapped and labelled, and samples of fronds bearing developing sporangia were taken in July so that the identification of each plant as DRYOPTERIS BORRELI could be checked cytologically.

The ripe spores were harvested by laying the fronds, sporangial side downwards, on sheets of white paper in the laboratory. After 24 hours the fronds were removed and the spores that had been shed onto the paper were transferred to specimen tubes. These tubes were then labelled according to the parent plant and the year of collection. 24 hour collecting periods were used because, although the spore yield could be increased by leaving the fronds on the paper for 48 or 72 hours, there was a danger that some of this increase might be due to the precocious liberation of unripe spores under these laboratory
conditions. This would have resulted in an increased spore yield only at the expense of the percentage germination achieved.

The specimen tubes containing the spores were then stored in a refrigerator at $4^\circ C$ until required for use. Unlike certain other work on ferns, it was not found necessary in this case to store the spores in a desiccator. In this investigation, preliminary experiments were carried out using spores from a single plant harvested in 1964 ($A_{1964}$). All later, quantitative work involved spores, again from a single plant, collected in 1966 ($B_{1966}$). No differences in germination percentages or rate or pattern of cell division, could be distinguished between these two spore samples. Each year the continued viability of the spores was assessed by comparing spores from the same plant harvested in successive years. (Again germination percentage and division rates were used as the criteria of viability). No decrease in viability was observed even after 4 years of storage.

**CULTURE MEDIUM**

The fern gametophytes were grown floating on the surface of an aqueous mineral medium. The medium was modified from that described in Mohr in 1956:

```
1) MgSO$_4$$\cdot$7H$_2$O  0.51 gm/litre  (0.25 gm anhydrous)
   KNO$_3$             0.12 gm/litre
2) FeCl$_3$$\cdot$6H$_2$O  0.017 gm/litre  (0.01 gm anhydrous)
   Ca(NO$_3$)$_2$$\cdot$H$_2$O  1.44 gm/litre  (1 gm anhydrous)
4) K$_2$HPO$_4$          0.25 gm/litre
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The constituents of this medium were made up and stored separately at a concentration 100 times that used in the eventual medium. Fresh batches of such stock solutions were made up every six months, and several times during
the investigations comparisons were made between the behaviour of ferns grown on different batches of medium.

In additions to these nutrients, the medium was supplemented by a fungicide Mycostatin (Squibb) at a concentration of 100 units per ml. This concentration was found by experiment to inhibit the growth of fungi such as Penicillium funiculosum which sometimes interfered with early experiments (cf MALTZAHN and MACQUAIRDIE 1958; HUTCHINSON and FAHIM 1958) without altering the growth characteristics of the ferns themselves. Suspensions of the fungicide were made up every month according to the directions of the manufacturers and kept at 4°C. Bacteria did sometimes contaminate the cultures still but they appeared to have no affect on the growth of the ferns.

This medium had a pH of 4.1 In experiments on the effects of pH on the growth of the prothalli, variations in pH were achieved by replacing the KH₂PO₄ with K₂HPO₄ to give a pH of 7, or by mixing the two salts to give a pH of 5.8.

CULTURE TECHNIQUE

The fern spores were sown by tapping them out of the specimen tubes through 5 sheets of lens tissue, onto the surface of about 80 ml. of medium in small glass crystalizing dishes (4" in diameter). The lens tissue filtered out pieces of sporangial wall harvested along with the spores. The inoculum for each dish was produced by tapping the inverted tube 30 times. This standardisation of the sowing technique avoided any differences in growth caused by variations in the density of the populations (cf HUREL-FY 1950; PIETRZYKOWSKA 1962 and 1962b).
The dishes were floated in a specially built Grant water bath in which the light and temperature conditions could be accurately controlled. A high water level was maintained in the bath so that the culture dishes floated in contact with sheets of glass laid over the top of the bath. These sheets of glass cut down evaporation from the dishes and prevented them from being swept round the bath in the current set up by the propeller.

TEMPERATURE CONTROL

The bath had two temperature controlling systems. Temperatures above the ambient were maintained by means of two thermostatically controlled heating coils; temperatures around and below the ambient were maintained by a continually running cooling unit operating in conjunction with a single heating coil under thermostatic control. This latter system could maintain a temperature of down to 1°C. Under either system the temperature fluctuations in the bath were ±0.25°C. In the dishes themselves no fluctuations could be detected.

LIGHT CONTROL

a) white light

A light rack holding six, two foot 40 watt Osram fluorescent and four, one-foot 60 watt tungsten tubes was supported at an adjustable height above the water bath. The wiring of these lights was arranged so that 2, 4, 6 fluorescent and/or 2 or 4 tungsten tubes could be switched on at any one time. This allowed a range of light intensities and qualities to be produced. 100 lumens/sq.ft (1080 lux) of fluorescent light, at the level of the glass sheets above the bath, was used as a standard. The energy content of this light was measured with a spectroadiometer (ASCO Model 3051) which characterized light
in terms of microwatts/cm²/nm. at sixteen different wavelengths. (see energy curves drawn from these measurements fig.1) (DOWNS et al 1964).

For experiments on the effects of light intensity, light with \(\frac{1}{4}\) and \(4 \times\) the energy of the standard 100 lumens/sq./ft was used. These are referred to as 25 and 400 lumens/sq./ft. white light respectively since it was found that provided the same fluorescent tubes were used and provided also that the light was only passed through clear glass, reducing the light intensity by \(\frac{1}{4}\) also cut the energy content by \(\frac{1}{4}\). Routine light checks during experiments were therefore done with a simple light metre instead of the much less convenient spectroradiometer.

b) **blue light**

Blue light was provided by replacing the clear glass sheets over the water bath, with blue glass plates (Flashed-blue sheet supplied by Cunningham, Dickson and Walker (Edinburgh). Measurements with a recording spectrophotometer (Unicam SP 800) showed that these sheets transmitted mainly in the range 382–516 nm and in the far-red above 690 nm. At wavelengths between these two regions of the spectrum, transmission was less than 1%. However the spectroradiometer curves show (fig. 1) that using white fluorescent tubes as the light source, the "blue" light had a single peak in the blue region, the energy then falling sharply to a very low level which is maintained through the red and far-red regions of the spectrum. For most experiments the blue light was standardised so that its energy was as close as possible to that of the blue component of the standard 100 lumen/sq./ft, white fluorescent light. This is referred to as "100 Blue" and had an energy peak at 440 nm of 0.71 microwatts /cm²/nm. (fig 1). In experiments on the effects of intensity on growth in blue light, the blue was standardised in a similar way but against other intensities of white light.
Fig. 1. Spectroradiometer measurements of the energy of 100 lm/sq.ft which fluorescent light ("100 White"), "100 Blue" and "100 Red".
c) **red light**

Red light was provided in a similar way to blue, using red glass sheets instead of blue (flashed Ruby sheet from Cunningham, Dickson and Walker (Edinburgh)). These glass plates transmitted light at all wavelengths above 580nm. but, using a solely fluorescent light source, the far-red content was very low. In most experiments this light was similarly standardised so that its energy content was brought as near as possible to that in the red region of the standard 1001m/sq.ft white. This "100Red" had an energy peak at 632nm of 1.5microwatts/cm² nm (fig. 1). Other intensities were obtained by standardising against other intensities of white light.

d) **far-red light**

Far-red was obtained using a combination of the red and blue filters and a tungsten source.

e) **green light**

Green light was obtained using a white fluorescent source and a Kodak Wratten filter (No. 59) which gave a single transmission peak at 525nm. The filter was enclosed in two clear glass plates and handled in the same manner as the glass filters.

These glass filters were 1-foot square, exactly half the surface area above the bath. This allowed two filters to be laid side by side over the bath. In order to do this it was important that the gametophytes received light only through the filter directly above them and to ensure this the outside of the culture dishes were painted black. When this was first done, it was feared that it might lead to a shading effect on the filaments close to the darkened walls of the dishes but comparisons of growth in unpainted, silver (reflecting) and black-painted dishes failed to produce any evidence of such an effect.
IMBIBITION

The cultures were all given an initial two day imbibition period in the dark at 22.5°C. The reason for this is discussed in the section on the "Standardisation of culture techniques".

GERMINATION

A standard germination procedure at 22.5°C was followed in all experiments. This consisted of 18 hours red light (15-184m/sq.ft) followed by 48 hours in the dark. (the evolution of this procedure is discussed below under "Standardisation of culture techniques").

ANALYTICAL METHODS

A) RATE OF CELL DIVISION:

Since during most of the filamentous phase of development, only the apical cell divide to give new green protonemal cells, the rate of increase in protonemal cell number per filament is a direct measure of mitotic rate at the apex. This rate of increase in green cell number was determined by finding the mean green cell number per filament in daily samples from the cultures. The products of divisions of basal cells to give rhizoids, and of interstitial divisions in the filament, were not included in these counts of mean cell number although the occurrence and frequency of such divisions were recorded.

Samples were gathered on a mounted dissection needle and placed on a microscope slide. Water or dilute acetic acid were the usual mounting media but in certain cases scoring was facilitated by staining the nuclei with lacto-propionic orcein. Sampling was always done from two dishes; two samples from each dish. In each sample, the number of cells in each of 50 filaments.
was scored under the low power of the microscope (x10 objective) (See account of the preliminary experiments on sample size in the section on "Standardisation of culture techniques").

At the end of the filamentous phase of growth the protonemata undergo a transition into prothalli in which divisions are no longer confined to a single cell or to a single plane. Growth and division during the filamentous stage is referred to as 1-dimensional. Division following the transition is referred to biplanar or 2-dimensional.

Sampling was discontinued once 50-60% of the gametophytes in a culture had undergone this transition.

B) CELL AND PROTONEMA LENGTH:
The lengths of the cells and protonemata were scored in the same samples as were used for scoring the mitotic rate and again the low power of the microscope was used. Camera lucida drawings of a sample of gametophytes were made and the lengths of these measured. For such measurements, a special "micron" ruler was prepared by tracing the Camera lucida projections of the scale of a micrometer slide. Using this device the actual lengths in microns, of the gametophytes could be measured directly.

The size of the fern sample traced, depended on the amount of variation in the cells/filament present in that culture. The size was adjusted so that the sample included five protonemata with the cell number represented in the culture with the lowest frequency. For example, in a culture containing

15% 3-celled filaments
65% 4-celled filaments
20% 5-celled filaments
a random sample of protonemata would be drawn until it included five 3-celled filaments. This rule was only applied to cell numbers representing 5% or more of the population. Filaments with a cell number occurring with lower frequency were considered atypical and not included in measurements of mean protonema length.

In most experiments, the mean protonema length was found by direct measurement and the mean cell length determined from this by dividing by the mean cell number per filament. In cases where the length or breadth of specific cells were required, these were measured on the same drawings that had been used for protonema length determinations.

C) CELL AND PROTONEMA VOLUME:

Cell and protonema volumes were measured after the method used for shoot apices by SUNDERLAND and BROWN (1956), by flattening the cells between two glass sheets and then determining their area. Two sheets of thick plate glass the size of microscope slides were used and between the ends of these were placed "separators" of a polyester film (Melinex type S gauge 12.7 microns (I.C.I)). The glass sheets were clamped together at both ends by means of pairs of brass bars, slightly longer than the width of the glass which could be screwed tightly together with bolts and wing-nuts. The space between the clamped glass sheets was then governed by the thickness of the spacer film. This was checked several times during the investigation by squashing a known weight of mercury between the plates and measuring the area of the metal.

The protonemata were mounted in liquid paraffin so that the contents of any broken cells were not dispersed.
The areas of the flattened protonemata were determined by tracing the Camera lucida projections of a sample of squashed filaments seen under the low power of the microscope, onto sheets of paper. These outline drawings were then cut and weighed and the area found by comparison with the weight of a known area of the same paper. The volume of the filaments was then taken to be this area \( \times \) the depth i.e. the gap between the two glass sheets (12.7 microns).

The size of the sample was decided in the same way as that used in the measurements of length. Again mean protonema volumes were found directly and mean cell volume derived from these but once again the same drawings could be used for finding the actual volume of specific cells by cutting out and weighing these cells separately.

D) FRESH AND DRY WEIGHTS:

The contents of a culture dish (or two dishes in samples from very early stages of growth) were collected on a millipore filter membrane (.45\(\mu\)) in a bacterial filter (carlson E.K. 3/1 "M" filter). The filter was connected to a vacuum water pump which was run for about 15 minutes in order to remove surface water from the gametophytes. After this draining period the gametophytes were removed from the membrane, divided into two portions and weighed. These weights are referred to as "fresh weight". One portion was placed in a centrifuge tube before weighing and used for chlorophyll and protein-N analyses. (see below). The second portion was weighed in a small specimen tube and used in dry-weight and cell count determinations. These gametophytes were dried for 24 hours in an oven at 95\(^\circ\)C., cooled in a desiccator and re-weighed - the "dry weight". From these weights the ratio of "fresh" to "dry" weights was found and this ratio used to calculate the dry weight of the portions used for biochemical analyses.
Early attempts to count the number of cells present in a weighed sample, showed that the well-known method of macerating the cells in chromic acid was too harsh for the ferns and resulted in a shattering of these highly vacuolate, thin-walled cells. The method eventually settled upon, therefore, was a modification of that used with fern gametophytes by (00TAKI 1965). The weighed, oven-dried cells were fixed for several weeks in a weak chromic-acetic fixative:

\(1 \text{ mg glacial acetic acid } \)   \[ \text{diluted to } 200 \text{ ml} \]
\(10 \text{ ml } 5\% \text{ chromic acid} \)

They were then washed thoroughly in water and dilute alkali and incubated overnight at 45°C in the bisodium salt of EDTA (0.01M). The EDTA separates the cells by attacking the pectic links between the cells of the filaments. The separation was completed and a homogeneous suspension obtained by passing the cells repeatedly through a hypodermic needle. The suspension was then made up to a suitable end-volume and the cells counted in a haemocytometer. Six grids were counted for each sample and the cells present in the sample calculated from these counts.

F) CHLOROPHYLL CONTENT:

Methanol was used to extract the chlorophyll from the samples that had been collected and weighed and described above. 1ml methanol was added to each sample in centrifuge tubes and the tubes placed in a refrigerator at 4°C. At the end of the experiment when all the daily samples had been collected and stored in this way, a second methanol extraction was carried out using 1 ml of hot methanol at 64°C. The decolourised gametophytes were then washed with two successive 1 ml portions of methanol. These washings and the two previous extracts were then combined and used in the estimation of chlorophyll,
while the residue was used for the determination of protein-nitrogen. The optical density of the chlorophyll solution at 650 and 665 nm. was found using a Unicam spectrophotometer sp 500. From this data, the total chlorophyll content was calculated using the formula of MACKINNEY (1941):

\[
\frac{2.55 \times 10^{-2} + 0.4 \times 10^{-2}}{D_{650}} = \text{chlorophyll in mg/cm}^3
\]

G) PROTEIN-NITROGEN CONTENT:

The methanol insoluble residue obtained above (chlorophyll estimation), was used to estimate the protein-nitrogen content of the gametophytes. This residue was extracted twice with perchloric acid; the first time in the cold (4°C) for two hours using a concentration of 0.2M perchloric acid, then at 70°C for 30 minutes using 0.5M acid. The residue from these extractions was then dried overnight in a vacuum desiccator, and the nitrogen content of this final residue was referred to as "protein-nitrogen".

The nitrogen estimation was carried out by means of the microdiffusion technique of CONWAY (1962), following the digestion of the perchloric acid insoluble pellet in an acid solution which converts organic nitrogen to an ammonium salt. The digest acid employed was 36N N-free sulphuric, to which had been added the equivalent of 0.2 gm/litre CuSO₄, the selenium acting as a catalyst in the digestion process. 0.2ml of this acid was added to each of the pellets in centrifuge tubes and the digestion was carried out on an electrically heated rack. The heating was continued until the solutions were completely colourless (60-90 minutes). The tubes were then covered with aluminium caps, cooled and the solutions diluted to 2ml with distilled water.
The microdiffusion process was done in no. 2 Gallengamp vessels. These were cleaned by boiling three times in detergent, rinsed thoroughly with dilute acid and distilled water and then dried overnight in an oven.

The ground glass rims of the vessels were painted with melted vaseline in order to ensure an air-tight seal when the lids were in position. Vaseline was also smeared around the central well to prevent creeping of the liquids, and a barrier of vaseline drawn across the outer vessel so that the reactants could be kept separate before the lids were secured.

0.5ml of the digest, i.e. 1/4 of the total, were pipetted carefully onto one side of the vaseline barrier and 0.5ml of 40% NaOH on the other. 0.2ml of indicator were placed in the central well. The indicator used was 0.0016% bromo-cresol green + 0.0002% methyl red in boric acid.

2 microdiffusion vessels were prepared from each digest and duplicated blanks prepared using distilled water. When the three solutions had been pipetted into each vessel, the flat ground glass lids were pressed on firmly and the vessels tipped and rotated so that the alkali and digest were thoroughly mixed.

The reaction vessels were then left undisturbed overnight to allow the ammonia, liberated by the alkali, to diffuse into the central well where it was absorbed by the boric acid solution. The next morning the lids were removed and the alkaline solution in the central well titrated against N/10 HCL in a Beckmann model 153 microtitrator. The Nitrogen content was then calculated from the titration value on the basis that:

1 microlitre N HCL = 14 micrograms Nitrogen
1) IMIBITION TIME

One of the advantages of *Dryopteris* as an experimental system is that, because the spores require light for germination, they can be kept in the dark and then growth "switched on" by bringing them into the light. The spores do not however, become light sensitive until they are fully imbibed and if the spores are allowed to imbibe in the light then, within any culture, variations in the time necessary for individual spores to imbibe results in a spread in the rate of germination and subsequent growth. A dark imbibition period long enough to render all spores equally light sensitive was found necessary in order to produce a uniform culture. A series of preliminary experiments was therefore carried out to determine the optimum duration of this dark period.

In the first experiment, imbibition times of 12, 18, 24, 36 and 42 hours at 22°C were used and the cultures were then given 40 lumens/sq. ft. white fluorescent light. The cultures given 12, 18 or 24 hours in the dark showed an initial "lag period" with a slow division rate. The lag phase in the division rate against time curves (fig. 2a) disappeared when an imbibition time of 36 hours or more was given. This suggested that the time necessary for complete imbibition lay somewhere between 24 and 36 hours.

A second experiment was then done in order to check this conclusion and also to see whether longer dark periods had any affect on subsequent growth. The results from this experiment showed that there was no difference in the growth rates of cultures given 36, 48 or 96 hours in the dark. The cell number against time curves (fig. 2b) for these cultures were parallel and exactly 12 and 48 hours behind each other respectively. Once again a lag
Fig 2. Effect of the duration of the dark imbibition period on the subsequent increase in filament cell number in "100 White" at 22°C.
phase in the growth rate curve showed that imbibition was not complete at the end of a 24 hour period. Because these data failed to indicate any inhibitory effects of long dark periods, 48 hours was chosen as a convenient standard period.

2) GERMINATION PROCEDURE

Data from previous workers on fern gametophytes indicated that while both germination and subsequent phases of gametophyte development require light, the optimal light wavelengths involved are quite different. Because of this, and because the investigation was primarily concerned with the post-germination stages, it was decided to give all the cultures a standard germination treatment. In this way uniform cultures would be obtained at the onset of the filamentous period. In order to avoid interference with subsequent experimental light treatments, it was important that only a minimum amount of light was used to induce germination. A series of preliminary experiments were designed to find a suitable germination-inducing procedure. These experiments were designed to determine:

(i) The most effective light quality.

(ii) The minimum amount of light necessary - including the possibility of splitting the inductive period into two shorter periods with a dark interval in between.

ISAKAWA and COHUSA (1956) had found in DRYOPTERIS CRASSIRHIZMA that such a split enhanced the inductive effect of the light.

(iii) Any possible interactions between light of different wavelengths.

All the experiments were done at 22°C and all the cultures were given an initial two-day imbibition period in the dark as already described.
Germination was scored on the fifth day following the light treatment.

The criterion used for germination was the appearance of a small, transparent, lens-shaped protuberance through the spore wall.

The results of these experiments are summarised in tabular form below:

<table>
<thead>
<tr>
<th>First light period</th>
<th>Dark</th>
<th>Second light period</th>
<th>final light regime</th>
<th>percentage germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>light</td>
<td>duration</td>
<td>light</td>
<td>duration</td>
<td>regime</td>
</tr>
<tr>
<td>200 lm/sq.ft</td>
<td>10 mins</td>
<td>-</td>
<td>-</td>
<td>Dark</td>
</tr>
<tr>
<td>white fluorescent</td>
<td>3 hours</td>
<td>-</td>
<td>-</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>12 &quot;</td>
<td>-</td>
<td>-</td>
<td>&quot;</td>
</tr>
<tr>
<td>30 lm/sq.ft Red</td>
<td>18 &quot;</td>
<td>-</td>
<td>-</td>
<td>&quot;</td>
</tr>
<tr>
<td>15&quot;</td>
<td>&quot;</td>
<td>-</td>
<td>-</td>
<td>&quot;</td>
</tr>
<tr>
<td>40&quot;</td>
<td>&quot; Blue</td>
<td>-</td>
<td>-</td>
<td>&quot;</td>
</tr>
<tr>
<td>30 lm/sq.ft Red</td>
<td>5 mins</td>
<td>18 hours</td>
<td>30 lm/sq.ft Red</td>
<td>5 mins</td>
</tr>
<tr>
<td>(Fl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red—far-red</td>
<td>5mins—5mins</td>
<td>&quot; red</td>
<td>5 mins</td>
<td>&quot;</td>
</tr>
<tr>
<td>(30 lm/sq ft)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red (30 lm/sq ft)</td>
<td>5mins</td>
<td>&quot; red—far-red</td>
<td>5mins—5mins</td>
<td>&quot;</td>
</tr>
<tr>
<td>30 lm/sq.ft Red</td>
<td>5 mins</td>
<td>18 hrs</td>
<td>Red</td>
<td>6 hrs</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
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<td>&quot;</td>
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</tr>
</tbody>
</table>

These results illustrate several of the findings reported in a recent paper on the germination of *Pteris Vittata* by Sugai and Furuya (1967).
(1) Red light was the most effective inductive light treatment (cf. BUNNING and MOHR 1955).

(2) White light also induced germination at much higher intensity used.

(3) Phytochrome was the photoreceptor involved. This was indicated by the reversal of the red light induction by far-red light. (cf. MOHR 1956a).

(4) Blue light was not merely ineffective as an inductive agent, it also inhibited the inductive effect of red light. Replacing the blue light with green led to an enhancement of germination, thus indicating that the inhibition was a specific blue light effect. (SUGAI and PURUTA (1967) also found that green light could induce a low % germination).

Quite apart from its very high efficiency, red light has an even greater advantage as the inductive agent in these experiments. Earlier work had established that one of the critical factors affecting development during the filamentous and prothallial stages, was the amount of blue light available. If this blue light requirement was to be investigated experimentally, then blue light should be excluded as far as possible from the standard light inductive period of germination. Red light of 15 lm/sq.ft was therefore decided upon as the inductive agent.

The results of the preliminary experiments also showed that 6 hours red light split into two inductive periods separated by 18 hours dark, was more effective than a single period of 6-12 hours (70% cf. 40-60%). The total length of the split treatment however was 24 hours, while a single period of 18 hours was sufficient to yield maximum germination (90%). Spores given an 18 hour inductive period and then left in the dark, germinated but underwent no further growth. It was judged from this observation, that, although this inductive treatment involved more than the absolute minimum light energy necessary for germination, it was unlikely to interfere with subsequent experi-
mental light treatments. On this basis, a single 18 hours period of low red light (15 \text{ w/sq ft}) was chosen as a simple and effective inductive treatment.

The experiments with blue and green light, showed that light given immediately after the inductive light could, on the other hand, interfere with the effectiveness of this induction. It was therefore necessary to impose a dark interval between the inductive and experimental light treatments. Such an interval had the additional advantage of increasing the uniformity of subsequent growth, by allowing slow-germinating spores to catch up with faster ones by suspending the growth of the latter.

The optimum duration of this dark period was determined by finding the time necessary for every spore in the culture to have undergone their first, rhizoidal, mitosis. This had been found to be 42 hours after the end of the inductive light period (DYER unpubl.). Increasing the interval to 48 hours did not however adversely affect subsequent growth, so 48 hours was settled upon as a convenient standard dark period.

The complete germination treatment eventually used after 48 hours dark imbibition was therefore:

18 hours 15 lumens/sq.ft red light followed by 48 hours in the dark - all at 22.5°C.

3) THE UNIFORMITY OF CULTURES

(A) THE USE OF MEAN CELL NUMBER/PROTONEMA AS A INDICATOR OF GROWTH RATE

It is a great advantage in any growth study to have a quick and reliable indicator of the rate of growth. In some systems, fresh weight or the area of tissue have been used for this purpose but in the young fern gametophyte, the rate of increase in cells/protonema provided the most convenient way of
assessing growth. Examples of its use as an indicator have already been referred to in the sections on "plant material" and "imbibition".

If it was to be a reliable indicator of growth, then it was essential that the cell number per protonema was determined accurately. Examination of samples from cultures grown under standard conditions indicated that a certain amount of variation in cell no/filament existed within these cultures. This variation was only of the kind expected in a sample of individuals sharing a fairly uniform rate of division but not undergoing synchronous mitoses. Nevertheless its existence meant that the samples used for the scoring of mean cell number/protonema had to be large enough to represent the variation within the whole culture. An experiment was therefore designed to investigate the amount of variation within and between samples and so determine a sample size suitable for the accurate evaluation of mean cell number/protonema.

The experiment was set up at 22°C using:

1) Two light treatments a) 40 lumens/sq. ft white light (432 lux)
   b) 100 " " " " (1080 lux)

2) Four dishes per treatment A, B, C, and D under 40 lumens/sq. ft.
   E, F, G, and H under 100 " " "

3) Four samples taken per dish A_i, A_ii, A_iii, A_iv etc

4) 100 sporelings counted in each sample.

A 24 hour imbibition period was given to all the cultures prior to the start of the light treatment. Daily sampling was started on fifth day of the light (day 5) and was continued until differentiation set in on day 9.

Mean daily values for the cells/protonema were found for each sample, dish and treatment. Histograms (fig 3) have been used to indicate the extent
Fig. 3. Histograms expressing the variation in the number of cells per filament within a sample of 1600 filaments, at 22\textdegree\ in white light

a) 40 lm/sq. ft

b) 100 "  "
of variation in cell number over the whole 1600 filaments counted per
treatment per day. These histograms show that variation increased over
the period of the experiment so it was the data from the last samples (day
9, 40 lumens/sq. ft) that were chosen to test the significance of the variation
per sample.

The 1600 individuals scored each day were taken to represent the total
variation within the whole population and an analysis of variance within and
between each culture dish was carried out to test the random distribution of
this variation. The figures used in this calculation are in an appendix of
this thesis. Application of SNEDECOR'S test indicated that the variation
ratio variation between dishes variation within dishes was significant at a 9% but not a 1% level.

One possible reason for variation between dishes was that there was a
variation in light intensity over the tank of 10-15%. In order to minimise
the effects of this and other sources of variation between dishes, it was
decided to take a mean of samples from more than one dish. The effectiveness
of this measure was tested by a second analysis of variance using "blocks"
of two samples, one from each of two dishes. Such an analysis showed that
here the variation within blocks was greater than between blocks i.e. the
combined samples were large enough to represent the variation that exists
within this population.

As a further precaution against non-random variation within dishes, two
separate samples were taken from each dish and 50 filaments scored in each
sample.
The cell numbers per protonema data quoted in this thesis are therefore means of 200 filaments; 50 filaments per sample, two samples per dish, two dishes per treatment.

(B) THE CONSTANCY OF THE GROWTH RATE IN REPLICATED EXPERIMENTS

An example of the kind of replication obtained with regard to the rate of increase in cell number/protonema using the sampling procedure described above, is illustrated in the graph (fig 4).
Fig. 4. Increase in filament cell number with time in replicated experiments in "100 White" at 22°C after standard imbibition and germination treatments.
Days after Germination

Cells per Protonema

△ Run 1

△ Run 2

○ Run 3
Introduction

This investigation set out to study the influence of certain environmental factors on the growth and morphogenesis of the gametophytes. The environmental factors were light quality and intensity, temperature, and pH. The growth parameters measured were:

1. Cell number per filament
2. Protonema length
3. Protonema volume
4. Dry weight per gametophyte
5. Protein-Nitrogen per gametophyte
6. Chlorophyll per gametophyte

In the following accounts, these experimental results have been recorded as changes in each parameter with time and they have then been used in the derivation of secondary data.

These include:

1. Derived parameters
2. Relationships between parameters
3. Rates of change

1) Derived parameters

(a) mean cell diameter

This is determined from measured mean filament volume and length by the equation:

\[
\text{Diameter} = 2 \sqrt[3]{\frac{\text{volume}}{\text{length}}} 
\]
(b) **Mean cell values**

Where filament values were measured, mean cell values were determined by dividing by mean cell number. For other parameters, such as dry weight, mean cell values were obtained during the calculation of filament values as described under "Methods".

2) **Relationships between parameters**

(a) **Ratio of protein-Nitrogen/dry weight**

On the assumption that the two major components of dry weight are protoplasm and cell wall, and that the protein-Nitrogen content is related to the amount of protoplasm, a change in the protein-N : dry weight ratio is taken as an indication of a change in the proportion of protoplasm to cell wall synthesised.

(b) **Length : Diameter ratio**

Changes in volume are the result of both lateral and longitudinal expansion and the length : diameter ratio expresses the extent to which the expansion is polarised longitudinally.

3) **Rates of change**

(a) **per unit time**

The slope of a graph of a parameter recorded against time is the rate of that parameter per unit time. The overall mean rate or the rate at any given time, determined from this slope, can then be plotted against an environmental variable such as temperature or light intensity.

(b) **per cell division**

The slope of a graph of a parameter plotted against cell number is the rate of change of that parameter per cell division. A slope of 1
indicates that the parameter is increasing in step with cell division and thus the mean cell value remains constant. Slopes of greater or less than 1 indicate respectively a rise or fall in mean cell values, as the parameter rises more or less rapidly than cell number. Where mean cell values, however seem to be of special significance, they have been plotted separately.

The mean slope of a graph plotted against cell number gives the average increase in the time taken for one cell division (a "mitochron"). Values can also be determined for particular cell division cycles during development.

Filament cell number is one way of assessing developmental stage since certain morphological events such as the formation of the second rhizoid and the first longitudinal wall occur in filaments of a particular cell number. Consequently, plotting a parameter against cell number reveal any changes that are associated with a particular developmental stage, even if different conditions have affected the duration of filamentous growth.

PATTERNS OF MORPHOGENESIS

The typical sequence of development in the gametophytes of DRYOPTERIS is common to many ferns and may be divided into four main phases: (see fig 5-6)

1) Germination
2) Filamentous growth
3) Transition from 1-dimensional filamentous to 2-dimensional prothallial growth
4) 2-dimensional growth

The criterion used to recognise germination was the appearance of a small lens-shaped projection from the spore-coat. This projection then elongates to become
Fig. 5. Pattern of germination and filamentous growth in "100 White" at 22°.
Germination

1. Germination

2. Filamentous Growth

Interstitial division in $P_{ii}$
the first rhizoid \(R_1\) of the gametophyte. The green spore cell then expands out of its constricting jacket and divides to give a second green cell, the main axis of this expansion being at right angles to the elongation of \(R_1\). The new apical cell \(P_2\) now divides again so that a filament of three green cells is formed, while the basal cell \(P_1\) usually undergoes a second rhizoid division. The apical cell continues to divide to give a filament of four or more cells, the number depending on the environmental conditions.

Transition from this filament to a prothallus with divisions occurring in two planes, may now be initiated by a re-orientated division – with the new cell wall at right angles to the long axis of the filament – in the third cell back from the apex \(P_{iii}\). This first re-orientated division is sometimes followed by another apical division in the old plane, this in turn being followed by a second re-orientated division in the new \(P_{iii}\). A re-orientated division then occurs in \(P_{ii}\) and the products of all these re-orientated divisions divide to give short files of cells ending in papillae. The original apical cell \(P_i\) meanwhile divides unequally first to the side away from these papillae and then alternately to one side and then the other, cutting off files of cells, most ending in papillae which form the 'wings' of the prothallus. Eventually this apical cell forms the apical initial cell of the heart-shaped prothallus.

This pattern of transition is the one usually considered in the literature to be typical of Dryopteris and so it is referred to here as TYPE 1 transition, (Plate la). Early on in the investigation however it became obvious that another quite distinct pattern of transition occurs. This second pattern is referred to as TYPE 2 and it may occur alongside type 1, even in a single culture.
Fig. 6. Cellular patterns during Type I and type 2 transition.
3. Transition

TYPE 1

\[ P_{iii} \rightarrow P_{ii} \rightarrow P_i \]

TYPE 2

\[ P_{iv} \rightarrow P_{iii} \rightarrow P_{ii} \rightarrow P_i \]
Plate Ia. Young prothallus after Type I transition ("100 White" 22°C).

Plate Ib. Young prothallus after Type 2 transition ("25 White" 25°C).
In TYPE 2 development, (Plate 1b) filamentous growth is terminated by the formation of an apical papilla from \( p_1 \). Further growth is then a function of the third or fourth cell back from the apex, \( p_{iii} \) or \( p_{iv} \). Thus in TYPE 2 development the apical meristem of the prothallus is derived from \( p_{iii} \) or \( p_{iv} \) whereas in TYPE 1 it is derived directly from \( p_1 \), the apical cell of the filament.

In these descriptions of TYPE 1 and TYPE 2 transition it has been assumed that only the apical cell divides to give new protonemal cells. Under certain conditions however, interstitial divisions may occur (first in \( p_{ii} \) then sometimes \( p_{iii} \)) as a modification of either a TYPE 1 or TYPE 2 development. In TYPE 2 such divisions may occur either before or after the formation of the apical papilla. (Plate 2)

Following both types of transition a typical heart-shaped prothallus is formed, but under rather more extreme environmental conditions such a high temperature, no such prothallial development takes place. In some cases the filamentous phase of growth is terminated by a papilla as in TYPE 2 transition, and a single re-orientated division may even occur. Subsequent development of a 2-dimensional mass of cells at the apex does not occur however, and further growth proceeds from filamentous branches which originate from cells along the main filament by divisions at right angles to the long axis of the filament (fig 7b).

In other cases no terminal papilla is formed and the end of the initial filamentous stage is marked only by the cessation of divisions in the apical cell and by the production of lateral branches along the filament.
Plate 2. Filament in which an interstitial division has occurred.
("100 White" 25°C)
All the developmental patterns described so far involve only a limited period of filamentous growth in the main filament, but the gametophyte may under certain conditions continue as a filament for long periods. Such filaments may be simple and unbranched (fig. 7a) or may be forked due to outgrowths from basal cells (fig. 7c). In such forked filaments, cells of both arms normally continue to grow and divide.

Under a few conditions, filaments may be formed that display both types of branching already described — the basal forking and lateral branching from cells along the filament (fig. 7d).

In summary then, the main variations in morphogenetic patterns are:

1) Germination and early filamentous growth is very similar in all cases except that sometimes only one basal rhizoid is formed and in others three or four, instead of the usual two.

2) A minimum of four filamentous cells are always formed prior to the onset of transition or "lateral" branching. Basal forking may occur at the two-three celled stage.

3) (A) Growth of the main filament limited:

   (i) transition to 2-dimensional growth (a) TYPE I transition. NO interstitials
       (b) TYPE I " + "
       (c) TYPE 2 " NO "
       (d) TYPE 2 " + "

   (ii) continued growth of lateral branches

       (a) involves "abortive" transition - either papilla, or papilla +
           I re-orientated division
       or (b) involves no transition, merely cessation of divisions in
           the apical cell.
Fig. 7. Variations in the pattern of filamentous growth.
Unbranched filament
Filament + Lateral Branches
Forked Filament
Lateral Branches
(B) Growth of main filament unlimited:
   (a) unbranched
   (b) + basal forking
   (c) + " " and lateral branching

In order to avoid repetition in descriptions of the pattern of morphogenesis found under any particular set of environmental conditions, a shorthand code has been devised. The very convenient (though not literally accurate) terms, one-dimensional and two-dimensional have already been explained and applied to the modes of growth and division found during filamentous and prothallial growth respectively. The other abbreviations which will be used are:

- $R_n$ referring to the basal Rhizoids where $n$ is the mean number of such rhizoids.
- $F_n$ referring to the Filamentous cells where $n$ is the mean number of cells at the onset of transition or branching.
- $t_n$ referring to the cells of the filamentous "Tail" i.e. the cells that are not involved in transition, where $n$ is the mean number of such cells.
- $I_x$ referring to Interstitial divisions where $x$ is the frequency of such divisions in a culture.
- $T_I$ and $T_{II}$ referring to TYPE I and TYPE 2 Transition respectively.

In mixed cultures the suffix $x_1 I + x_II = T_1$ is the relative frequencies of the two transition types.

- $XP$ referring to filaments terminated by Papillae where $x$ is the frequency of such papillae in the culture.
- $P_{+I}$ referring to a filament undergoing abortive transition.
\( B_{x(f)} \) and \( B_{x(I)} \) refering to basal Forking and Lateral Branching respectively.

Again the suffix \( x = \) frequency of branching.

\( B_{x(f)} = \) filaments displaying both types of branching.

Specific cells in the filaments are designated in two ways:

(a) Capital \( P \) with a suffix of Arabic numerals \( 1, 2, 3 \) etc. describe the cells from the base towards the apex so that \( P_1 \) is the basal cell and in a 3-celled filament \( P_3 \) is the apical cell.

(b) Small \( p \) with a suffix of the type \( i, ii, iii \) etc. describes the cells from apex to base so that \( p_1 \) is the apical cell and \( p_{iii} \) is the third cell back from the apex.
Preliminary observations indicated that the developmental pattern of the gametophytes is extremely sensitive to temperature and that shifts of only 2 - 2\degree C could result in changes in both the rate and the pattern of development. A series of experiments was devised to investigate more fully the influence of temperature on morphogenesis and to look for correlated effects on the growth parameters.

For this series 100 lumens per sq. ft. of white fluorescent light and a range of five temperatures at 2\degree intervals were employed. 22\degree C was chosen as the standard, central point in the temperature range because, under these light conditions:

1. the division rate was high.
2. there is an appreciable phase of filamentous growth (around 5-6 cells) which gave scope for both the lengthening and shortening of this phase.
3. TYPE 1 transition occurs in nearly all the gametophytes.

The complete temperature range used was 17\degree, 20, 22\degree, 25, 27\degree C.

Exploratory cultures were also set up to investigate the effects of temperature beyond the range of this main experiment:

a) 30\degree C - no growth at all occurred at this temperature.

b) 15\degree C - the rate of division was considerably lower than at 17\degree C but the pattern of development was the same.

It seems likely therefore that the range of temperatures used, was sufficient to encompass the main variations in growth patterns found under these light and nutritional conditions.
EXPERIMENTAL RESULTS

(1) The number of cells per filament

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>slope of cell no./time</th>
<th>mitotic cycle time</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 1/2</td>
<td>0.64</td>
<td>37.5 hours</td>
</tr>
<tr>
<td>20</td>
<td>0.65</td>
<td>37 &quot;</td>
</tr>
<tr>
<td>22 1/2</td>
<td>0.9</td>
<td>26.7 &quot;</td>
</tr>
<tr>
<td>25</td>
<td>0.7 - 0.87</td>
<td>34 &quot;</td>
</tr>
<tr>
<td>27 1/2</td>
<td>0.7 - 0.55</td>
<td>34.5 - 44 hours</td>
</tr>
</tbody>
</table>

The data show that the mitotic rate is considerably affected by temperature and that 22 1/2 °C is the optimum for division. At this temperature the rate of division remains remarkably constant throughout the filamentous growth phase. At higher temperatures (25 and 27 1/2 °C) the rate of division changes at the 2 - 3 celled stage of development. This 2 - 3 celled stage appears to be in some way critical since similar "kinks" occur in the curves of other parameters at equivalent points.

The extreme constancy of the division rate in replicated experiments and hence the validity of the differences shown here, has already been demonstrated in the section on the "uniformity of cultures". (page 25).

(2) Morphogenesis pattern

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>F_n</th>
<th>T_n</th>
<th>Transition type</th>
<th>I_x</th>
<th>Day of 50% transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 1/2</td>
<td>4 - 5</td>
<td>1 - 2</td>
<td>T_I</td>
<td>0.3</td>
<td>9</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>2</td>
<td>T_I</td>
<td>0.2</td>
<td>9.5</td>
</tr>
</tbody>
</table>
Fig. 8. Effect of temperature on the increase in filament cell number with time in "100 White" (in this and all subsequent figures, time is expressed in days after the end of the standard germination treatment)
At temperatures such as 17° and 22° C with a variable filament cell number, the lowest number occurred in the protonemata that underwent the earliest transition i.e. there was an increase in Fₙ with time. Similarly at 27° C, the first formed papillae were in cell P₆.

(3) **Protonema length**

fig 9. shows that the rate of elongation is influenced by the temperature and that the maximum rate occurred between 22° and 25° C. At the lower temperature (17° - 22°) the rate was more or less constant throughout the filamentous period of growth while the curves of elongation at 25 and 27° are slightly sigmoid.

(4) **Protonema volume (fig 10)**

The rate of expansion was also affected by the temperature and the optimum again lay between 22° and 25° C. Here however, a constant rate of

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>Fₙ</th>
<th>Tₙ</th>
<th>Transition type</th>
<th>Iₓ</th>
<th>Day of 50% transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>22°</td>
<td>5-6</td>
<td>2-3</td>
<td>T.98 I + .02 II</td>
<td>0.18</td>
<td>8</td>
</tr>
<tr>
<td>25</td>
<td>6</td>
<td>3</td>
<td>T.91 I + .03 II</td>
<td>0.16</td>
<td>10.75</td>
</tr>
<tr>
<td>27°</td>
<td>6-9</td>
<td>3-9</td>
<td>.05F + .01P + B₁</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**KEY**

- Fₙ = filament cell number at transition
- Tₙ = cells of filamentous "tail"
- xP = frequency of filaments + papillae
- xP⁺₁ = "" + abortive transition
- B₁ = lateral branching
- Iₓ = frequency of interstitial divisions
Fig. 9. Effect of temperature on the increase in protonema length with time, in "100 White".
DAYS AFTER GERMINATION

PROTONEMA LENGTH IN MICRONS

- - - - 17½ °C
□□□□□ 20°
▼▼▼▼ 22½°
 keeps 25°
○○○○○ 27½°
Fig. 10. Effect of temperature on the increase in protonema volume with time, in "100 White".
DAYS AFTER GERMINATION

PROTONEMA VOLUME IN CU J X 10^4

- 17.5°C
- 20°C
- 22.5°C
- 25°C
- 27.5°C

..
increase was given at the higher temperatures (25 and 27°C) while the curves of expansion at 17°C - 22°C indicate that initial slow expansion was followed by a sharp rise in the rate. This higher rate then remained constant up to the onset of transition.

(5) **Dry Weight per Gametophyte**

The dry weight per cell was found by estimating the number of cells in a known dry weight of gametophytes; this multiplied by the mean number of cells per filament already determined for each culture, gave the dry weight per gametophyte.

Several of the curves on the graph (fig. 11) show an initial decrease in dry weight. This is caused mainly by the shedding of the outer spore coats. At the start of the sampling period most of the protonemata were still enclosed in these coats and both were harvested and weighed together. Later, when the coats had been shed and lay free in the culture medium, they tended to be retained on the filter membrane when the protonemata were removed for weighing.

After this early loss in dry weight, all the cultures displayed the expected increase with the highest once again occurring at around 22°C although the final dry weight achieved was very similar in all the cultures grown at temperatures between 17°C and 25°C. Only the 27°C culture showed significantly lower dry weight throughout the sampling period.

(6) **Protein-N per Gametophyte**

Protein-N per gametophyte was determined indirectly first finding the protein-N content of a known weight of gametophytes. The number of cells per unit weight was then estimated as above, and the protein-nitrogen per cell and thence per gametophyte found from this.
Fig. 11  Effect of temperature on changes in dry weight per gametophyte with time, in "100 White".
The graph (fig. 12) reflects an increase with time in the protein-N content of gametophytes grown at temperatures between $17\frac{1}{2}$ and $25^\circ$, the greatest increase being at $22\frac{1}{2}^\circ$. At $27\frac{1}{2}^\circ$ the gametophytes increased their protein-N contents up to a maximum on Day 7. After this the protein level decreased markedly although the reason for this is not clear.

CHLOROPHYLL PER GAMETOPHYTE

The chlorophyll was measured in a sample of known weight and the number of cells (and thence protonemata) per unit weight found for a second sample from the same culture. These results show that the changes in chlorophyll content of the gametophytes followed the same pattern as the changes in protein content. The main feature of interest shown in the graph (fig. 13) is that the rate of increase was much slower in cultures grown at $27\frac{1}{2}^\circ$ and that at this temperature a maximum value was reached at Day 7, whereas at the lower temperatures a steady rate of increase was maintained throughout the sampling period.

DISCUSSION

Similarities in the response of these growth parameters to temperature can be demonstrated when the relative "rate of change" at different temperatures are compared graphically (fig. 14). Here the slope of each growth curve has been averaged out over the sampling period as a whole, and plotted against temperature. Obviously, since the units were different in each case, only the shape of the curves and not their absolute values can be compared. Most show an optimum at $22\frac{1}{2}^\circ$, only volume having a peak at a slightly higher temperature. The curves also indicate that most of the parameters changed less between $17\frac{1}{2}$ and $20^\circ$ than between any other pair of temperatures.
Fig. 12  Effect of temperature on changes in protein-N per gametophyte with time, in "100 White".
Fig. 13 Effect of temperature on changes in chlorophyll per gametophyte with time, in "100 White".
Fig. 14  Effect of temperature on "rates of change" of growth parameters.
The graphs of basic data against time reveal two distinct types of response. The influence of temperature on volume and length of the protone-mata parallels its influence on the rate of division, while the changes in dry weight protein-N and chlorophyll resemble each other closely. The results can therefore be discussed in relation to two distinct aspects of cell growth, cell expansion and cell synthesis.

**Cell Expansion**

(1) **Volume**

Although the rates (per unit time) of increase in protonema volume were markedly different at different temperatures, the increases per division cycle (mitochron) were similar (fig. 15). In fact at the 4-celled stage the mean volume in all the cultures were remarkably alike. The increases per mitochron in the 17.3, 25 and 27.2°C cultures were more or less constant throughout the sampling period but at the other temperatures - 20 and 22°C there was a sharp rise at the 2-3 celled stage. The greatest final volume (i.e. at the onset of transition) occurred at 22°C so, in this respect, this can be considered the optimum for expansion.

Mean cell volumes plotted against filament cell number (fig. 16) indicate that at 17.3, 25 and 27.3°C there was a slight increase in volume over the whole sampling period. This suggests that expansion just compensated for the effects of division upon cell volume. At 20 and 22°C the mean cell volume fell, initially then after the 3-celled stage, rose rapidly to give high final values. This demonstrates that expansion compensated for and exceeded the effects of division, only after the filament comprised several potentially expanding cells.
Fig. 15. Effect of temperature on the increase in protonema volume with increase in number of cells per filament, in "100 White"
PROTONEMA VOLUME IN CU μ X 10^4

CELLS PER FILAMENT

- ○ 17½ °C
- □ 20 °C
- ▽ 22½ °C
- ▼ 25 °C
- ○ 27½ °C
Fig. 16. Effect of temperature on changes in mean cell volume with increase in number of cells per filament, in "100 White".
(a) lateral expansion (fig. 17)

The cross-sectional area of the gametophytes grown at the lower temperatures showed a slight increase during filamentous growth, although at 20 and 22.5°C this increase was again not apparent until the 2 - 3-celled stage had been reached. Grown at 25°C the mean cross-sectional area of the gametophytes remained fairly constant while at 27.5°C it fell with time. Judged in terms of the final values achieved, the maximum cross-sectional areas occurred at 22.5°C and the minimum at 27.5°C.

(b) longitudinal expansion

As was the case with volume, the protonema length data indicate that while the rate of increase was clearly influenced by temperature, the increases per mitochon were very similar throughout the temperature range, with perhaps a slight indication that 25°C was the optimum. Increase in length (fig. 18) against cell number also shows a linear relationship i.e. a constant increment per mitochon, throughout filamentous growth.

The changes in mean cell length per mitochon (fig 19) on the other hand showed a pronounced dependence upon temperature. Between 17.5 and 22.5°C the mean cell length decreased during development, indicating that longitudinal expansion was not sufficient to offset the effects of division. At 25°C there was a gradual increase in cell length while at 27.5°C the cell length values started to rise rapidly at the 2 - 3-celled stage, so that the final values show an increase with increasing temperature. From these data it is clear that elongation and lateral expansion are independently variable and that in addition to variation in total expansion over the temperature range examined, there was also variation in the polarity of this expansion.
Fig. 17. Effect of temperature on changes in cross-sectional area with increase in number of cells per filament, in "100 White"
Fig. 18. Effect of temperature on the increase in protonema length with increase in number of cells per filament, in "100 White"
Fig. 19. Effect of temperature on changes in mean cell length with increase in filament cell number in "100 White".
MEAN CELL LENGTH IN μ

CELLS PER FILAMENT

- 17½ °C
- 20 °C
- 22½ °C
- 25 °C
- 27½ °C

80
70
60
50
40
30
20
10
0
This polarity of expansion is reflected in the length : diameter ratios (fig. 20). From the graph one can see a clear temperature effect at least after the 2-3-celled stage. Up to this time all the curves have slopes of around 1.6 showing that most of the early increase in volume was due to elongation. Later the slopes of the lower temperature curves fell while that of $27^\circ$ rose considerably. The final slopes were:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>$17^\circ$</td>
<td>0.5</td>
</tr>
<tr>
<td>$20^\circ$</td>
<td>0.8</td>
</tr>
<tr>
<td>$22^\circ$</td>
<td>1.3</td>
</tr>
<tr>
<td>$25^\circ$</td>
<td>1.8</td>
</tr>
<tr>
<td>$27^\circ$</td>
<td>4.0</td>
</tr>
</tbody>
</table>

These results indicate that at the lower temperatures the cells were (on average) getting shorter, while at the highest temperatures expansion was strongly polarised along the long axis of the filament.

It must be emphasised that these ratios refer only to the average shape of the cells along the filament. They do not take any account of possible thickening or tapering. Reference to the volume of individual cells of the filaments (fig. 21) indicates that in 5-celled filaments a tapering effect is apparent at all the temperatures. At $17^\circ$ the tapering occurred steadily from the basal cell $P_1$ to the apex $P_5$, but at the other temperatures the largest cell in the filaments were the sub-basal ones $P_2$ and the filaments tapered from there. Such a tapering effect might indeed be expected from the graph of the expansion of a single filamentous cell (fig. 22) which shows that this expansion extends over several cell cycles.

**Synthesis of cellular materials**

The graphs of increase in dry weight per gametophyte against filament cell number (fig. 23) indicate that the increment per mitochron was more or less constant for each temperature over the filamentous period but that the total
Fig. 20  Effect of temperature on changes in length:diameter ratios with increase in number of cells per filament, in "100 White"
Fig. 21. Effect of temperature on the volume of individual cells along a 5-celled filament, in "100 White".
CELLS FROM BASE → APEX
Fig. 22  Effect of temperature on changes in the volume of cell P2

with increase in number of cells per filament, in "100 White"
Effect of temperature on changes in dry weight per gametophyte with increase in number of cells per filament, in "100 White"
Fig. 24  Effect of temperature on changes in mean dry weight per cell with increase in number of cells per filament, in "100 White"
Fig. 25  Effect of temperature on changes in protein-N per gametophyte with increase in number of cells per filament, in "100 White".
CELLS PER FILAMENT

PROTEIN-N PER GAMETOPHYTE IN µG X 10⁻³

- 17½°C
- 20°
- 22½°
- 25°
- 27½°
Fig. 26  Effect of temperature on changes in the ratio of protein-N: dry weight with increase in number of cells per filament, in "100 White"
dry weight for a given cell number i.e. at equivalent stages in development, decreased as the temperatures increased. The early apparent fall in dry weight has already been explained in the previous section. Expressed on a per cell basis (fig. 24) the dry weight values at most temperatures show a tendency to rise slightly, while the values for the 273°C cultures showed no such tendency. Both graphs show that towards the end of the sampling period the dry weight values for 172°C - 25°C cultures were becoming more similar and a gap was widening between these values and those of the 273°C culture. This observation points to a difference between gametophytes that were about to undergo transition and those that were to remain filamentous.

Increase in dry weight is principally due to the synthesis of cell wall material and new cytoplasm. A measure of this latter component is given by the protein-N data (fig. 25). Here there was an even sharper difference between 273°C and the lower temperatures. While all the cultures showed an initial increase, at the 2 - 3-celled stage where the lower temperatures gave an increase in the increment/mitochron, at 273°C protein-N first stopped increasing and then decreased. This means that there must have been breakdown of cytoplasmic material and the slight net increase in dry weight at this stage must have been concentrated in cell wall synthesis. This results in an abrupt difference in the ratios of protein-N: dry weight (fig. 26) between the high and low temperatures. At 273°C there was a peak in the ratio at 2 - 3-celled stage whereas at lower temperatures there was a trough. Further, at 172°C - 25°C the final protein-N per gametophyte values were very similar while at 273°C they were very much lower.
There is a marked resemblance between the results for protein-N and chlorophyll content (fig. 27, 28). Chloroplasts have a high protein content and can comprise a high proportion of the cell's organic nitrogen. This suggests that failure of chloroplast replication and some degeneration could contribute to, or at least parallel, changes in total protein-Nitrogen. Any reduction in the number of chloroplasts would be expected both to lower the nitrogen content and reduce the synthetic potential of the cells.

**SUMMARY**

1) Temperature had pronounced effects on morphogenesis. With increasing temperature there were:
   a) An increase in filament cell number at the onset of transition ($F_n$)
   b) A shift from Type I towards type II transition and then to a failure of any transition at 27°C.
   c) A decrease in the frequency of interstitial division.

2) The rate of increase in cell number and all the other basic growth parameters showed a maximum at intermediate temperatures (22°C - 25°C).

3) The rate of total expansion and elongation per mitochron was not altered by temperature.

4) The polarity of expansion was temperature dependent and there was a marked increase in the degree of longitudinal polarisation with increasing temperature.

5) "Kinks" occurred in the growth curves of certain parameters at the 2 - 3-celled stage:
   a) At low temperatures (17°C - 22°C) there was an increase in the rate of total expansion per mitochron (i.e. in mean cell volume).
Fig. 27  Effect of temperature on changes in chlorophyll per gametophyte with increase in number of cells per filament, in "100 White".
Fig. 28 Effect of temperature on changes in the ratio of chlorophyll: dry weight with increase in number of cells per filament, in "100 White".
in cross-sectional area and in the rate of increase in protein-N while there was a decrease in the slope of length : mean diameter against cell number.

b) At 27° there was a fall in the rate of increase in cell number, and in the protein-N content and a rise in the mean cell length and the increase per mitochron in the length : mean diameter ratios.

6) By the end of the sampling period there was a distinct difference between the levels of dry weight and more especially protein-N in gametophytes about to undergo transition and those which were to remain filamentous.

7) The close resemblance between the patterns of change in chlorophyll and protein-N content suggests that much of this difference between protein-N levels at 27° and the lower temperatures could be explained on the basis of differences in the number or size of the chloroplasts.

8) Results show a correlation between shift away from type I (to type II) transition and increased polarisation of elongation.
In the experiments to investigate the influence of temperature on the development of the gametophytes, the light used was 100 lumens/sq. ft. from a white fluorescent source. The series was then extended to include light of intensities \( \frac{1}{4} \) and \( 4 \times \) that of this standard (i.e. 25 and 400 lm/sq. ft). In this way the influence of intensity at each of the 5 temperatures could be assessed.

**EXPERIMENTAL RESULTS**

(1) **Cell number per filament**

(fig 29)

Table - Slope of cell number/time curves

<table>
<thead>
<tr>
<th>Intensity</th>
<th>( 17^{1/2} )</th>
<th>( 20^\circ )</th>
<th>( 22^{1/2} )</th>
<th>( 25^\circ )</th>
<th>( 27^{1/2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 lm/sq.ft</td>
<td>0.57</td>
<td>0.65</td>
<td>0.5-0.8</td>
<td>0.55-0.72</td>
<td>0.45</td>
</tr>
<tr>
<td>100 &quot; &quot;</td>
<td>0.64</td>
<td>0.65</td>
<td>0.9</td>
<td>0.7-0.87</td>
<td>0.7-.55</td>
</tr>
<tr>
<td>400 &quot; &quot;</td>
<td>0.72</td>
<td>0.6-.93</td>
<td>0.8-1.0</td>
<td>0.6-.93</td>
<td>0.8-.55</td>
</tr>
</tbody>
</table>

This table shows that the mitotic rate tends to increase with intensity at all temperatures. At \( 20^\circ \) there was little difference between 100 and 25 lm/sq.ft. cultures, while at higher temperatures 100 lm/sq.ft. gave a division rate very similar to that under 400 lm/sq.ft. From this it seems that intensity only has an effect below a certain threshold value, and that this threshold varies with temperature.

(2) **Morphogenetic pattern.**

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Intensity</th>
<th>( T_n )</th>
<th>( T_{n} )</th>
<th>Transition type</th>
<th>( T_i )</th>
<th>Day of 50% transition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 lm/sq.ft</td>
<td>5</td>
<td>2</td>
<td>( T_i )</td>
<td>0.35</td>
<td>11</td>
</tr>
<tr>
<td>( 17^{1/2} )</td>
<td>100 &quot; &quot;</td>
<td>4-5</td>
<td>1-2</td>
<td>( T_i )</td>
<td>0.3</td>
<td>9</td>
</tr>
</tbody>
</table>
Fig. 29  Effect of white light intensity on the increase in number of cells per filament with time
Time after Germination in Days

Mean Cell Number per Filament

- - - - 25 lm/sq ft
- - - - 100 " "
- - - - 400 " "

17½ °C
20°C
22½ °C
25°C
27½ °C
(2) Morphogenetic pattern (continued)

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Intensity</th>
<th>$F_n$</th>
<th>$T_n$</th>
<th>Transition type</th>
<th>$I_x$</th>
<th>Day of 50% transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 lm/sq.ft.</td>
<td>4-5</td>
<td>1-2</td>
<td>$T_I$</td>
<td>0.3</td>
<td>8.75</td>
<td></td>
</tr>
<tr>
<td>25°C</td>
<td>100</td>
<td>5-6</td>
<td>2-3</td>
<td>$T_I$</td>
<td>0.35</td>
<td>11.5</td>
</tr>
<tr>
<td>20°C</td>
<td>100</td>
<td>5</td>
<td>2</td>
<td>$T_I$</td>
<td>0.2</td>
<td>9.5</td>
</tr>
<tr>
<td>25°C</td>
<td>400</td>
<td>5</td>
<td>2</td>
<td>$T_I$</td>
<td>0.16</td>
<td>8</td>
</tr>
<tr>
<td>22°C</td>
<td>25</td>
<td>6</td>
<td>3</td>
<td>$T_{35I+.65II}$</td>
<td>0.3</td>
<td>10</td>
</tr>
<tr>
<td>22°C</td>
<td>100</td>
<td>5-6</td>
<td>2-3</td>
<td>$T_{98I+.02II}$</td>
<td>0.18</td>
<td>8</td>
</tr>
<tr>
<td>25°C</td>
<td>400</td>
<td>5</td>
<td>2</td>
<td>$T_{99I+.01II}$</td>
<td>0.1</td>
<td>7.25</td>
</tr>
<tr>
<td>25°C</td>
<td>25</td>
<td>6</td>
<td>3</td>
<td>$T_{11I+.9II}$</td>
<td>0.2</td>
<td>9.75</td>
</tr>
<tr>
<td>25°C</td>
<td>100</td>
<td>6</td>
<td>3</td>
<td>$T_{7II+.5II}$</td>
<td>0.16</td>
<td>9.5</td>
</tr>
<tr>
<td>27°C</td>
<td>400</td>
<td>5-6</td>
<td>2-3</td>
<td>$T_{6II+.4II}$</td>
<td>0.35</td>
<td>8</td>
</tr>
<tr>
<td>27°C</td>
<td>25</td>
<td>6-9</td>
<td>5-9</td>
<td>0.06P + $B_I$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>27°C</td>
<td>100</td>
<td>6-9</td>
<td>5-9</td>
<td>0.05P + $B_I$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>400</td>
<td>6-9</td>
<td>5-9</td>
<td>0.12P + $B_I$</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Key:
- $F_n$ - filament cell number at transition
- $T_n$ - cells of filamentous tail
- $T_{IorII}$ - transition type I or II
- $I_x$ - frequency of interstitial divisions
- $xP$ - frequency of filaments + papillae
- $xP+1$ - frequency of filaments + papillae + abortive transition
- $B_I$ - lateral branching
Plate 3a  Filaments grown in "100 Red" at 22° C, displaying basal forking.

Plate 3b  Filaments grown in "400 White" at 27° C, displaying lateral branching.
(3) **Protonema length**

(fig 30) In terms of ultimate length, 25 lm/sq.ft appeared to give maximum elongation although the slopes of the 25 and 100 lm/sq.ft. length/time curves were very similar. Increasing the intensity to 400 lm/sq.ft. tended to depress the increase in length at 17\(\frac{1}{2}\), 22\(\frac{1}{2}\) and 25\(\circ\). At the other 2 temperatures intensity appeared to have no effect on the rate of elongation.

(4) **Protonema volume**

(fig 31) The rates of increase in protonema volume by the intensity of the light. Only at 27\(\frac{1}{2}\)\(\circ\) was there an increase in the rate of expansion with increasing light intensity. At lower temperatures the final protonema volume tended to be greatest at low intensities. This is consistent with the finding that the filamentous phase was extended (in terms of cell number) under these low light conditions.

(5) **Dry weight per gametophyte**

(fig 32) The data here is difficult to interpret as it shows no consistent trends over the whole temperature range but it seems likely that the available energy became more critical at higher temperatures. At 17\(\frac{1}{2}\)\(\circ\) there was no change in the dry weight pattern with increasing intensities, while at 27\(\frac{1}{2}\)\(\circ\) the increase in dry weight was much more rapid under 400 lm/sq.ft. than at lower intensities.

(6) **Protein-N per gametophyte**

(fig. 33) These data resembles that concerned with dry weight in that intensity appears to have been more critical at 27\(\frac{1}{2}\)\(\circ\) that at lower temperatures.

(7) **Chlorophyll per gametophyte**

(fig 34) Here again intensity has a noticeable effect at 27\(\frac{1}{2}\)\(\circ\) whereas at lower temperatures the rate of increase in chlorophyll was very similar under all three intensities.
Fig 30  Effect of white light intensity on the increase in protonema length with time.
Fig. 31  
Effect of white light intensity on the increase in protonema volume with time.
Days after Germination

Protonema Volume

20°C

22½°C

25°C

27½°C

17½°C

25 lm/sq ft

100

400
Effect of white light intensity on changes in dry weight per gametophyte with time.
Fig. 33  Effect of white light intensity on changes in protein-N per gametophyte with time.
Effect of white light intensity on changes in chlorophyll per gametophyte with time.
Chlorophyll per Gametophyte in μg x 10^-3

Days after Germination

- 20°C
- 22½°C
- 25°C
- 27½°C

- 25 lm/sq ft
- 100 " "
- 400 " "

17½°C
DISCUSSION

Decreasing the light intensity to 25 lm/sq.ft. extended the filamentous phase of growth in terms of:

1. time
2. filament cell number at the onset of transition
3. elongation

Dry weight, protein-N and chlorophyll only show a clear intensity effect at \(27\degree C\), when each is greater in 400 lm/sq.ft that at lower intensities.

Volume also increases very slightly with intensity at \(27\degree C\) but at lower temperatures show the same inverse relationship as filament cell number and length. The cell expansion and cell synthesis again respond differently.

Cell expansion

The increase in volume per mitochron shows little response to intensity, mean cell volume remaining more or less the same (i.e. the graphs fig 35 have slopes of around 1). The lengths of the protonemata however increased as intensity decreased, at all temperatures and though the rate (against time) of elongation was only increased slightly, the amount of elongation between each division was conspicuously higher at the lowest intensity (fig 36).

The mean cell length per mitochron data (fig 37) emphasises these differences, and shows that the mean cell length decreased at 400 lm/sq.ft. while it was maintained or increased at lower intensities.

The cross-sectional areas showed a reverse trend, increasing with intensity at all temperatures (fig 38). As a result of these opposite effects of intensity on length and width, there was a marked correlation between the length:diameter ratios and intensity at all temperatures (fig 39).
Fig. 35  Effect of white light intensity on the increase in protonema volume with increase in number of cells per filament.
Fig. 36  Effect of white light intensity on the increase in protonema length with increase in number of cells per filament.
Fig. 37 Effect of white light intensity on changes in mean cell length with increase in number of cells per filament.
Fig. 38  Effect of white light intensity on changes in mean cross-sectional area with increase in number of cells per filament.
Fig. 39  Effect of white light intensity on changes in length:diameter ratios with increase in number of cells per filament.
At all three intensities the length:diameter ratios decreased at low temperatures and the combined temperature and intensity data indicate that at lower temperatures and higher intensities, the average cells got shorter and fatter with time, whilst at higher temperatures and lower intensities the cells got longer and relatively thinner.

The graph (fig 40) of individual cell volumes at the 5-celled stage shows that all the gametophytes tapered towards the apex but while the basal cell was the largest at lower temperatures and higher intensities, at higher temperatures and lower intensities, cell $P_2$ had the greatest volume. The tapering of the gametophytes in 25 and 100 lm/sq.ft were more or less identical. The volume of cells under 400 lm/sq.ft were consistently smaller than at lower intensities except at $273^\circ$ where neither the total volume nor the volume per cell was affected by intensity.

The graph (fig 41) expressing the expansion of cell $P_2$ per mitochondrion suggests that at 25 lm/sq.ft, the distribution of expansion was slightly less wide than under higher intensities since the volume of $P_2$ did not increase after the 4-5 celled stage under these low light conditions.

**Synthesis of cellular materials**

Only at the highest temperature - $273^\circ$ - was there any evidence that cellular synthesis was limited by the light intensity. At this temperature, neither of the lower intensities - 25 or 100 lm/sq.ft. led to any significant increase in either the protein-N or chlorophyll levels (fig 42, 43) during the sampling period. Late in this period the dry weight content however, increased at $273^\circ$ even in these low intensities (fig 44). At low intensities this increase was presumably due solely to the synthesis of cell wall material whereas at 400 lm/sq.ft. synthesis of new cytoplasm also contributed to the increase in dry weight.
Fig. 40  Effect of white light intensity on the volume of individual cells in a 5-celled filament.
Fig. 41  Effect of white light intensity on the increase in volume of cell P₂ with increase in number of cells per filament
Fig. 42  Effect of white light intensity on changes in protein-N per gametophyte with increase in number of cells per filament.
Fig 43  Effect of white light intensity on changes in chlorophyll per gametophyte with increase in number of cells per filament.
Fig 44  Effect of white light intensity on changes in dry weight per gametophyte with increase in number of cells per filament.
The protein-N:dry weight ratios at $27^\circ_C$ remained fairly constant with time at high intensity and actually fell at 25 and 100 lm/sq. ft. (fig 45). This indicates that even at the high intensity, the proportion of the total dry weight increase due to new cytoplasm was very different from that at lower temperatures where the ratios rose at the end of the sampling period. The intensity data thus emphasises a point already made in the account of temperature effects on growth; that just prior to transition there was a sharp rise in the protein-N:dry weight ratios that was completely absent in gametophytes that were to remain filamentous.

**SUMMARY**

(1) Increasing the light intensity over a certain threshold increased the mitotic rate. The threshold varied with the temperature.

(2) Filamentous phase increased at low intensities in terms of:
   a) time
   b) cell number per filament.

(3) The frequency of interstitial division decreased with increasing intensity.

(4) The proportion of gametophytes undergoing type II transition at $22^\circ_C$ and $25^\circ_C$ was increased by lowering the intensity below 100 lm/sq. ft. The frequency of filaments forming papillae at $27^\circ_C$ was increased by raising the intensity.

(5) Intensity only affected the expansion per mitochron at $27^\circ_C$, when increasing the light increased the volume.

(6) Polarisation of expansion was greatest at low intensities and Li:D ratios increased with decreasing light at all temperatures.

(7) Expansion was completed earlier (in terms of division cycles) at low intensity.
Fig 45  Effect of white light intensity on changes in the ratio of protein-N; dry weight with increase in number of cells per filament.
Intensity was more critical for cellular synthesis at 27°C where protein-N and chlorophyll only increased in 400 lm/sq.ft. At lower intensities dry weight increase was confined to cell wall synthesis.

Protein-N: dry weight ratios emphasize that there is a different pattern of synthesis in gametophytes about to undergo transition from those remaining filamentous.
EFFECT OF LIGHT WAVELENGTH ON GROWTH AND DEVELOPMENT

It has already been emphasised in the introduction to this thesis, that most of the work done in the past on morphogenesis in fern gametophytes, has been concerned with comparing the contrasting growth under light of different wavelengths. This present work was aimed not at making direct comparisons between growth in red and blue light but rather at comparing the effects of red and blue light when given separately, with their effects when combined in white light.

For this type of comparison, instead of standardising the energy contents of the red and blue light against one another, as had been done by MOHR and others (MOHR and OHLENROTH 1964) the energies where standardised against the standard 100 lm/sq.ft. white light. Thus the energy of the standard blue approximated as closely as possible to the blue-light component of the standard white. Similarly, the energy of the standard red was as close as possible to that of the red component of the white (see fig 1).

The series of experiments of the effects of light wavelength to be described here were done at 22°C and used red, blue and white light standardised as above. Growth was analysed in terms of the same parameters as had been used in previous experiments on the effects of light wavelength and intensity.

EXPERIMENTAL RESULTS

(1) Cell number per filament

The rate of mitosis is shown graphically (fig 46) as cell number per filament against time. The slopes of these curves are given in the table below:
Fig 46  Increase in filament cell number with time, in "100 White", "100 Red", "100 Blue".
<table>
<thead>
<tr>
<th>Light</th>
<th>$\text{slope}$</th>
<th>Mitotic cycle time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>0.5-0.2</td>
<td>48 - 120 hours</td>
</tr>
<tr>
<td>Blue</td>
<td>0.5-0.61</td>
<td>48 - 40 &quot;</td>
</tr>
<tr>
<td>White</td>
<td>0.9</td>
<td>26.7 &quot;</td>
</tr>
</tbody>
</table>

It must be remembered throughout this comparison that the total energy content of the white light, and therefore its photosynthetic potential, is much higher than the energy of either the red or blue light.

(2) **Morphogenesis**

<table>
<thead>
<tr>
<th>Light</th>
<th>$T_n$</th>
<th>$F_n$</th>
<th>transition type</th>
<th>$I_{x}$</th>
<th>Day of 50% transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>-</td>
<td>1-2</td>
<td>0.5B</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blue</td>
<td>4-5</td>
<td>1-2</td>
<td>$T_{0.11+0.11}$</td>
<td>0.3</td>
<td>10</td>
</tr>
<tr>
<td>White</td>
<td>5-6</td>
<td>2-3</td>
<td>$T_{0.98+0.02}$</td>
<td>0.18</td>
<td>8</td>
</tr>
</tbody>
</table>

Key

- $F_n$ — filament cell no. at transition
- $I_{x}$ — frequency of interstitials
- $T_n$ — cells of filamentous tail
- $B_f$ — basal forking
- $T_I$ or $T_{II}$ — type I or II transition

1) No transition occurs under red light. Branching occurs from the basal or sub-basal cell after the 2-3 celled stage. By Day 12 (5-celled) 32% of the filaments were branched and some branches had several cells. By this time there was no longer a strict relationship between the number of divisions of the apical cell ($F_n$) and the number of cells in the protonema. Per gametophyte data which depended on the existence of such a relationship between $F_n$ and cells counted per sample, have been confined to the early period when most of the filaments were still unbranched.
2) In red light only a single, basal rhizoid was formed instead of the "typical" two, found in blue and white light.

3) Protonema length
   (fig 47a) The rate of elongation plotted against time was greatest in red and least in blue light. In red light however, the rate started to fall after the onset of branching.

4) Protonema volume
   (fig 47b) The volumes of red- and white-grown filaments plotted against time were very similar up to the onset of transition in white light. Blue-grown filaments were smaller throughout the sampling period and there was an initial phase of several days during which no appreciable expansion occurred.

5) Dry weight, Protein-N, Chlorophyll per gametophyte.
   (fig 48) The patterns of increase in dry weight, protein-N and chlorophyll were very similar, the rates being highest in white and lowest in red.

DISCUSSION
The experimental results show that expansion was greatest under red light despite its lower energy content, while increases in such parameters as dry weight, protein-N and chlorophyll were greater in white than either red or blue. This probably reflects the higher photosynthesis activity. Once again therefore, cell expansion was responding independently of overall synthesis.

Cell expansion
The mean cell volume (fig 49a) in blue light showed an initial fall suggesting that division got underway before expansion, whilst under red light it appears the considerable expansion, or elongation at least was achieved prior
Fig 47a  Increase in protonema length with time, in "100 White", "100 Red", "100 Blue".

Fig 47b  Increase in protonema volume with time, in "100 White", "100 Red", "100 Blue".
Fig 48a Changes in dry weight per gametophyte with time, in "100 White", "100 Red", "100 Blue".

Fig 48b Changes in protein-N per gametophyte with time, in "100 White", "100 Red", "100 Blue".

Fig 48c Changes in chlorophyll per gametophyte with time, in "100 White", "100 Red", "100 Blue".
Fig. 49 Changes in volume with increase in number of cells per filament in "100 White", "100 Red", "100 Blue".

a) Protonema volume

b) Mean cell volume
Cells per Filament

Protonema Volume in cuμ × 10^4

- ▼ white
- ■ blue
- ○ red

Cell Volume in cuμ × 10^4
to the first division. Apart from this early fall in blue light, the mean cell volume remained fairly constant in red and blue light and only showed a slight rise in white. Both these mean cell values and those for protonema volume (fig 49a) were greatest in red and least in blue. The initial rates of expansion were similar in red and white, and the greatest final volume in red was due entirely to accelerated elongation and occurred despite a reduction in mean cross-sectional area (fig 51a). The areas in blue and white light started off at a similar level but separated when white-grown filaments showed a sudden increase in area at the 3-celled stage. Filament and mean cell length (which remained constant) were very much greater in red, with white giving only slightly higher values than blue light (fig 50).

Examination of all the volume, area and length data demonstrates that expansion was polarised along the long axis of the filament under red light, whilst under blue and white light, expansion was isodiametric. This is confirmed by the length:diameter ratios (fig 51b) which indicate that the average shape of the cells in a white or blue grown filament were very similar, while in red light the cells were very much more attenuated. The slopes of these curves show that in white and blue light the mean cell shape remained fairly constant throughout the filamentous period whereas in red light the cells got considerably more elongated with time. By the end of the sampling period, the length:diameter ratio in red was nearly 4 x that in blue or white.

The graph of individual cell volumes (fig 52b) shows that tapering occurred under all light wavelengths but that in blue light the basal cell $P_1$, in white $P_2$ and in red $P_3$ was the largest in a 5-celled filament.
Fig 50  Changes in length with increase in number of cells per filament in "100 White", "100 Red", "100 Blue"

a) Protonema length

b) Mean cell length
Fig 51a  Mean cross-sectional areas in "100 White", "100 Red", "100 Blue"

Fig 51b  Changes in length:diameter ratios with increase in number of cell per filament in "100 White", "100 Red", "100 Blue"
Fig 52a  Increase in volume of cell P₂ with increase in number of cells per filament in "100 White", "100 Red", "100 Blue".

Fig 52b  Volume of individual cells along a 5-celled filament in "100 White", "100 Red", "100 Blue".
Cells per Filament

Volume $V_2$ in cm$^3 \times 10^4$

Cells from base $\rightarrow$ apex

- $\triangledown$ white
- $\square$ blue
- $\bullet$ red
The expansion of cell $P_2$ plotted against cell number (fig 52a) shows that in red light there was little or no expansion of this cell in a 4-celled filament. In blue and white light $P_2$ seemed to be still expanding at the end of the filamentous period. This means that here expansion was extended over several cell division cycles.

Synthesis of new cellular materials

The patterns of increase in protein-N, dry weight and chlorophyll (fig 53, 54, 55) appeared to mimic one another closely and the rate of synthesis were lower in blue than white light, and lower still in red. When the data are plotted against cell number per filament, the increases per mitochon are seen to have been very similar in white and blue-grown filaments and to have maintained a more or less constant value. In red the increases were much less and the mean cell values fell.

The chlorophyll:dry weight ratios seem to have been little affected by wavelength; whilst the results for protein-N expressed as a percentage of dry weight are difficult to interpret, there was probably no significant difference between the values in red and white light up to the onset of transition. The fluctuations in protein-N: dry weight ratios in blue-grown filaments (which were reproduced in replicated experiments) suggests that during the initial stages when, under these conditions the protonema volume did not increase, the cell cytoplasm became denser. Then, when expansion started, cell wall synthesis became appreciable and the protein-N: dry weight ratio fell. Later, at the onset of transition, the protein-N % again rose in both white and blue light. In red where there was no transition, there was no corresponding rise in the protein-N: dry weight ratio.
Fig 53 Increase in dry weight per gametophyte with increase in filament cell number in "100 White", "100 Red", "100 Blue".
Cells per Filament vs. Dry weight in μg x 10^-1

- V--V White
- ■■■■ Blue
- ○○○○ Red
Fig 54a  Increase in protein-N per gametophyte with increase in number of cells per filament in "100 White", "100 Red", "100 Blue".

Fig 54b  Changes in the ratio of protein-N: dry weight with increase in filament cell number in "100 White", "100 Red", "100 Blue".
Fig 55a  Increase in chlorophyll per gametophyte with increase in number of cells per filament, in "100 White", "100 Red", "100 Blue".

Fig 55b  Changes in the ratio of chlorophyll:dry weight with increase in number of cells per filament in "100 White", "100 Red", "100 Blue".
The similarity between the changes in dry weight, protein-N and chlorophyll, considered alongside the constancy of chlorophyll:dry weight ratios, implies that the differences in dry weight under the three wavelength conditions could be attributed largely to increases in chloroplast protein in blue and white light.

**SUMMARY**

1) These experiments confirm the well known effects of light wavelength on the transition from a filament to a prothallus. In red light transition fails to occur and the filamentous phase is prolonged indefinitely. In this case half the filaments branched near the base. In blue light transition was predominately type II and in white it was type I. In terms of filament cell number the filamentous stage was longer in white than blue light.

2) The absolute rates of division and increase in dry weight, protein-N and chlorophyll showed a correlation with the higher energy content of the white light.

3) The rates of increase in dry weight and protein-N per mitochron on the other hand, were very similar in blue and white light, and both were higher than in red. The rise in protein-N:dry weight ratio at the end of the filamentous period in blue and white light, was related to transition and did not occur in red light.

4) The total expansion per mitochron was, despite lower energy content, greater in red than white, and greater in white than blue. Expansion in red was highly polarised and the cells were very long and thin, while in blue and white expansion was isodiametric and the cells were smaller and more rounded.

5) In blue light division was initiated before appreciable expansion had occurred. In red light elongation was already considerable, prior to the first protonemal division.
EFFECT OF TEMPERATURE ON GROWTH IN LIGHT OF DIFFERENT WAVELENGTHS

This set of experiments was designed to discover whether temperature modified the patterns of development found to be characteristic of gametophytes growth under light of different wavelengths at 22°. In the experiments red and blue light, standardised as in the earlier experiments against 100 lm/sq. ft. of white fluorescent light ("100" red and blue), and temperatures of 17°, 22°, and 27° were employed.

A) BLUE LIGHT

EXPERIMENTAL RESULTS

No growth occurred in germinated spores placed in blue light at 27°. The spores were not killed by these conditions as some elongation of the rhizoids was evident and growth could be re-started if the spores were returned to a lower temperature. Since no protonemal growth however could be detected, no results for growth at 27° could be included in the account below.

(1) Cell number per filament

Table (fig 56a)

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>slope of division rate curve</th>
<th>mitotic cycle time</th>
</tr>
</thead>
<tbody>
<tr>
<td>17°</td>
<td>0.5 - 0.2</td>
<td>48 - 120 hours</td>
</tr>
<tr>
<td>22°</td>
<td>0.5 - 0.61</td>
<td>48 - 40</td>
</tr>
</tbody>
</table>

These results indicate that temperature affected the division rate only after the 2-3 celled stage had been reached. After this stage, 22° appeared to be nearer the optimum.
Fig 56a Effect of temperature on the increase in filament cell number with time, in "100 Blue"

Fig 56b Effect of temperature on the increase in protonema length with time, in "100 Blue"
(2) Morphogenetic pattern

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>Fn</th>
<th>Tn</th>
<th>transition type</th>
<th>I&lt;sub&gt;x&lt;/sub&gt;</th>
<th>Day of 50% transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>17&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.5</td>
<td>1-2</td>
<td>T&lt;sub&gt;II&lt;/sub&gt;.2&lt;sub&gt;II&lt;/sub&gt;I</td>
<td>0.4</td>
<td>17</td>
</tr>
<tr>
<td>22&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.5</td>
<td>1-2</td>
<td>T&lt;sub&gt;II&lt;/sub&gt;.1&lt;sub&gt;II&lt;/sub&gt;.9&lt;sub&gt;II&lt;/sub&gt;</td>
<td>0.3</td>
<td>10</td>
</tr>
</tbody>
</table>

This shows that while temperature had no effect on the length of the filamentous phase in terms of cell number, transition was completed considerably earlier at 22<sup>3</sup>° because of the higher division rate at this temperature. Lowering the temperature from 22<sup>3</sup> to 17<sup>2</sup>°, switched the pattern of transition sharply from predominately type II to predominately type I. At both temperatures, the interstitial divisions in gametophytes undergoing type II transition, occurred after the formation of the apical papilla.

(3) Protonema length

(fig 56b) Temperature appeared to have no effect on the rate of elongation in blue light.

(4) Protonema volume

(fig 57a) Again, temperature seemed to have no effect on the increase in protonema volume per unit time.

(5) Dry weight per gametophyte

(fig 57b) Temperature appeared to have no significant effect on the rates of increase in dry weight.

(6) Protein-N per gametophyte

(fig 58a) There was no temperature effect on either the rate of increase per gametophyte, during most of the filamentous period or, on the final protein level
Fig 57 Effect of temperature on growth in "100 Blue":

a) Increase in protonema volume with time.

b) Changes in dry weight per gametophyte with time
Fig 58 Effect of temperature on growth in "100 Blue":

a) Changes in protein-N per gametophyte with time

b) Changes in chlorophyll per gametophyte with time
on the day of 50% transition. The only difference was found at the onset of transition which at 22°C, occurred rapidly and led to a rapid rise in the protein level while at 17°C both transition and the rise in protein-N was more gradual.

(7) Chlorophyll per gametophyte

(fig 58b) The graph shows that there was no significant difference between the rate of increase in chlorophyll at the two temperatures studied.

DISCUSSION

Cell expansion

The graphs of protonema and cell volume (figs 59a and b), protonema and cell length (fig 60a and b) and cross-sectional area (fig 59c), show no evidence that temperature affected either the rate of elongation or lateral expansion per mitochron.

The length: diameter data confirms the conclusion that might be inferred from these previous results, that temperature had no effect on the shape of the cells in blue light (fig 60c).

Synthesis of cellular materials

Temperature had little apparent affect on cell synthesis. The data for dry weight and protein-N plotted against cell number per filament, indicates that during the early part of filamentous growth, the rate of increase per mitochron was very slightly higher at the lower temperature but the final levels were very similar (fig 61). There is no evidence of any temperature effect, at any stage, on the chlorophyll content, (fig 62b).
Fig 59  Effect of temperature on expansion with increase in number of cells per filament in "100 Blue":

a) Protonema volume

b) Mean cell volume

c) Mean cross-sectional area
Fig 60  Effect of temperature on changes in length with increase in number of cells per filament in "100 Blue"

a) Protonema length

b) Mean cell length

c) length:diameter ratio.
Fig 61  Effect of temperature on synthesis in "100 Blue":-

a) Changes in dry weight per gametophyte with increase in number of cells per filament.

b) Changes in protein-N per gametophyte with increase in number of cells per filament.
Temperature did not affect the ratio of protein-N: dry weight and the rather pronounced fluctuations of this ratio during development already noted at 22\textdegree\textsubscript{0} were closely followed at 17\textdegree\textsubscript{0} (fig 62a). There was some indication, although only very slight, that the ratio of chlorophyll:dry weight was higher at 22\textdegree\textsubscript{0} than 17\textdegree\textsubscript{0} over the first two division cycles (fig 62c). This is in accord with the finding that at this stage, the dry weight levels were lower at the higher temperature.

Interpretation of these results

The lack of any apparent difference in any parameter under two sets of conditions has two possible interpretations:

a) That the two environmental conditions lay either side of an optimum.

b) That these variations in the environment did not, in fact, affect the parameter.

In the case of blue light growth at different temperatures, only two temperatures were involved in the study and so it is not possible to distinguish between these two alternatives. The data does however show that the response of blue-grown filaments to temperature is different from that of white-grown ones. Either the temperature optimum is lower - around 20 instead of 22\textdegree\textsubscript{0} - 25\textdegree\textsubscript{0}, or there is a considerably lower sensitivity to temperature under blue light.

SUMMARY

1) No division or growth occurred at 27\textdegree\textsubscript{0}. 22\textdegree\textsubscript{0} seemed nearer the optimum for division than 17\textdegree\textsubscript{0}.

2) There was no evidence that either the rate or the polarity of expansion was temperature dependent.
a) Changes in the rates of protein-N:dry weight with increase in number of cells per filament.

b) Changes in chlorophyll per gametophyte with increase in number of cells per filament.

c) Changes in the ratio of chlorophyll:dry weight with increase in number of cells per filament.
3) Temperature had little apparent effect on cell synthesis and both the final dry weight levels and the ratios of protein-N and chlorophyll:dry weight were very similar at the two temperatures studied.

(B) RED LIGHT

EXPERIMENTAL RESULTS

(1) Cell number per filament (fig 63a)

Table

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>slope of cell no./time curve</th>
<th>mitotic cycle time</th>
</tr>
</thead>
<tbody>
<tr>
<td>17_2^1^</td>
<td>0.5 - 0.2</td>
<td>48 - 120 hours</td>
</tr>
<tr>
<td>22_2^2^</td>
<td>0.42 - 0.16</td>
<td>55.6 - 150 &quot;</td>
</tr>
<tr>
<td>27_2^3^</td>
<td>0.38 - 0.06</td>
<td>63.2 - 400 &quot;</td>
</tr>
</tbody>
</table>

This indicates that 17_2^1^ - 22_2^2^ was nearer the optimum but that the temperature differences did not become pronounced until after the 3-celled stage.

(2) Morphogenetic pattern

No transition occurred in red light at any of these temperatures. At 17_2^1^® the filaments were simple and unbranched, while at 22_2^2^® 50% of the filaments branched at, or near, the base. Raising the temperature further raised the frequency of branching to around 78% by the end of the sampling period. In addition to this form of branching, 5% of the filaments also branched from the cells near the apex (lateral branching).

(3) Protonema length

(fig 63b) The elongation rate at 27_2^3^® fell behind those at the lower temperatures during the latter half of the sampling period.
Fig 63  Effect of temperature on growth in "100 Red":-

a) Increase in cell number per filament with time.

b) Increase in protonema length with time.
Fig 65  

Effect of temperature on growth in "100 Red":

a) Changes in protein-N per gametophyte with time.

b) Changes in chlorophyll per gametophyte with time.
(4) **Protonema volume**

(fig 64a) There was no difference in volume between gametophytes grown at \(17^\circ\) and \(22^\circ\). At \(27^\circ\) however, expansion became very slow after the first week of culture.

(5) **Dry weight per gametophyte**

(fig 64b) The dry weight level per gametophyte was very slightly higher at \(27^\circ\) than at either of the lower temperatures.

(6) **Protein-N per gametophyte**

The graph (fig 65a) of protein-nitrogen against time shows no clear temperature effect on protein synthesis.

(7) **Chlorophyll per gametophyte**

Again the graph (fig 65b) shows that temperature had little effect on the rates of increase in chlorophyll. The graph does however, suggest that the chlorophyll content fell at the higher temperatures towards the end of the sampling period. This apparent anomaly was caused by the high frequency of branching in the filament in the way already discussed in the earlier section on light wavelength effects.

**DISCUSSION**

**Cell expansion**

The rates of increase in protonema volume i.e. total expansion per mitochron were very similar at all three temperatures and while the mean cell volumes were rather lower at \(27^\circ\), the differences were only slight and became hardly significant by the end of the sampling period (fig 66). The polarity of expansion was not altered by temperature either. This can be seen by reference
Fig 64  Effect of temperature on growth in "100 Red":

a) Increase in protonema volume with time

b) Changes in dry weight per gametophyte with time.
Fig 65  Effect of temperature on growth in 7100 Red:

a) Changes in protein-N per gametophyte with time.

b) Changes in chlorophyll per gametophyte with time.
Fig 66  Effect of temperature on expansion in "100 Red":

a) Increase in protonema volume with increase in filament cell number.

b) Changes in mean cell volume with increase in filament cell number.

c) Changes in mean cross-sectional area with increase in filament cell number.
to the graphs of protonema and cell lengths (fig 67) and mean cross-sectional area (fig 66c) none of which show any dependence upon temperature.

The length:diameter ratios (fig 68) again confirms that the mean cell shape was not altered by altering the temperature over the range employed.

**Synthesis of new cellular materials**

As mentioned before, the graph of dry weight plotted against time gave some slight indication that 27\(^\circ\) was the optimum for the synthesis of new cellular materials. When these dry weight data were plotted against filament cell number (fig 69a) this point is seen more clearly. It also appears that the levels of protein-nitrogen and chlorophyll, plotted on a per mitochron basis, (fig 69b, 70a) rise faster at 27\(^\circ\) that at the lower temperatures. Unfortunately, once a significant proportion of the filaments had branched, the data could no longer be plotted accurately and so it was not possible to determine whether this early high rate of increase in dry weight, protein-nitrogen and chlorophyll was maintained throughout the later part of the experimental period.

The ratios of protein-N and chlorophyll to dry weight on the other hand show no dependence upon temperature (fig 69c, 70b). This suggests that the faster, early increase in dry weight at 27\(^\circ\) comprised an increased rate of synthesis of chloroplast protein.

**SUMMARY**

1) The mitotic rate was highest in the range 17\(^\circ\) - 22\(^\circ\).

2) At the above 22\(^\circ\), the filaments branched and the frequency of branching rose with temperature. At 27\(^\circ\) both types of branching - basal forking
Fig 67  Effect of temperature on elongation in "100 Red":

a) Increase in protonema length with increase in filament cell number.

b) Changes in mean cell length with increase in filament cell number.
Fig 68  Changes in length:diameter ratios with increase in filament
cell number in "100 Red" at different temperatures.
Fig 69  Effect of temperature on synthesis in "100 Red":

a) Changes in dry weight per gametophyte with increase in filament cell number.

b) Changes in protein-N per gametophyte with increase in filament cell number.

c) Changes in the ratio of protein-N:dry weight with increase in filament cell number.
Cells per Filament

- Dry wt./Gametophyte µg x 10^7
- Protein-N/Gametophyte µg x 10^-3
- Protein-N/Dry wt. %

Different symbols represent different temperatures: 17°C, 22°C, and 27°C.
Fig 70  Effect of temperature on chlorophyll levels in "100 Red":

a) Changes in chlorophyll per gametophyte with increase in filament cell number.

b) Changes in ratio of chlorophyll: dry weight with increase in filament cell number.
and lateral branching near the apex occurred.

3) Expansion was not altered by temperature.

4) There is some evidence that the rates of synthesis of cellular materials per mitochron was higher at $27^\circ$C than at lower temperatures.

**COMPARISON OF THE EFFECTS OF TEMPERATURE ON GROWTH IN LIGHT OF DIFFERENT WAVELENGTHS**

1) These results suggest that at least some aspects of growth in red or blue light were less sensitive to temperature than is growth in white light. This is particularly well illustrated by the length : diameter ratios which showed a clear temperature dependence in white but were unchanged by temperature in red or blue light.

2) When temperature did seem to have an affect, the optimum in blue appeared to be lower and in red (except for mitotic rate) to be higher than in white light.
THE EFFECT OF INTENSITY ON GROWTH AND DEVELOPMENT IN LIGHT OF
DIFFERENT WAVELENGTHS

BLUE LIGHT

One of the ideas put forward to explain the effects of light wavelength on growth, is that development is sensitive to the differences in photosynthetic efficiency of red and blue light. The experiments described earlier in this thesis on the influence of light intensity on growth in white light, showed that changes in energy levels can bring about variations in the morphogenesis pattern. This suggests that photosynthesis can at least modify morphogenesis and, more especially the rate of growth. On the other hand it has also been noted that expansion and elongation were greater in red than white of a considerably higher energy content. This suggests that more than the level of nutrition is involved in controlling at least some aspects of growth.

If the growth pattern was determined entirely by photosynthetic potential, then very low intensities of blue light should give the long narrow cells characteristic of red light culture. In order to test this and also to determine the threshold energy for growth in blue light, cultures were set up under intensities of blue equivalent to 400, 100, 10 and 5 lm/sq.ft. of white light. All the experiments were done at the standard temperature of $22\frac{1}{2}^{\circ}$ and all cultures were given the standard germination treatment.

EXPERIMENTAL RESULTS

In "5 Blue" (i.e. equivalent to 51m/sq.ft white) 98% of the spore cells failed to divide at all while the other 2% developed into 3-4 celled filaments after which growth ceased. Therefore as this intensity supported growth in such a
very low proportion of the spores, it was considered to be as close to the threshold for growth as it would be feasible to get. The energy peak at this threshold intensity was 0.35 micro watts/cm². m at 440nm. The frequency of the dividing cells was too low to permit a complete analysis under these conditions but the shape of the cells may be judged from the Camera lucida diagrams (fig 7a).

In "10 Blue" the great majority of the spore cells divided and filaments of up to 4-5 cells were achieved, although no transition was seen over the experimental period of several weeks.

(1) Cell number per filament (fig 72a)

<table>
<thead>
<tr>
<th>Intensity blue</th>
<th>slope of cell no./time curve</th>
<th>mitotic cycle time</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;10&quot;</td>
<td>0.36</td>
<td>67 hours</td>
</tr>
<tr>
<td>&quot;100&quot;</td>
<td>0.5 - 0.61</td>
<td>48 - 40 hours</td>
</tr>
<tr>
<td>&quot;400&quot;</td>
<td>0.5 - 1.07</td>
<td>48 - 22.4</td>
</tr>
</tbody>
</table>

(2) Morphogenetic pattern

"10" not included in the table as it gave no transition.

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Fₙ</th>
<th>Tₙ</th>
<th>type of transition</th>
<th>Iₓ</th>
<th>Day of 50% transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;100&quot;</td>
<td>4-5</td>
<td>1-2</td>
<td>T₀.₉I⁺.₉II</td>
<td>0.8</td>
<td>10</td>
</tr>
<tr>
<td>&quot;400&quot;</td>
<td>4-5</td>
<td>1-2</td>
<td>T₀.₃₃I⁺.₆₇II</td>
<td>0.6</td>
<td>8.2</td>
</tr>
</tbody>
</table>

Key

Fₙ - cells per filament
Tₙ - cells of filamentous tail
Iₓ - interstitial divisions
Tᵢ or II type I or II transition
Fig 71 Camera lucida drawings of filaments grown under lowlight intensities (22\textdegree C)

a) "5-Blue"

b) "2-Red"
The type I transitions occurred earlier than type II both in terms of filament cell number and time. Thus under "400 Blue" the first transitions recorded were type I and on Day 8 the ratio between types I and II was 1.

Most of the interstitial divisions were recorded after the formation of the apical papilla.

(3) Protonema length
The graph (fig 72b) shows that the rate of elongation rose with increasing light intensity.

(4) Protonema volume
(fig 72c) The expansion rate also showed a dependence upon the intensity of blue light.

(5) Dry weight per gametophyte
The results shown in the dry weight against time graph (fig 72a) give no indication of any intensity effect on dry weight accumulation.

(6) Protein-N per gametophyte
(fig 73b) The only indication that intensity affected the rate of protein-N synthesis in blue light was that just prior to transition, the gametophytes in "400 Blue" showed a sudden rise in their protein-N contents that was not followed in "100 Blue".

(7) Chlorophyll per gametophyte
(fig 73c) Here there was a definite intensity affect, the chlorophyll levels being higher in the high intensity cultures throughout the filamentous growth phase.
Fig 72   Effect of intensity on growth in blue light:

a) Increase in number of cells per filament with time.

b) Increase in protonema length with time.

c) Increase in protonema volume with time.
Fig 73  Effect of intensity on rate of synthesis in blue light

a) Changes in dry weight per gametophyte with time.

b) Changes in protein-N per gametophyte with time.

c) Changes in chlorophyll per gametophyte with time.
DISCUSSION

Cell expansion

The protonema volume data plotted against time indicated that expansion was enhanced by increasing the intensity of light. The same data plotted against filament cell number shows that there was a difference in expansion per mitochron between "400" and "100" but not between "100" and "10 Blue". (fig 74a and b).

The protonema length data on the other hand shows that only the absolute elongation rate and not the rate per mitochron was dependent upon the light intensity (fig 74c and d). The cross-sectional areas of the filaments were slightly greater at the higher intensity, this reflecting the difference in the degree of intensity dependence of volume and length (fig 75a). This slight difference however, in the effect of intensity on filament length and width was not sufficient to have a significant effect on the mean cell shape as represented by the length:diameter ratios (fig 75b).

This is an important result as it demonstrates that there was no appreciable tendency for the cells to become attenuated i.e. "red type" under very low blue light.

Synthesis of cellular materials

The rates of increase in dry weight and protein-N per mitochron were very similar (fig 76a and b) in "400" and "100" Blue and gave no indication that intensity had any significant influence on the net synthesis of cellular materials. Following from this data it is not surprising that the variations in protein-N expressed as a percentage of dry weight and plotted against filament cell number (fig 76c) were similar in the two intensities of blue light.
Fig 74  Effect of intensity on expansion in blue light:

a) Increase in protonema volume with increase in filament cell number.

b) Increase in cell volume with increase in filament.

c) Increase in protonema length with increase in filament cell number.

d) Increase in cell length with increase in filament.
Fig 75  Effect of intensity on expansion in blue light

a) Changes in mean cross-sectional area with increase in filament cell number.

b) Changes in the ratio of length:diameter with increase in filament cell number.
Fig 76  Effect of intensity on cell synthesis in blue light:

   a) Changes in dry weight per gametophyte with increase in filament cell number

   b) Changes in protein-N per gametophyte with increase in filament cell number.

   c) Changes in the ratio of protein-N:dry weight with increase in filament cell number.
In contrast, chlorophyll synthesis does seem to have depended upon light intensity, and plotted against filament cell number as well as against time (fig 77a), the chlorophyll levels per gametophyte were consistently higher in the higher light conditions. The ratios of chlorophyll to dry weight plotted against cell number, (fig 77b) indicate that there was a great intensity effect on these ratios during the 1-4 celled stage although by the onset of transition the ratios had become very similar under the two light conditions.

**SUMMARY**

1) The division rate was increased by raising the light intensity. "5" Blue was at the threshold for growth and division occurred in only 2% of the spores.

2) No transition occurred in "10 Blue" during the experimental period. At higher intensities, the ratio of type I:II changed from 1 : 9 in "100 Blue" to 1 : 2 in "400 Blue".

3) Expansion was slightly enhanced by raising the light intensity but the length: diameter ratios give no indication of any attenuation of the cells under very low blue light.

4) Protein-N and dry weight per gametophyte were little affected by the blue light intensity.

5) Chlorophyll synthesis was higher at high intensity although by the onset of transition, the differences were not sufficient to produce a noticeable effect on the chlorophyll:dry weight ratios.

2. **RED LIGHT**

Because of the low percentage transmission through the red filter it was unfortunately not practicable to raise the red light intensity significantly above the "100 Red" level. The intensity study was therefore confined to a
Fig 77  Effect of intensity on chlorophyll levels in blue light:

a) Changes in chlorophyll per gametophyte with increase in filament cell number.

b) Changes in chlorophyll:dry weight ratio with increase in filament cell number.
determination of whether the rate and nature of growth was altered by lowering the light intensity. For this the light was standardised so that the energy at its 532nm peak was equal to the energy peak in the threshold blue light ("5 Blue") i.e. 0.035 microwatts/cm².nm ("2.33 Red"). In this way the threshold levels for growth in the two light qualities could also be compared.

The results from this experiment is summarised below: (fig 71b);

1) Both the initial and the final rates of division were very similar in "100" and "2.33 Red" although the "fall off" occurred earlier in the lower intensity.
2) Elongation was increased by lowering the light intensity.
3) Total expansion was decreased and the polarity of expansion consequently raised under the lower light conditions but this alteration in cell shape only took place after the 3-celled stage had been reached:

<table>
<thead>
<tr>
<th>Cells per filament</th>
<th>&quot;100&quot;</th>
<th>&quot;2.33&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>18.4</td>
<td>17.7</td>
</tr>
<tr>
<td>4</td>
<td>27.3</td>
<td>55.9</td>
</tr>
</tbody>
</table>

This red light experiment thus provided a further example of apparent buffering against the environment up to the 3-celled stage.

3. WHITE LIGHT

Having discovered that cells grown under blue light maintained their characteristic shape right down to the threshold of growth and that the threshold energy necessary was lower in red than in blue light, the question that remained was how white-grown filaments would behave at very low intensities. This was resolved by setting up a culture of gametophytes under 5 lm/sq.ft. white light, an intensity that would support growth in red but formed the threshold for growth in blue light.
The results of this experiment is shown in the photograph (plate 4). It shows that the cells were extremely elongated and resemble typical "red" rather than "white" growth. From this it seems that while at high intensities the blue component of white light predominates and the cells are isodiametric, at low intensities the red component prevails and cell expansion is highly polarised.
Plate 4  Filaments grown under very low white light ("5 White") at 22°C.
The standard medium used in this investigation was a slightly modified version of that quoted by Mohr in 1956 and had a pH of 4.1. For his more recent work on _Dryopteris filix-mas_ however, Mohr changed the pH of his medium to 5.2 after finding that this was more favourable for germination in his species. Because of this discovery that pH can affect the induction of growth in ferns, it was decided to investigate whether it also affected the rate and pattern of growth.

The variations in pH reported here were produced by replacing the KH$_2$PO$_4$, in whole or in part, with KH$_2$PO$_4$, but experiments done in this laboratory (Dyer unpub) have demonstrated that identical results can be obtained by using NaOH as a means of raising the pH. In addition to the standard 4.1, the pHs used were 5.8 and 7.0. The experiments were done at 22°C under 100 lm/sq ft. white fluorescent light.

**Experimental Results**

Samples taken from the culture dishes at intervals during these experiments, showed that the pH values tended to converge towards pH 6. In considering the data to be described below therefore, it must be remembered that while the initial pHs were varied, the final pH was similar in all the cultures.

(1) **Cell number per filament** (fig 78)

The rate of increase in cell number (i.e. the rate of division of the apical cell) was not altered significantly by pH.
Fig 76  Effect of pH on growth in "100 White" at 22° C.
(2) **Morphogenetic pattern**

<table>
<thead>
<tr>
<th>pH</th>
<th>$F_n$</th>
<th>$T_n$</th>
<th>transition type</th>
<th>$T_\infty$</th>
<th>Day of 50% transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>5-6</td>
<td>2-3</td>
<td>$T_{98+023}$</td>
<td>0.18</td>
<td>8</td>
</tr>
<tr>
<td>5.8</td>
<td>5-6</td>
<td>2-3</td>
<td>$T_{77+23}$</td>
<td>0.38</td>
<td>8.35</td>
</tr>
<tr>
<td>7.0</td>
<td>5-6</td>
<td>2-3</td>
<td>$T_{77+23}$</td>
<td>0.48</td>
<td>7.5</td>
</tr>
</tbody>
</table>

2) At pH the "typical" two basal rhizoids were formed almost invariably but this number was raised to three in nearly all gametophytes at pH 5.8 and in most of those at 7.0.

(3) **Protonema length**

(fig 79a) The filaments grown at pH 5.8 were slightly longer than at other pHs but the differences were too slight to be of much significance.

(4) **Protonema volume**

(fig 79b) The results show very little difference up to day 5 but after this, pH 4.1 seemed most favourable for expansion.

(5) **Dry weight per gametophyte**

(fig 80) The dry weight values were higher and rose faster at pH 4.1 than at either of the other pHs.

(6) **Protein-N and chlorophyll per gametophyte**

(figs 81a, 81b) The patterns of increase in these two cellular constituents were very similar and show that no pH effect was apparent up to day 4-5 but that after this the rates increased more rapidly at pH 4.1.
Fig 79  Effect of pH on growth in "LOO White" at 22°C.

a) Increase in protonema length with time.

b) Increase in protonema volume with time.
Days after Germination

Protonema length in μ

Protonema volume in cu μ x 10^4

pH 4.1
pH 5.8
pH 7.0
Fig 80  Effect of PH on changes in the dry weight per gametophyte with time in "100 White" at 22.1°C
Dry wt./Gametophyte in μg x 10^-1

Time in Days

- pH 4.1
- 5.8
- 7.0
Fig 81 Effect of pH on the synthetic rate in "100 White" at 22°C

a) Increase in protein-N per gametophyte with time.

b) Increase in chlorophyll per gametophyte with time.
DISCUSSION

Because the mitotic rate was not significantly affected by pH changes, there was no appreciable difference between graphs of results plotted against time and cell number. Consequently, the data already presented against time has not been replotted on a per mitochron basis.

The results show that once again synthesis and elongation have responded independently but in this case volume varied along with synthesis. Both total volume and cross-sectional area (fig 82a) were greatest at pH 4.1 and, in the case of area least at 5.8, by the end of the filamentous period. Length was not altered by pH and expansion therefore was polarised to a greater extent at pH 5.8 than at the other pHs. This is confirmed by the length:diameter ratios (fig 82b) and means that lateral expansion was more sensitive than elongation to pH and, unlike the light wavelength effects on growth, increased polarisation was not here accompanied by an increase in total expansion.

It is worth noting that both volume and the length:diameter ratios showed a maximum pH effect at the 4-celled stage and the values for the different cultures then tended to re-converge. This is in accord with the changes in pH known to be occurring within the cultures.

As already pointed out, pH 4.1 appeared to be nearest the optimum for both synthesis and expansion. The rate of increase in dry weight was consistently higher at this pH throughout the sampling period, while the differences in protein-N and chlorophyll became apparent rather later. Taken over the whole period therefore, the ratios between these cell components did not vary consistently, or significantly with pH (fig. 83).
Fig 82  Effect of pH on cell expansion in "100 White" at 223°C

a) Changes in mean cross-sectional area with increase in filament cell number.

b) Changes in length:diameter ratios with increase in filament cell number.
Fig 83  Effect of pH on cell synthesis in "100 White" at 22°C:

a) Changes in the ratio of protein-N:dry weight with increase in filament cell number.

b) Changes in the ratios of chlorophyll:dry weight with increase in filament cell number.
SUMMARY

1) The rate of division was not dependent on the pH.
2) Raising the pH increased the frequency of type II transition and of interstitial divisions.
3) At pH 5.8 and 7.0 three not two basal rhizoids were formed.
4) Polarisation of expansion was most pronounced at pH 5.8 while total volume and cell synthesis were greatest at pH 4.1 i.e. the degree of polarisation (from the length: diameter ratio) varied inversely with the dry weight.
The results presented in this thesis confirm well known light wavelength effects on morphogenesis in ferns. In blue light the gametophytes develop into prothalli in which division occurs in several planes (2-dimensional growth), whilst in red light the 1-dimensional, filamentous phase is prolonged indefinitely. In the language of workers in this field, blue-grown filaments are said to undergo morphogenesis whereas red-grown filaments do not. From this usage the term morphogenesis has come to be almost completely identified with a single stage in development - the transition from filament to prothallus. Transition in turn, is generally treated as if it was controlled by a single "all or none" switch-mechanism and involved only a single event, the re-orientation of division.

In this present work, the scope of the experimental conditions has been widened to include environmental factors such as temperature and pH. It has also included the use of light of mixed wavelength (white) in addition to that of restricted wavebands i.e. red and blue. In the course of this investigation it became clear that the concepts of transition and morphogenesis as outlined above are considerably over-simplified. Varying the environmental conditions led to variations in not one but several aspects of transition and also features of earlier filamentous growth all of which could be properly described as morphogenetic. These will be considered in turn:

a) **TRANSITION**

(1) The type of transition

The type of transition found in *Dryopteris borreri* under the range of conditions studied - type I, type II and abortive - have already been described
in the section on "patterns of morphogenesis". It is perhaps worth commenting here that the distinction between type ll and type 1, the presence or absence of an apical papilla, has in the past been regarded as rigid enough for use as a diagnostic character in ferns (ORTW 1936).

Type 1 transition occurred at low temperatures (17° - 22°) and/or high intensity of both white and blue light. Then, with increasing temperatures and decreasing intensities there was a sequence type 1 - type ll - abortive transition - no transition at all. The stages in this sequence are not always clearly defined and some type 1 gametophytes were almost always found alongside type ll within a single culture. In the same way abortive and non-transition often occurred in mixed cultures.

The actual light intensities and temperatures corresponding to the steps in the sequence were different in different light qualities. Type ll transition occurred at lower temperatures and higher light intensities in blue than white light. This seemed rather anomalous at first sight since transition is generally considered to be controlled by blue light and type 1 is the only pattern of transition that has previously been referred to in this context. One might therefore have expected that "100 Blue" would be as effective as "100 White". In practice the relative frequencies of the transition types in "25 White" were similar to those in "400 Blue" which had both a higher blue and a higher total energy content. The implication of this with regard to the photoreceptors involved in the control of transition, will be discussed later. It may however be remembered that there was some evidence from other growth parameters, that the "optimum" temperature for growth was lower in blue than white light.
Type II differs from type I in that, in the latter the apical cell continues to divide after transition and the apical dividing cells of the prothallus are derived directly from it. In type II, these cells are derived from the filamentous cell $p_{III}$ while the apical cell $p_{I}$ forms the apical papilla and then divides no more. Thus type II may be considered intermediate between type I and abortive transition not only because it occurred in intermediate environmental conditions but also because it has intermediate morphological characteristics.

In abortive transition, as in type II, an apical papilla forms but then, instead of an ordered pattern of re-orientated divisions giving a young prothallus, there are either no further divisions or, a single oblique which occurs apparently at random either in the cell immediately below the papilla or in one further back. These abortive transitions usually formed a minority in cultures in which the rest of the gametophytes showed no signs of transition at all. Thus abortive transition occurred in threshold conditions and shared characteristics of both "blue" and "red" type growth. Filamentous growth was terminated (at least in the main filament) as in blue light but as in red light there was no prothallial development. In this way abortive transition demonstrates that transition is not an all or none response, some filaments forming apical papillae and then ceasing to develop further while others continue one additional stage, undergoing one re-orientated division but even then not forming a prothallus.

(2) $F_n$ - the filament cell number at the time of transition

This includes only those protonemal cells cut off from the apical cell i.e. it excludes the products of interstitial divisions. The number increased
with decreasing and increasing temperature in white and blue light. This means that there was a correlation between the sequence type 1 - type II - abortive transition - no transition and an increase in the filamentous phase in terms of cell number. A comparison between development in blue and white light however, shows that there can not be a strict relationship between cell number and transition type since \( F_n \) was lower in "100 Blue" at 22\(^{\circ}\), which gave mostly type II transition, than "100 White" in which transition was almost entirely type 1 at this temperature.

This observation is relevant to the theory that there is a critical cell number at which transition will occur. If such a critical number does exist it would evidently only apply to a specific set of conditions, a point that is emphasized by a consideration of growth in red light. Here the filament may have 30-40 cells and still show no signs of transition. MOHR (1964) and DYER (un. publ.) have indeed both found that a very low proportion of red-grown filaments do eventually undergo some sort of transition, suggesting that there may be a very high critical cell number that in most culture periods is never reached.

Further evidence that for a specific set of environmental conditions a critical cell number does exist, has been obtained here in Edinburgh by BURNS and INGLE (1968). They found that under constant conditions of light and temperature, transition invariably occurred as soon as the 4-5 celled stage had been reached even when growth and cell division were partially inhibited by the addition of RNA-base analogues.
(3) Branching

Previous descriptions of the filaments of DRYOPTERIS have always implied that they were simple and unbranched (e.g. MOHR 1965) but in this study two types of branching - basal and lateral - have been observed.

a) Basal forking was characteristic of red-grown filaments at temperatures of, or above 22.5°C. The frequency of such forking increased with increasing temperature.

b) Lateral branching from cells along the filament occurred at 27°C. In red light at this temperature both types of branching were found.

These two forms were quite distinct in origin. The basal branches (plate 3) were initially really outgrowths or outpushings, from the cytoplasm of the basal cells. Only rarely was a cell wall formed across the base of these outgrowths i.e. the original cell forked and remained forked. The nucleus of this parent cell then migrated into the outgrowth and mitosis occurred some way up the new branch.

The first signs of lateral branching in contrast to this, were re-orientated divisions in sub-apical cells of the filaments. The branches thus originated as cells cut off from the main filament. In this way, lateral branching involved initially a re-orientation and re-distribution of division whilst basal forking involved a re-distribution and orientation of expansion.

In both these cases, branching represented some kind of escape from the strict apical control that seemed to exist in the unbranched filaments. Lateral branching usually only started after the apical cell of the main filament had stopped dividing, suggesting that perhaps the apical dominance was dependent upon mitotic activity at the apex. NAKASAWA and COTAKI (1961) have found that if
apical growth in DRYOPTERIS VARTA was arrested by placing the gametophytes in the dark, the normal polarity was upset and, on re-illumination growth was frequently continued from sub-terminal branches.

In the present study, the first branches were in the majority of cases formed from cell p_iii, the cell that normally shows the first signs of transition.

Basal forking, on the other hand, occurred at the base of actively growing filaments. Growth then continued on both sides of the fork. Since this branching is characteristic only of very elongated red-grown filaments, it is possible that some distance factor is involved in the lessening of apical control. This cannot however explain the situation completely, since the frequency of the branching was temperature dependent whereas expansion was not. It may not be coincidental that the forking was initiated at the same developmental stage as the second basal rhizoid was formed in the presence of blue light. In red light the stimulus for the differentiation of such a rhizoid appears to be missing but with increased temperature the synthetic rate was also increased and even in the absence of the apical stimulus the basal cell may synthesise the materials necessary for the formation of a new cell.

(4) **Interstitial divisions**

These divisions, in which the new cell walls are formed perpendicular to the long axis of the filament, provide exceptions to the rule that only the apical cell divides to give new, prototermal cells. They occurred in high frequencies in all conditions giving type II transition and in mixed cultures of type I and II, the interstitials usually occurred in filaments undergoing the latter type. The frequency of such divisions in filaments undergoing type I transition in "100 White" decreased with increasing temperature. In general there was an inverse correlation between the frequency of interstitial
divisions and the rate of mitosis in the apical cell. (in mixed cultures the slower dividing filaments were usually those which then underwent type II transition). This relationship is demonstrated in the table below:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Light</th>
<th>slope of cell number/time curve</th>
<th>( \frac{1}{x} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>17(^\circ)</td>
<td>100 White</td>
<td>0.64</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>100 Blue</td>
<td>0.5 - 0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>20(^\circ)</td>
<td>100 White</td>
<td>0.65</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>0.9</td>
<td>0.18</td>
</tr>
<tr>
<td>22(^\circ)</td>
<td>100 Blue</td>
<td>0.5 - 0.61</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>100 White</td>
<td>0.7 - 0.87</td>
<td>0.16</td>
</tr>
</tbody>
</table>

During type II transition, the interstitial divisions sometimes occurred before but more commonly after, papilla formation. In the latter case the divisions served to protract the transitional stages and to separate the termination of filamentous growth (the formation of the papilla) and the re-orientation of division. Interstitial divisions therefore provide additional evidence that transition involved several distinct stages under separate control.

(5) Rhizoids

The rhizoids referred to here are basal ones originating from cell P. Those formed from more apical cells at the time of transition are not included. Spore germination led directly to the formation of the first rhizoid and all gametophytes entered the experimental period with this primary rhizoid. In red light no post-germination rhizoids were cut off but in both white and blue light a second was formed opposite the first, at the 2-3 celled stage. When the pH of the medium was raised from 4.1 to 5.8, a third (and sometimes a fourth) basal rhizoid was formed in almost all gametophytes.
Consideration of these various aspects of development gives several indications that decreasing the light intensity and/or increasing the temperature in white light, shifted the growth pattern towards that characteristic of red grown filaments. High intensity, low temperature white light cultures resulted in growth very similar to typical blue-grown filaments.

b) FILAMENTOUS CELL GROWTH

(1) Cell division rate

The rate of cell division has been shown to be highly sensitive to the environmental conditions particularly during the later stages of filamentous growth. In general, increasing the light energy led to an increase in division rate and "100 White" gave higher rates than either "100 Blue" or "100 Red". The optimum temperatures for division also differed slightly in different light qualities, the fastest rate in white light being found at 22° - 25° and in both red and blue, between 17° and 22°.

(2) Cell synthesis

Cellular synthesis was measured in this study in terms of dry weight, protein-N and chlorophyll. The protein-N values served as an estimation of the protoplasmic content of the filamentous cells and rhizoids. The ratio of protein-N: dry weight formed a measure of the ratio between protoplasm and cell wall materials. In the majority of experiments these three parameters showed parallel responses to environmental variations e.g. fig 33A shows that the variation in the patterns of net chlorophyll synthesis brought about by light intensity at the two temperatures closely resembles the variation in patterns of both protein-N and dry weight increases. Because of the similarity of their responses, these parameters may conveniently be considered together.
Fig 83A  Effect of white light intensity on chlorophyll, protein-N and dry weight at 20° and 22°C.
The absolute rates of synthesis were higher in white light with its high total energy content but the increments per mitochron were very similar in white and blue. The rate of synthesis in red light was lower both in absolute terms and per mitochron. The synthesis per mitochron had different optima in different light conditions, being faster at $27^\circ_2$ than at lower temperatures in red whilst the optimum in white light was close to $22^\circ$°. In blue light, the rates were either unaffected by temperature or had an optimum midway between two sampling temperatures i.e. around $20^\circ$°.

Intensity had little effect on synthesis in blue, or in white light except at $27^\circ_3$° where it seems that at the lower intensities, only cell wall materials continued to be synthesised after the 3-celled stage.

The protein-N:dry weight ratios showed a sharp rise at the end of the sampling period in both white and blue light between 17° and 25°. This rise was absent from cultures at $27^\circ_2$, or in red light. The connection between this rise and transition has already been pointed out and will also be discussed later.

(3) Cell expansion

a) The total expansion did not seem completely dependent on the total light energy since it was greater in red than in white light and the intensity of white only showed any influence at $27^\circ_3$. In blue light expansion was even less than in white but here, increasing the energy did lead to some slight increase in total expansion.

b) The polarity of expansion as represented by the length:diameter ratios, showed a clear light/temperature interaction. The ratios in red light were very high, whilst those in blue were low. In neither case were the ratios
affected significantly by either intensity or temperature. By contrast, the ratios in white light were intermediate and very sensitive to both these factors. At low temperatures and high intensity the ratios were similar to those in blue. Then, as the temperature was raised or the intensity lowered, the ratios moved upwards towards those characteristic of red-grown filaments.

c) Examination of the data on the duration and distribution of expansion contribute further evidence of parallels between the patterns of growth in red and white light of low intensities, at high temperatures. The expansion of each cell under such conditions was confined to a much narrower developmental period than under the converse conditions of high intensity white or blue light, at low temperatures. For example in red light, expansion of cell \( P_2 \) had already been completed in 4-celled filaments (i.e. within two mitochondria) see fig 52a and in 5-celled ones, \( P_3 \) was already the largest cell (fig 52b).

In white of low intensity (or at high temperatures) \( P_2 \) was usually the largest cell in such a filament, while in high intensity/low temperature white or blue light, \( P_1 \) remained the largest cell and expansion of cell \( P_2 \) continued throughout the filamentous perin. There was no evidence that expansion was completed in this cell by the onset of transition i.e. expansion extended through more than three mitochrons (fig 40).

d) One cellular process that is closely associated with expansion, is vacuolation. This process is reflected in the ratios of total volume:protoplasm and can therefore be assessed from the ratios of volume:protein-N. The graph (fig 84) gives an indication of the degree of vacuolation just prior to transition at different temperatures, light qualities and quantities. It shows that the cells were densest in blue, and most highly vacuolated in red light.
Fig 84 Ratio of volume:protein-N just prior to transition in various conditions of light and temperature.
It also indicates that in white light vacuolation, in common with both total expansion and the polarisation of expansion, was increased both by lowering the intensity and by raising the temperature. Again the values for red and blue light demonstrate that growth under these restricted wavebands was less sensitive to factors such as temperature, than it was in white (mixed wavelength) light at 25 or 100 lm/sq.ft. Higher intensities ("400 White") however, gave a growth pattern that showed a stability equal to that in blue light.

These accounts of variations in cell synthesis and cell expansion, provide several examples of similarities between red light, low intensity white light and high temperature cultures:

1) High total expansion
2) High L:D ratio
3) Expansion completed within few cell cycles i.e. filaments include few expanding cells at any one time.
4) Low synthetic rate and highly vacuolate cells.

An additional environmental factor that has been shown to affect all these aspects of cellular growth was pH although it must be remembered that this differed from the other factors in that it did not remain constant throughout the sampling period. However, raising the initial pH from 4.1 to 5.8 led to a shift towards a "red-type" L:D ratio, this being accompanied by a relative decrease in the dry weight. Total volume on the contrary, did not respond in the same way as L:D in this instance and this suggests that perhaps the inverse relationship between L:D and the dry weight content of the gametophytes was more fundamental than the relationship between total expansion and the L:D ratios.
(1) Cell synthesis

There have been a number of attempts to establish links between the pattern of morphogenesis and biochemical parameters such as RNA, protein, chlorophyll and dry weight. Most of these attempts have aroused controversy and, while it has been convincingly shown these parameters change during transition, it has proved much more difficult to establish any causal relationships.

One of the clearest features standing out from the data assembled in this present investigation is the rise in protein-N/dry weight percentage, associated with the end of filamentous growth in gametophytes about to undergo transition. Similar increases in relative protein content during transition were found to occur in Dryopteris erythrosora by Hotta and Osawa (Hotta and Osawa 1958, Hotta 1960) and, from experiments with amino-acid analogues and 8-azaguanine, these authors claimed that there was a causal relationship between protein synthesis and the transition to prothallial growth. Bell and Zafar (1961) however, questioned this conclusion after showing that in Dryopteris borreri the protein-N per gametophyte rose steadily throughout development. They suggested that the fluctuations in the relative protein levels (protein/dry weight) associated with morphological change were caused by changes in non-proteinaceous cell wall materials. They pointed out that filamentous cells are large and highly vacuolate whilst much of the prothallus immediately after transition comprise small, dense cells of the type characteristic of meristems. More recently Sobota and Partenem (1967) have calculated
that while the protein/dry weight ratios increase after transition, the protein content per cell decreases.

Certainly in this present study, the cells formed at the time of the rise in relative protein-N i.e. just prior to transition, were considerably smaller and denser than those at the apex of filaments grown at high temperature or in red light from which the rise in protein was absent.

On the other hand OHLENROTH and MOHR (MOHR and OHLENROTH 1962; OHLENROTH and MOHR 1963) have shown by eliminating dry weight differences between red and blue-grown gametophytes of DRYOPTERIS FILIX-MAS, that the increase in relative protein in this case, reflected a genuine increase in protein and not a decrease in other cellular materials. Furthermore BERGFELD found (1963) that the size of the chloroplasts increased (reversibly) when gametophytes were transferred from red to blue light and this could be prevented by the addition of an inhibitor of protein synthesis - 5 Methyltryptophane (BERGFELD 1965).

Although both BERGFELD and MOHR believed at first (OHLENROTH and MOHR 1964; BERGFELD 1963a) that this correlation between protein synthesis and transition was in fact a direct causal relationship, later light transfer experiments by MOHR et al (MOHR 1966) showed that the morphological changes were visible prior to changes in protein-N level. It seems therefore that blue light has two distinct effects:

1) induced protein synthesis

2) controlled morphogenesis

Moreover, from examination of the relationships existing between the relative chlorophyll and protein contents, KASHMIR and MOHR (1965) have suggested that
the bulk of the blue-induced protein synthesis was located in the chloroplasts, a conclusion that was drawn from a comparison of their results with those of PIRSON and BOGER for CHLORELLA (BOGER 1964; PIRSON and BOGER 1965). Such a conclusion is in accord with the findings in this thesis, that variations in environmental factors led to parallel changes in chlorophyll and protein-N. An example of this is shown in fig 85.

Several workers have also demonstrated correlations between RNA levels in transition. (HOTTA, OSAWA and SAKAKI 1959; HOTTA 1960; RAGHAVAN 1965a and b; RAGHAVAN and HENG FONG TUNG 1967). DRUM and MOHR (1967) have established from transfer experiments that these changes in RNA occur as rapidly as morphological changes, and more rapidly than the changes in protein. From this they have postulated that blue light leads to the activation of specific genes and thus to the synthesis of new RNA. This RNA then directs both the morphological responses and protein synthesis within the plastids.

All this work of MOHR and his collaborators involved comparisons of 2-D (blue-grown) gametophytes with 1-D (red-grown) ones and, since most of the transition stages in the study came after transfer from red to blue light there was no attempt to compare the filamentous and prothallial stages of cultures grown under constant conditions. (In high intensity blue-light the filamentous stage is virtually eliminated).

Work done under conditions more comparable with those described in this thesis is that of DAVIS and POSTLETHWAITE (1965; DAVIS 1968a and b). In this, gametophytes of PTERIDIDIUM AQUILINUM grown under white fluorescent light of varying intensities were used to study correlations between protein-N and transition. The increase in percentage of gametophytes with 1 oblique
Fig. 85  Effect of temperature on protein-N and chlorophyll levels in "100 White"
(re-orientated) division was plotted against time and the changes in both protein-N and chlorophyll studied over the same period.

In contrast to both the results of KOHR and those presented here, DAVIS observed constant chlorophyll:dry weight ratios and in his experiments the protein level fell during the later part of this transition period. One obstacle to an evaluation of these conflicting results, is that DAVIS was sampling a mixture of 1 and 2-dimensional gametophytes instead of either the "either/or" cultures of KOHR or the primarily 1-d ones sampled in the present work. Most of DAVIS' reported fall in protein-N in fact came after the 50% transition stage i.e., after the end of the sampling period involved here.

DAVIS also observed that while increasing the light intensity led to a large increase in the percentage transition at any one time, the protein level was unchanged and that of chlorophyll actually fell. From this data he concluded that no strict relationship existed between the relative protein level and 2-d development. (DAVIS 1968a). Once again these results differ from both the present and previous workers who have found a correlation between increasing intensities and protein but again the differences in sampling methods make direct comparisons difficult.

On the other hand, from experiments described in the same paper (DAVIS 1968a) the author claimed that when protein synthesis was inhibited by RNA-base analogues, anti-metabolites or anti-biotics, there was a decrease in the percentage transition even when division per se was not affected. This result is in line with those obtained by other workers (HOTTA and OSAMA 1958; RAGHAVAN and HEUNG FONG TUNG 1967) although the method used to estimate cell division rates is not entirely satisfactory because it rests on the assumption that all filaments within a culture have identical division rates even when
the rate of reaching transition showed considerable variation (the 1st re-orientated divisions in individual filaments were recorded over a period of five days).

If, as is the experience of the present author, there is a correlation between the rate of division and transition in specific gametophytes, then DAVIS' data would not have shown up any partial inhibition had this occurred. These doubts have been substantiated by the work in this laboratory by BURNS and INGLE, (1968) which indicates that the inhibition of transition brought about by RNA-base analogues is indeed due to general inhibition of cell division.

DAVIS himself does not attempt to reconcile what he describes as a specific necessity for protein synthesis at transition, with the decrease in relative protein content that he described. Such a contradiction might however, be resolved if the "protein" inhibition was in fact a reduction in total synthesis leading to a slower, overall growth rate.

The second, unsolved problem raised by DAVIS' work, is the difference between his results for relative protein levels, and those of other workers. The difficulties implicit in any comparison involving results from experiments done under different cultural conditions and on different species, have already been mentioned. All that can be added here is a reminder that where the most direct comparison could be made, i.e. between the data obtained in this present investigation and that from the red-blue light transfer experiments of MOHR, both sets of results show a rise in the protein-N/dry weight ratios associated with filaments about to undergo transition from 1-D to 2-D growth.
(2) Cell division and expansion

Despite the reservations just expressed about the sampling technique employed by DAVIS in his estimation of cell division rate, the work has great interest since it represents one of the few published attempts to utilize cell number in an analysis of morphogenesis. SOBOTA and PARTANEN (1966) have made a fairly extensive study of this kind, in which they tried to correlate the relationship between cell division and expansion with morphogenesis in PTERIDIUM ACQUILINUM. These authors have expressed the view (based on the ideas of STEBBINS 1965) that the distinct patterns of growth associated with red and blue light cultures, are dependent upon the different rates of cell division under the two light regimes.

Thus, in red light the mitotic rate is low, therefore there is a long intermitotic time available for expansion to occur and so very elongated cells are formed. Then, on the assumption that cell division tends to be orientated so that the new walls form parallel to the short axis of the cell, the result of having elongated cells will be strictly polarised division.

In blue light by contrast, the mitotic rate is high and the intermitotic expansion time short. As a result (according to the theory) isodiametric cells in which division may occur in any direction are formed and thence a 2-dimensional prothallus.

This theory is based on the assumption that only cell division and not expansion is in any respect directly dependent on the environment. In the present work on DRYOPTERIS BORBERIK it became very clear that both the rate and the polarity of expansion can be modified.
i) The expansion rates varied throughout the range of experimental conditions employed but if comparisons are made between instances when the division rates were the same e.g. "100 Red" at 22°0 and "100 Blue" at 17°2, then it can be seen that the expansion rate was independent of the rate of division:

At the 4-celled stage the mean volume of the red-grown filaments were 40.3 x 10⁴ cu microns; in sharp contrast with the mean for the blue-grown ones, 14.75 x 10⁴ cu microns.

ii) Polarity of expansion. SOBOTA and PARTANEN assume that "expansion" in their system was synonymous with "elongation" i.e. that the mean cross-sectional area remains constant under all conditions. The data already given in this thesis indicates that this is not so. While expansion in red light may be strictly polarised, that in blue or white light is not. Moreover MöHR (1966) showed that on transference from red to blue light, the apical cell of the filaments becomes trumpet-shaped even before the first division in blue-light, a result that has been confirmed in this laboratory (DYER un.publ). This indicates that there was a direct wavelength effect on expansion. There is also evidence from other systems that expansion and division can vary independently (HABER 1962; VAN'HOFF and YING 1965).

The polarity of expansion seems to depend upon the area of the expanding portion of the cell wall (fig 86). In white, or blue light, expansion occurs over several cell cycles (see data on the volume of cell P₂ and also TAKAHASHI 1961). This implies that the side walls of 2-3 sub-apical cells must be still plastic allowing outward, in this case lateral expansion (fig 86a). The mean cross-sectional areas of such filaments may thus become greater than that of the
Fig 86 Distribution of expansion and its effect on the shape of cells under different light wavelengths

(a) Blue or white
(b) Red

(Arrows indicate direction of expansion.
--- areas of expanding wall)
of the original spore. In red light MOHR has postulated that there is only a small expanding area at the tip of the apical cell which means that filaments narrower than the spore are formed (fig 86b).

Even if SOBOTA and PARTANEN's claim that the mitotic rate controls expansion and hence morphogenesis, is false, there is considerable evidence that the polarity of expansion i.e. the L:D ratios (rather than total expansion) does have great morphogenetic significance.

**L:D ratios as a morphogenetic index**

The idea of using the relationship between length and width as an index of morphogenetic pattern, came from MOHR (MOHR and BARTH 1962). In filamentous, 1-D growth (red light) the maximum width is the width of a single cell, the length is the sum of several, highly elongated ones and the L:W ratio is therefore very high. In a 2-D prothallus (i.e. in blue), the gametophytes are several cells wide giving a high value for W and therefore a relatively low L:W ratio.

The ratio suggested as a morphogenetic index in this thesis differs in several ways from that of MOHR and his co-workers.

1) Instead of referring to all gametophytes the L:D ratios refer only to filaments and the diameter is therefore the diameter of a single cell. Thus the ratio is comparable to MOHR's L:W ratios for red but not blue-grown filaments.

ii) MOHR uses maximum width in deriving his index. The diameter used here is an average value obtained from measurements of filament volume. (For convenience, the term "width" is used here for direct micrometer measurements, "diameter" for these derived values). For practical reasons a true average for width would be difficult to obtain and even
the maximum width of a filament may be distorted significantly by even slight pressure on the filaments under a cover-slip. The use of mean, instead of maximum diameter, also eliminates the need to assume that the shape of the cells in cross-section remains constant in all conditions. iii) MOHR's L:D ratio provides a useful device for describing established morphogenetic patterns and changes in patterns under different conditions. The L:D data presented here applies to filamentous growth prior to transition. If there is a real correlation between these ratios and the eventual pattern of transition, then this index would have a predictive as well as a descriptive function.

Examination of all the data collected in this investigation indicates that the L:D ratios did indeed relate more closely to the characteristics of subsequent transition than any other parameter measured. Not only did the two extreme environmental conditions, blue and red light, provide the two extremes in L:D ratios (low and high respectively) but in white light at varied intensities and temperatures, the sequence type 1, type II, abortive and no transition and the parallel increase in $F_n^1$ was also accompanied by an increase in the L:D ratio.

The one set of data in which the L:D ratios failed to show a simple correlation with transition came from a comparison of "100 White" with "100 Blue" at $22^\circ$C. Here a shift from type 1 to type II was accompanied by a decrease in L:D (fig 88a). The two types of transition have already been shown to be difficult to separate by environmental adjustments, into "pure" cultures and it is likely that the control of transition type is a complex one involving several factors.
The effect of light intensity on the L:R ratio in white light, suggests that the nutritional level may be one of these interacting factors (fig 88a). The available parameter most closely related to nutrition is dry weight. A correlation between L:R, dry weight and transition can indeed be demonstrated. (fig 87).

The point marked with an arrow on this diagram, represents the one occasion where the correlation does not seem to exist. This point refers to a high temperature, high light intensity culture where the initial rate of dry weight increase was high but then (at around the 3-5 celled stage) fell off sharply while elongation and the L:R ratio continue to rise steeply.

One further point that is indirectly relevant to a discussion of MOHR's L:R and this L:R data is that SCHNARRENBERGER and MOHR (1967) include a comparison of red and "dark"-grown filaments. The data on the threshold blue light energy required for growth (page 69) emphasises that in this present investigation "dark-growth" was not possible. In all these experiments on DRYOPTERIS BORRERTI, germination was induced by an 18 hour period of red light whereas MOHR and his co-workers, gave the closely related species DRYOPTERIS FILIX-MAS a 4-day induction treatment. Following such a treatment, an increase in protein-N (but not dry weight) was recorded over the first 4-days in the dark but after this, "growth" consisted of an increase in length without any net synthesis. (OHLENROTH and MOHR 1964).

**Changes in Growth Rate at the 2-3 Celled Stage**

One feature of the growth parameter curves that recurred several times during the survey was the occurrence of a "kink" at the 2-3 celled stage. For example, at this stage in "100 White":

Fig 87  Scatter diagram showing correlations between L:D ratios; dry weight values and transition type.
The total expansion per mitochondrion increased

- The total area of the cross-sectional area increased.
- Protein-N decreased.
- The slope of the L:D ratio decreased.

b) $25^\circ$
- The mitotic rate increased.
- The slope of the L:D ratios increased.

c) $27^\circ$
- The mitotic rate decreased.
- The slope of the L:D ratios increased.

Similar kinks also occurred in both red and blue light e.g. the mitotic rate decreased in red while both mitotic rate and expansion increased in blue. Chlenuoth and Mohr (1963) also showed that in red light the rate of increase in protein-N slowed down at the 3-celled stage.

This kink corresponded to the stage at which the second rhizoid appeared under both "100 White" and "100 Blue" but although this coincidence may not be completely fortuitous, the fact that kinks also occurred in red light where no $R_2$ forms, implies that there is no direct causal relationship between the two events.

An alternative explanation for the kink is that certain food substances are stored in the spore and only when these are exhausted is the filament completely dependent upon photosynthesis. In red and low white light, and at high temperatures where photosynthetic rate was low, the kink existed as a downward bend i.e. to a decreased growth rate. At low temperatures and high light intensity, chlorophyll and presumably photosynthesis was high and the kink was an upward one.

Light and electron-microscope studies carried out in this laboratory (Cran u.nplub.) have shown that the germinating spore contains large amounts
lipids which then disappear during filamentous growth. In addition there may of course be other substances such as amino acids that are stored and then used up but which are not visible in the same way.

On rare occasions throughout the investigation, filaments in one culture dish would fail to develop or would develop very much more slowly than those in neighbouring dishes. These "rogue" cultures remained apparently normal until the 2-3 celled stage and then growth fell off suggesting that the sensitivity of the cultures to some adverse factor was enhanced at this stage. Such cultures therefore emphasise that gametophytes were buffered from the environment during the initial stages of filamentous growth.

Both the "rogue" cultures and the kinks during normal growth point to the existence of correlations between increases in the growth parameters and a specific developmental stage other than the transition from 1 - to 2-dimensional growth.

e) CONTROL OF MORPHOGENESIS

The photoreponses

The literature on transition, the only aspect of morphogenesis considered to any extent previously, has produced three alternative interpretations of the general mechanism for the light wavelength dependence of transition induction they are:

1) That nutrition alone is involved, light wavelength acting through differences in the products of photosynthesis.

2) That there is a blue sensitive system which alone is responsible for initiating transition.

3) That there are red and blue sensitive systems interacting in their
control of morphogenesis.

In all cases, intensity may act as a modifying influence through its effect on photosynthetic rate and thence nutritional level.

In these three models for transition induction, three basic photoreceptors are implicated: chlorophyll (and other photosynthetic pigments), a red-sensitive and a blue-sensitive pigment. Now that two other clear photoresponses, the type of filamentous growth and the pattern of transition, have been studied, the observations can be re-considered for evidence of the activity of one or more of these systems in each response.

1) Filamentous growth

Certain parameters studied, varied in ways that could not be attributed solely to the level of nutrition. Foremost amongst these were volume and, more especially, length, which were greater in red than white light of a considerably higher total energy. Thus the photomorphogenetic effects on cell expansion clearly outweighed the modifying influence of photosynthetic rate.

However, the "blue-photosystem" is not the only one that is active in morphogenesis. Evidence for this comes from a comparison of the L:D ratios of filaments grown in white or blue light. In white light, the ratios increased as the total energy was decreased (fig 88a). From this one might expect that, if cell shape was completely controlled by blue light, the L:D ratio in white light should be equal to, or less than, that in the corresponding blue component alone. Fig 88b however, shows that in fact the ratio was greater in "25 White" than "10X Blue" even though the white light had both a higher total energy and a higher blue content, some component of this white light must therefore have a modifying effect on the blue, resulting in longer, thinner filaments.
Fig 88  

A) Effect of light intensity on changes in L:D ratios in white light at 22°C

b) L:D ratios in white and blue.
Further evidence of this, comes from filaments in which the blue-absorbing spore wall was retained on the apical rather than the basal cell. Such "capped" filaments were present as a very low proportion of all cultures and in white light, they were conspicuous in being highly elongated and very reminiscent of red-grown filaments (plate 5). As the spore wall transmits very little blue-light (fig 89 in appendix), this "red-type" behaviour has been interpreted as evidence that the blue-photoreceptor is active only at the apex. On the other hand in blue-light culture, the capped filaments were not elongated and this suggests that the "red behaviour" of white capped filaments, depends not only on the absence of a blue stimulus at the apex but also on the presence of longer wavelength light.

The observations described above demonstrate that both red and blue light are morphogenetically active. Filaments grown in white light display characteristics intermediate between those grown in red and blue light which implies that the two photosystems can interact to affect filamentous growth. Data from cultures grown under different intensities and at different temperatures, suggest that these two systems display differential sensitivities to such factors and thus the relative activities of each system can vary (cf. germination in LACTUCA SATIVA - JACOBI and KANDELER 1965).

At low temperatures and high intensities, growth in white light resembles that in blue, so presumably the effects of the blue-sensitive system predominates. With increasing temperature and decreasing intensities, the early development becomes more like that in red light implying that the red system now has a proportionately greater effect.
Plate 5  Filaments grown in "100 White" at 22° C showing effects of apical "capping" of filament on the pattern of growth.
At 27.5°C, growth in "100 White" remains filamentous, while growth in "100 Blue" is completely inhibited. Apparently only the red system still functions. Preliminary experiments have indicated that growth does occur in higher intensities of blue, suggesting that the blue system is not completely inactivated but that, at this temperature, the threshold energy is considerably raised.

Thus it appears that both blue and longer wavelength light over a certain threshold level can stimulate growth during the filamentous phase but that the different wavelengths each result in different growth characteristics. The nutritional level can also modify filamentous growth.

2. **Induction of transition**

A considerable body of evidence against the proposal that nutrition is the only controlling factor in the induction of transition, has been compiled by Mohr and his co-workers. These workers standardised their red and blue light so that the same dry weight increases were achieved in gametophytes grown under either of the light regimes (Mohr and Chilenroth 1962). Under these conditions, photomorphogenetic distinctions were maintained.

The main disadvantage of this standardisation technique is that it makes no allowance for qualitative differences in photosynthetic products under different light conditions. Such differences have been found in other plants e.g. *Chlorella* (Hauschild, Nelson and Krotkov 1965).

Drumm and Mohr (1967) have however, also shown that $^{14}$C incorporation during photosynthesis was the same under the different wavelengths. Furthermore, transition has been shown to occur in CO$_2$-deficient atmospheres where photosynthesis is correspondingly low (Chilenroth and Mohr 1963; Schnarrenberger and Mohr 1967).
One of the observations in the present investigation, that seems to point firmly away from a purely nutritional basis to the wavelength effects, concerns the "capped" filaments already described. In white light such filaments are not only highly elongated but also, fail to undergo transition. Thus it seems that "blue growth" requires a specific apical stimulus and is not controlled by light absorbed by the majority of chloroplasts along the filament.

One source of some confusion however, has been experiments on the effects of sucrose on development. MOHR et al found in DRYOPTERIS FILIX-MAS that sucrose cannot replace blue-light in the induction of transition (MOHR and OHLENROTH 1962) but KATO (1967) found that in red-light, transition would occur if sucrose was added to the medium. Such confusing results may reflect species differences since MOHR himself has found transition occurring in red-grown gametophytes of ALSOPHILA AUSTRALLIS whereas in DRYOPTERIS FILIX-MAS, transition is very rare under these conditions. (MOHR and BARTH 1962).

Therefore, while it appears unlikely that the induction of transition has a purely nutritional basis, photosynthesis does seem to have some modifying influence. MILLER and MILLER (1961) showed in ONOCLEA SENSIBILIS, that sucrose added to the medium enabled gametophytes grown under low light conditions to resemble those characteristic of higher intensities.

Taken as a whole, MOHR's work has led towards the conclusion that short wavelength light alone induces transition. There are however, a few observations that suggest that other wavelengths can also have an effect e.g. DAVIS (1968b) found that the rate of transition under 200 lm/sq.ft. of White fluorescent light was equivalent to that in 400 lm/sq.ft. blue. Evidence that red light alone can also have some inductive effect has already been touched upon above.
In *Alsophila australis* (Mohr and Barth 1962), transition occurs in red light and even in *Dryopteris*, a very low percentage transition has been observed at the end of long periods of red light culture (Mohr 1964, Dyer unpubl).

The behaviour of red-grown filaments at high temperatures (27°C) suggests that the termination of apical growth in the filament has a less specific blue-light requirement than later stages of transition. This implies that the induction of transition is not a switch-mechanism and that either, distinct photoreactions are involved in the consecutive steps in transition or, light is required for the accumulation of some substance for which there is a higher requirement during the later stages of transition. If this second alternative is the correct one and if accumulation is rapid in blue and very slow in red-light, then the species differences could be caused by differences either in the accumulation rates or in the amount of the active substance necessary for transition.

Prothalli transferred from blue to red light may revert to filamentous growth, but only if the intensity of the red is low (Dyer unpubl.). Such reversion implies that the "prothallial substance" is labile and must be continuously replaced under the stimulus of light, if prothallial growth is to continue. High intensities of red light would seem to be sufficient to fulfill this replacement requirement whilst under low intensities the concentration of this hypothetical substance falls below the necessary level and reversion to I-D growth follows from this.

These observations indicate that while blue-light is the most active in initiating transition, other wavelengths, probably red, can have a synergistic effect. Furthermore, red light alone can have some stimulatory effect. Again,
as in filamentous growth, the level of nutrition can act as a modifying influence.

3. \underline{Type of transition}

Just as the results discussed above, indicate that wavelength light encourages transition, there are even clearer indications that once transition has been initiated, the pattern of transition is affected by wavelengths other than blue. The comparative responses to white and blue light can be adequately explained neither on the basis of energy in the blue region of the spectrum, nor total energy:

At \(22.5^\circ\), "100 Blue" induced mainly Type II transition while "25 White", with a lower blue component but a similar total energy, induced about 30 per cent Type I. That Type I is not simply the result of low energy, is shown by the fact that increasing the blue (to "400 Blue"), also produces about 30 per cent Type I. Increasing the intensity of white light similarly provides Type I. Thus, in the presence of some blue, an increase in the available energy encourages Type I but, as white is more effective than blue light of approximately the same total energy, this response is less readily induced by blue than by some other component of white.

Temperature also affects the response. At \(17.5^\circ\) "400 Blue" and "100 White" (again of almost equal total energies), both induced a considerable frequency of Type II transition. Here apparently, as in its control of filamentous growth, the activity of blue light is very sensitive to temperature. At \(17.5^\circ\) the blue-photosystem is very efficient and blue alone is sufficient as a stimulus for Type I transition.
It seems therefore, that blue light is necessary for transition but that other wavelengths, possible red or including red, can exert a strong influence over the type of transition. Since total energy is also important, it is likely that once again the nutritional level may also be involved.

Thus a consideration of these three photoresponses, filamentous growth, transition induction and transition type indicates that blue light is active in all of them; that red light can antagonise in one (filamentous growth), be synergistic in another (transition induction), and that certain wavelengths, possibly red are also synergistic in the third (transition type). Nutrition can modify all these responses.

The photoreceptors

The nature of the pigments lies rather aside from the main direction of this thesis, especially since the light sources used, comprised relatively wide wavebands and no attempt was made to establish action spectra for the various aspects of morphogenesis described. The problem has however, been at the centre of several other investigations and a great deal of argument and this present account has led towards the conclusion that morphogenesis involves at least three distinct photosystems.

(1) Photosynthesis. There is no evidence that the mechanism of photosynthesis or the pigments involved (chlorophylls and carotenoids) are directly concerned in the morphogenetic variations described and so this photosystem will not be further discussed here.

(2) Blue-sensitive photoreceptor. Blue-dependent morphogenesis in ferns is a
"high energy" photoreaction which in the past has been classed with the high energy reactions (H.E.R) of higher plants (MOHR 1957; SIEGELMAN and HENDRICKS 1957). The identity of the pigment involved in H.E.R. rests largely on circumstantial evidence and conclusive physiological demonstrations of its existence are difficult because of interactions with the low energy phytochrome system; interactions sometimes synergistic (MOHR 1959; MOHR and Van-NESS 1963) and sometimes antagonistic (MOHR and NOBLE 1960). Nutrition can also modify the interactions between photoreactions in higher plants (MOHR and PINNIG 1962) as well as ferns.

Recently the existence of H.E.R. as a distinct photosystem i.e. with a specific photoreactive pigment, has been questioned and several models have been proposed which interpret these high energy phenomena on the basis of phytochrome which does absorb blue-wavelengths (BERTSCH 1963; WAGNER and MOHR 1966; HARTMAN 1966). In some cases however, the high energy responses appear less compatible with such interpretations (GRILL and VINCE 1966) and this raises the possibility that the photosystems may vary in different species.

The blue-photomorphogenetic system in ferns differs from other, related, phenomena in that the action spectrum of the induction of transition lacks the peak in the far-red region (MOHR 1956b) which is present in higher plants, high energy systems (MOHR 1959; HEATH and VINCE 1962). Recognising this difference, SCHNARRENBERGER and MOHR (1967) have postulated that in ferns there is a specific blue-absorbing pigment, possibly (from the action spectrum) a flavoprotein. YEOH and RAGHAVAN (1967) have also suggested that riboflavin can stimulate the transition to prothallial growth although recently MILLER (1968) has questioned the validity of the methods of analysis used by these authors.
(3) Red-sensitive photoreceptor. The results obtained in this study suggest that other wavelengths (in the region 500-700 nm) in addition to blue, are morphogenetically active. The work of KATO (1967), ETZOLD (1965) and MOHR (1964) implies that red light specifically, has such activity and therefore the longer wavelength system found in this investigation will be referred to as "red-sensitive".

The most obvious candidate for the pigment involved in this system is phytochrome. Phytochrome has already been implicated in the induction of germination (MILLER and MILLER 1963; MOHR 1963; KING AND DYER unpubl.) and, in conjunction with a blue-sensitive pigment, in phototropism (MOHR 1964; ETZOLD 1965; HAUPT 1965). A number of authors have also suggested that elongation of the protonemata is stimulated by phytochrome (MILLER and MILLER 1967; SCHMANNBERGER and MOHR 1967). In addition MILLER and MILLER have claimed (1963) that growth and cell division during prothallial development are under red/far-red control. Thus it appears likely that phytochrome is present and active throughout gametophytic development but no direct evidence of the involvement of this pigment in either the induction, or the type of transition has been put forward.

On the contrary, two aspects of morphogenesis are not in accord with previously documented, red-light phytochrome responses:-

a) Both the induction and type of transition appear to be "high energy" reactions.

b) There is a requirement for continuous (or repeated) stimulation, both for the completion of transition and the maintenance of 2-D growth. Typically, red-light acts via phytochrome as a trigger for some response that may be completed in the absence of the red-light.
One alternative explanation of the induction of transition by red light is that the blue photoreceptor has an absorption "tail" in the red-part of the spectrum. This would explain why red is less efficient than blue light in the induction.

**Interactions between photosystems**

If this last interpretation is correct, then this implies that red light can influence morphogenesis through two distinct photoreactions (apart from photosynthesis). In a similar way ETZOLD's (1965) studies with polarised light have shown that two separate blue-sensitive pigments in addition to phytochrome and chlorophyll, are involved in the orientation of expansion in *DROUSPERIS FILIX-MAS*. Further more MILLER and MILLER (1967) have produced evidence of yet another pigment, sensitive to yellow light (and to a lower extent far-red) which is highly active in promoting elongation. This pigment has an absorption peak at 580 nm. Light of this wavelength is present as a significant component of the white but not the red light used in this investigation. Such a pigment therefore offers an explanation for the difference between white and red-grown filament at 27°C when the blue-system was apparently inactive. The evidence for this pigment was however, obtained in short irradiation experiments and, as investigations into photosystems in other plants have shown (MÄHR 1959; STEINER 1963; BORTHMICK, TOOLE and TOOLE 1964; NEGBI and KÖLLER 1964), the response of a tissue to brief, and prolonged irradiation can be very different.

Nevertheless, the results from these experiments contain hints of complex interactions between light of different wavelengths (MILLER and MILLER 1964a, 1967) as well as differential temperature (MILLER and MILLER 1966) and
age effects (MILLER and WRIGHT 1961). Moreover other, albeit rather isolated, observations have indicated that various other factors such as the osmotic status of the cells (KATO 1964, 1967) and exogenous growth factors such as I.A.A. and gibberellic acid (MILLER 1961; MILLER and MILLER 1965; KATO 1965) can also interfere with the effects of light.

**Interacting pigment systems in other plants**

Examination of the literature on the light responses of plants, suggests that certain helpful parallels may be drawn between the photoresponses of fern gametophytes and interacting pigment systems in a variety of plants, more especially lower ones.

STEINER showed that morphogenesis in the liverwort SPHAEROCARPUS DONNELLI, as in ferns, involved three photosystems (STEINER 1963):-

Phytochrome; a blue-dependent system similar to that in ferns; and photosynthesis. Here also, the quantitative effects of red and blue light depended upon photosynthetic rate. The situation differs from that in ferns in that here red light is responsible for normal development and also, in this case, blue light predominates in low intensity, red in higher intensity white.

One additional source of complexity exists in the liverwort system that has not been demonstrated in ferns: a diurnal fluctuation in the sensitivity of the phytochrome system which results in corresponding changes in the balance between the two photosystems (STEINER 1965).

In CHLORELLA, both red and blue light stimulates cell division (SENGER and SCHUSER 1964) but while the "blue response" is photomorphogenetic and involves a specific pigment, a carotenoid, the "red response" is apparently mediated (non-specifically) through chlorophyll and photosynthesis.
These two examples both involve morphogenesis directly but much of the work done on the pigment systems in lower plants has been concerned with the phototaxis of the plastids. In all the plants studied, the plastids moved towards the light (positive phototaxis) under low intensity stimuli and away from the light (negative phototaxis) under a high energy stimulus but the photoreactions involved, varied slightly from species to species. In SELAGINELLA (MAYER 1965) and VAUCHERIA (FISCHER-ARNOLD 1963) a blue-sensitive pigment, thought to be a flavoprotein, seems to be implicated in both responses. In SELAGINELLA, red light also stimulates the negative response although this is thought to be via a distinct primary reaction. In VAUCHERIA red light can interact synergistically with blue in the induction of positive phototaxis.

In MUGEE-TIA by contrast, (MUGEELE 1962; FISCHER 1963; SCHÖNBOHM 1963) positive phototaxis is stimulated only by red light, while blue and red light affect the negative response although much higher energy is required from red than blue wavelengths and the percentage response is lower in red. The positive response is induced by a short "trigger" irradiation and the pigment involved is probably phytochrome. The "high energy" response on the other hand, requires long or repeated irradiation. Irradiation with blue alternated with red or far-red suggests that here the "phytochrome-status" of the cells (i.e. whether the pigment is present as \textit{P}_{739} or \textit{P}_{660}) can affect its response to blue light.

Consideration of the effects of temperature on the responses of cells or plastids to light indicate that in some cases the photoreactions limit the response while in others chemical reactions are limiting. Thus in SELAGINELLA, (MAYER 1967) a high \textit{Q}_{10} indicates that the positive and negative responses are both limited by chemical not photoreactions. Whilst in VAUCHERIA and MUGEE-TIA...
the negative responses appears to be limited by the light stimulus. In the case of the low energy system in MUGEDTIA (MUGELE 1962), the induction appears to be limited by the red light stimulus but the response by chemical reactions.

These temperature studies serve as reminders therefore, of the danger of equating a (secondary) photoresponse with the primary photoreactions taking place. They demonstrate how closely the photoresponses are bound up with the metabolic activity of the cell and suggest that differences in the relative importance of separate photosystems in different plants may be governed by physiological factors rather than differences in primary photoreactions.

A further reason for differences between photoresponses in different plants, or even separate photoresponses in the same plant or cell, may be slight variations in the pigments involved. For example TAYLOR and BONNER (BONNER 1966) observed that phytochrome from an alga has absorption peaks some 17 nm shorter than classical, higher plant phytochrome. In this way, phytochrome and flavoprotein may refer to a class of pigments rather than a simple, uniform one and, just as a single cell may contain several anthocyanins, chlorophylls or carotenoids, it may contain more than one member of these classes also.

The site of receptor and response

Apical control of development

The observations on the development of "capped" filaments already described, show that filtering off most of the blue light from the extreme apex (50 microns or so) prevents "blue-type" development. This points firmly towards the apical region as the site for the active photoreceptors.
Further evidence that there is apical control of cell division and differentiation is that secondary rhizoids, which form at the time of transition, and both intercalary divisions and the first re-orientated division, which occur in initials show strict positional relationships with the apex (and not the base). This suggests the existence of some type of cytoplasmic or biochemical gradient.

Lateral branching in red and white filaments at high temperature, after filamentous growth at the apex has ceased, suggests that the apical control is related to mitotic activity at the apex. In support of this idea are the findings that factors that inhibit this mitotic activity e.g. chlchicine (NAKAZAWA 1959) upset the polarity of the filament. Placing gametophytes in the dark so that normal apical growth is suppressed, can also result in branching from subterminal cells (NAKAZAWA and OCTAKI 1961; OCTAKI 1965).

It is perhaps significant that the lateral branching observed in the present investigation, occurred in conditions in which the blue-photosystem is believed to be inactive. Possibly, therefore, there is a direct relationship between the apical site of the photoreceptors and apical control of development. If this is so, then the problem remains as to if and how apical control exists in red-grown filaments. In such filaments cell division and expansion is strictly polarised and confined to the extreme apex. This suggests some sort of apical dominance does exist although increasing the metabolic activity by raising the temperature or the red light intensity, seems to break this down to some extent; the pattern of expansion is altered and the filaments branch.
There is circumstantial evidence that extension of the cell walls occurs only in the close vicinity of a potentially mitotic nucleus. In red grown filaments, the nucleus of the apical cell lies close to the tip. The basal outgrowths that initiate the forking of the filaments are always preceded by migration of the nucleus to that side of the cell. This is certainly one aspect of development that especially merits further study.

Wherever the red-sensitive receptors are located along the filament, work with polarised light has led to the conclusion that within the cells, both the red and blue photoreceptors active in phototropism in DROPERUS are dichroic molecules located close or within the cell wall (ETZOLD 1965; HARRIMAN, MENZEL and MOHR 1965) with the axis of maximum absorption parallel to the cell surface (Jaffe and ETZOLD 1962).

The phototropic and polarotropic responses referred to above, are carried out at the point of maximum light absorption i.e. the site of receptor and response are coincident. In other photoreactions this is not so. For example, comparison of normal and "capped" filaments indicate that, in the presence of blue-light at the apex a re-orientated division occurs in cell P_{iii}. It seems indeed, that this cell is in some way more sensitive to the blue light stimulus than other cells for while some responses such as the formation of R_{2} and cell slope do occur in more basal cells, it is in P_{iii} that the most critical morphogenetic changes are initiated.

This remoteness of receptor from response implies that the stimulus can be transmitted in some way. One possible pathway for intercellular transmission is the plasmodesmata and indeed, breaking these by plasmolysis can induce "suppressed" cells to divide and form branches (DYER un-publ).
The very definite polarity of the filaments suggests that this transmission involves the setting up of some kind of basipetal gradient although a simple gradient would not explain the changes in polarity that occur during transition. Furthermore other evidence suggests that the response of the filamentous cells to a light stimulus depends not only on their position in the filament but also on their history. When gametophytes are transferred from red to blue, rhizoids are initiated in red-formed cells and transition only occurs after several division cycles i.e. when \( p_{iii} \) is a blue-formed cell.

From such evidence it seems that having now analysed the growth and development of the filaments and concluded that morphogenesis is controlled by three interacting photosystems, further elucidation of the primary mechanisms of the various photoresponses requires a detailed cytochemical analysis of changes occurring in specific cells.

The Gametophyte as a system for investigating cell polarity

The work described in this thesis has surveyed and documented in some detail, the variations in rate, orientation and distribution of cell division and expansion during early gametophyte development, induced by environmental variations. It has established the specific conditions that result in each of these developmental variants and has thus laid the foundations necessary for further studies of the control of cell polarity and other aspects of cellular growth and differentiation. The experiments have confirmed the great potential of fern gametophytes for use in such studies and in addition to the very considerable assets already ascribed to this system, it has now been established that its natural polarity can indeed be modified in similar ways by several distinct environmental factors; light quality, light quantity and temperature.
Quite aside from its value as groundwork on which to base further physiological and cytological studies, this investigation has already yielded a good deal of detailed and specific information about the pattern of early gametophyte development in DRYOPTERIS BORRERI.
1. **Table** of mean cell number values for analysis of variance within and between culture dishes.

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<th>100 lm/sq.ft</th>
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<td>Sample</td>
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</tr>
<tr>
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<tr>
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<td>H 1.02 1.02 1.02 1.02 1.03</td>
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<td></td>
<td><strong>DAY 5</strong></td>
<td><strong>DAY 6</strong></td>
</tr>
<tr>
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<tr>
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<td>G 1.95 1.92 2.02 1.94 1.96</td>
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<td>1.98 1.95 1.96 1.97 1.97</td>
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## DAY 9

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<td>H - 3.76 3.78 3.67 3.74</td>
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</tr>
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</table>

### ANALYSIS OF VARIANCE

Variation greatest at the end of the experiment therefore the analysis was done for the last day of sampling (day 9).

1. Four dishes and the four samples from each dish taken separately:
   - Grand total = 77.14
   - number of samples = 16
   - correction factor = 371.91122
   - total sum of squares = 372.0978 - 371.91122
     = 0.18658
   - "between dishes" sum of squares
     = \( \frac{1}{4} (19.15^2 + 19.45^2 + 19.70^2 + 18.86^2) - 371.91122 \)
     = 372.0978 - 371.91122
     = 0.09798
"within dishes" sum of squares

= "total" - "between"
= 0.18658 - 0.09798
= 0.0886

variance "between" = 0.03266
= 0.007384
\[ F = \frac{0.03266}{0.007384} = 4.42 \]
d.f between = 3

d.f within = 15 - 3 = 12

i.e. variance is significant at the 5% but not at the 1% level.

2. If 1 sample from each of 2 dishes are taken together then this variance is lowered

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<th>C</th>
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</tbody>
</table>

-- correction factor and total sum of squares as before

"between blocks" sum of squares

= \( \frac{1}{2} (9.88^2 + 9.52^2 + 9.64^2 + 9.59^2 + 9.8^2 + 9.59^2 + 9.58^2) - 37191122 \)
= 371.96930 - 371.91122
= 0.05808
"with blocks" = 0.12891

variance "between" = 0.01936
"within" = 0.010709

F = 1.808

This is well below the 5% level

9.0. differences are not significant
Fig. 29. Microdensitometer measurements (with standard deviations) of the relative absorbance of light of different wavelengths by the spore walls.
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