THE UNIVERSITY OF MANITOBA

THE EFFECT OF POLARIZED LIGHT ON GERMINATION, WITH SPECIAL REFERENCE TO THE CONIDIA OF BOTRYTIS CINEREA

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ABSTRACT

A study was made of the effects of polarized blue light on the germination of conidia of *Botrytis cinerea*. It was confirmed that the polarization axis of the light orients the direction of germination. It was found that spores do not germinate at a faster rate in the light than they do in the dark. The light appeared to be effective in directing germination only in the time immediately prior to the germination.

Electron microscope studies of *Botrytis* conidia at various times after wetting indicated that there exist more highly organized membranes in conidia which have been fixed shortly after wetting than those which have been fixed a few hours after wetting.

A model of the physical aspects of germination is developed, the general features of which can be used to explain many phenomena which have not previously been satisfactorily explained.

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INTRODUCTION

INTRODUCTION

The many effects of light upon living organisms have interested physiologists for a long time. That the reactions are complex is illustrated by the volume and calibre of attention that has been directed towards solving the problems of photosynthesis and vision.

In these cases the response bears a distinct relationship to the intensity, and is associated with light absorbing compounds and complex cytological fine structure, so that possible mechanisms may be envisaged, but no such structures have been reported in those transparent spores that are known to be sensitive to the plane of polarization of light. Here, possible mechanisms are not obvious and it was felt that the responses and structure of the conidia of *Botrytis cinerea* would be worth further study in the hope of elucidating the mechanism involved.

LITERATURE REVIEW

LITERATURE REVIEW

PHOTOBIOLOGY - General References

Much work has been done on the interaction between light and biological systems, and some good general reviews of photobiology have been written. The basic format of most of these reviews is similar, with the main differences being the areas which are emphasized. The reviews begin with an explanation of the physical and chemical processes involved in photobiology, i.e. the nature of excitation of molecular systems and energy conversion within them. They also set forth the chemical aspects of reaction mechanisms and rate determining factors, and then describe how these processes affect those photosensitive biological systems which have been extensively studied. These systems include photosynthesis, vision, phototaxis, phototropism, photomorphogenisis and photoperiodism, as well as biolumine sence.

One of the better reviews in this category is "Primary Photoprocesses in Biology" (1965) by Thomas. The book is divided into two parts. The first part, deals with the physical, chemical, and biological processes. The second part, discusses those biological systems mentioned above by examining the pigments involved, the photoreceptor structures, and the nature of the process, as far as is known; except,

of course, in the case of biolumineseence, where it discusses the emitting system of luminesent organisms.

A collection of articles contributed by many authors and edited by Geise was published in two volumes entitled "Photophysiology." The emphasis in these volumes, as the title suggests, is a description of the photosensitive biological systems mentioned earlier, as well as the mutagenic and other effects of ultraviolet radiation.

In 1961 McElroy and Glass edited a book containing the papers and informal discussions of a symposium on "Light and Life" held at John Hopkins University under the sponsorship of the McCallum-Pratt Institute. The emphasis at this symposium was on the physical and chemical aspects of photobiology, especially with respect to photosynthesis, although there were papers dealing with other photosensitive biological systems. The book concludes with a lucid summary of the symposium by Glass.

The book by Seigler and McElroy entitled "Light! Physical and Biological Action" (1965) emphasizes the practical techniques which are used in the study of interactions of light and biological systems.

PHOTOTROPISM

Tropisms are defined by Geise (1962a) as growth movements or growth curvatures in response to a stimulus. Photo-

tropism, therefore, can be defined as growth movement or curvature in response to light. Jaffe and Etzold (1965) expand on this definition and define cellular phototropism as the localization and hence orientation of cell expansion by light. They add that "in some cases light redistributes expansion between two sides of an elongating structure while in others...it determines the site of initiation of a new outgrowth."

The study of phototropism was initiated by Darwin in 1881 when he demonstrated that for certain dark grown grass coleoptiles, the site of sensitivity for phototropic induction was the coleoptile apex. Briggs has reviewed the work that has been done on higher plant phototropism in Photophysiology Vol. 1 (1962).

A second area where major work has been done on phototropism is in the study of light responses of the sporangiophore of the Phycomyces. This work has been reviewed by Thimann and Curry (1960), Shropshire (1963), Dennison (1965), and Castle (1966). Zankle, Burke, and Delbruck (1967), discuss the location of photoreceptors in the Phycomyces sporangiophore.

The mature sporangiophore is essentially a cylinder (with a spherical sporangium on top) which elongates vertically under uniform light conditions at a constant rate and exhibits two seemingly independent responses to light. Fir-

stly, if there is a point source of blue light, it bends towards the point source; and to a change in the angular position of the light, it bends in such a way as to reaim itself towards the new position of the light source. However, if the light is in the ultra-violet range it will bend away from, instead of towards the light source. Secondly, if there is a change in the intensity of the light upon it, the sporangiophore reacts by accelerating its rate of growth temporarily. If while exhibiting positive phototropism in the presence of an asymetrical light distribution there is a large change in the intensity of the light, it reacts with a temporary inversion to negative phototropism and then back to positive phototropism.

Another complicating effect is the interaction of the phototropic response and the tropic response to gravity as referred to by Varju, Edgar, and Delbruck (1961) and by Dennison (1961). Also, Dennison and Roth (1967) discovered a tropic response in the Phycomycetes to a mechanical force. This response also interacts with that of the phototropic and geotropic responses.

In spite of the work done on this system there is still no general agreement as to the solution of the three problems involved in any such study which are: the location and orientation of the photoreceptor, the identification of the photoreceptor pigments, or the way in which the photoreceptors af-

fect the growth of the sporangiophore (Zankle et al, 1967).

A third area in which work has been done on phototropism is the directional germination of germlings from the spores of algae, ferns, moss, fungus, and horsetails in response to asymetrical illumination. The germ tubes are found to emerge from the darkest or lightest part of the spore. Those germinating from the darkest part of the spore are the algae Fucus, the fern Osmunda, and the moss Funaria (when the light is of high intensity), and the horsetail Equisitum. Those germinating from the brightest part include the fungus Botrytis cinerea and the moss Funaria when the light source is of low or medium intensity, (Jaffe and Etzold, 1965).

POLAROTROPISM

Another aspect of phototropism discovered by Jaffe in 1956 in the egg of the brown algae Fucus and soon found to be general, is that of polarotropism. Polarotropism, as the name implies, can be defined as a tropic response to unilaterally applied plane polarized light. This has been studied mainly in single cell systems and especially in germination of spores, although work has been done in other areas.

Polarotropism has been found, and probably only looked for, in organisms which respond phototropically to asymetrical illumination. The effect was observed in the study of the phycomycetes, where it was found that horizontally polarized

light was from 10% to 15% more effective in illiciting the phototropic response than vertically polarized light (Shropshire 1963). Castle interpreted this as being due to differences in Fresnel reflection losses from the front surface of the cell for the two directions of polarization. Jaffe, using results obtained from germinating Fucus zygotes in 1958, reopened the question of differential sensitivity to plane polarized light and suggested that photoreceptors of the phycomycetes were stress oriented and highly dichroic and located near the cell wall. In 1960, he published a paper on "The Effects of Polarized Light on the Growth of a Transparent Cell" in which he used Shropshire's data published in 1959, which measured the polarization dependence of the tropic response in air and extended the measurements to include the growth response in air while immersed in a flurochemical which greatly reduced the surface reflection to nearly the same value for both directions of polarization. Jaffe's paper shows that the effect cannot be accounted for by Castle's explanation of differential reflections and that oriented dichroic photoreceptors would yield such a result.

PHOTOTROPIC AND POLAROTROPIC SYSTEMS

In 1962, Jaffe and Etzold published a paper entitled "Orientation and Locus of Tropic Photoreceptor Molecules in Spores of Botrytis and Osmunda." Three experiments were per-

formed on <u>Botrytis.</u> One experiment illuminated the spores vertically with plane polarized light, another partially illuminated the spores with unpolarized light and the last, illuminated spores horizontally with unpolarized and horizontally polarized light.

Partial illumination of the spores was obtained by immobilizing the spores on a substrate of fused quartz striped with 40 μ wide bands of chromium laid down at 40 μ intervals and then illuminating them vertically from below. Those spores which were lying upon the bands' edges were so shadowed as to be subject to almost 100% differences in light intensity.

The outgrowths were projected into the horizontal plane with the aid of a Wild goniometer eyepiece, modified by replacing the rotatable pointer with a series of fine parallel lines. Angles were recorded in 10 degree intervals. The directions of the most basal parts of their germ tubes were measured. In all cases only spores lying more than one long spore axis from any other spore were counted. If, at the time of counting a spore had more than one germ tube (occasionally there are two), the longer one was used for the measurement.

To characterize the degree of bipolar alignment the following parameter was calculated

$$V_2 = \Sigma \ P \ \mathrm{Cos}\ 2\theta$$

where p is the percent of all outgrowths lying at an angle θ to a reference axis (either the vibration axis of the polar-

ized light or the axis of the spores outline).

As a preliminary study a spectral survey was taken and it was found that *Botrytis* reacted to the range of wave-lengths which were isolated with a Wratten 45A filter which peaked at 470 $m\mu$ and fell to one half maximum intensity at 445 and 515 $m\mu$ and 1% maximum intensity at 415 and 550 $m\mu$.

The average refractive index of the wall of a living *Botrytis* spore was found to be $1.47 \pm .002$ at 470 $m\mu$ and the average refractive index of the cytoplasm was determined at $1.3 \pm .005$ at 470 $m\mu$. The thickness of the wall of a living, swollen, but as yet ungerminated spore was measured at $.55 \mp 0.1 \mu$.

It was found that when illuminated from above unilaterally with plane polarized light, *Botrytis* spores germinate parallel to the plane of polarization. The degree of the response depends upon the light intensity. As mentioned earlier *Botrytis* spores germinate from their brightest part when subjected to asymetrical unpolarized illumination.

Comparisons were made at high intensities between the polarotropic responses and a whole series of relative differences of partial illumination. It was found that only differences of about 100% orient growth as completely as does polarized light. Depolarization by the cell wall was neglected since inspection showed that the birefringence of the

cell wall was feeble.

Jaffe and Etzold consider and exclude three possible explanations of the polarotropic response. Firstly, the possibility of a dichroic shell around the photoreceptor is excluded since empirical observations with a polarizing microscope do not reveal the types of patterns one would expect if these dichroic shells existed. Secondly, differential scattering can be excluded since scattering is found to be negligible, and thirdly, they neglected the effect of internal reflection by virtue of the argument presented in Jaffe's paper on the "Effect of Polarized Light on the Growth of a Transparent Cell," (1960).

The conclusion arrived at is that the polarotropic response is due to highly dichroic oriented photoreceptors. Since Botrytis grows from the brightest region, when partially illuminated with unpolarized light, these molecules must be oriented anticlinally or radially, i.e. the axis of maximum absorption is perpendicular to the cell wall. Thus the model explains that Botrytis spores tend to germinate in the region where the photoreceptors absorb the most light.

Using horizontally directed unpolarized and horizontally polarized light, Jaffe and Etzold get further evidence for their radially oriented dichroic photoreceptor model. Since the polarization vector of a photon is perpendicular

to the axis of propagation of the photon, one would expect maximum absorption at an angle of 90° to the direction of propogation, assuming that the model is correct. However, it was observed that the angular distribution of germ tubes peaked at about 10° from this direction towards the opposite side of the spore which was being illuminated. This indicates that the photoreceptor molecules lie at about 90 ± 5% of one radius of a spore from the center. This corresponds to the inner half of the spore wall or the interface of the cell wall and the cytoplasm.

Jaffe and Etzold found two results which were unexpected.

1) In some groups of spores the polarotropic response at low intensities was much less than the effect of partial illumination. In other groups there was no observed difference. This result was attributed to the tendency of some spores to germinate perpendicular to the axis of their own outlines, whereas most spores do not.

2) When Botrytis spores were illuminated horizontally with unpolarized light of high intensity, there appeared a distinct tendency to germinate away from the light source. The authors state that they believe this effect is not caused by a change in the photoreceptor system, but in a change in the subsequent mechanism.

In 1965 Jaffe and Etzold published a paper entitled

"Tropic Responses of Funaria Spores to Red Light." One noteworthy aspect of this paper is that it is the first cellular phototropic response to red light to be extensively studied.

In this paper they examine responses of spores of Funaria hygrometrica to continuous illumination with red light (610 - 690 mμ) over the intensity range from 10^{-5} to 10^{6} ergs per cm^{2} per second using both plane polarized light and partial illumination. They infer from the relative frequency of outgrowth origins in different directions the following conclusions:

1) The germination direction of chloremal filiaments is directly influenced by red light over the whole intensity range studied, while that of the rhizoids tends to be the opposite of that of the chloremas. The argument used to support this is that the degree of orientation of the chloremas is greater than that of the rhizoids and that chloremas and rhizoids tend to germinate from opposite ends of the spore under any conditions. They also found that the polarotropic responses of the chloremal filaments were similar to those directing their origins.

2) Three photoreceptor systems, whose effects overlap to some extent direct chloremal primordia; (i) a low intensity system favoring growth from a cell's illuminated end. Its photoreceptors are disoriented, excited by the electric vector

and are probably disoriented phytochrome molecules. (ii) A medium intensity system also favoring growth from the cells illuminated end. Its photoreceptor molecules are also electrically excited but are tangentially oriented. (iii) A high intensity system which favors growth of the chloremas from a cells darkest part. In the abstract Jaffe and Etzold state that the receptors are probably tangentially oriented and magnetically excited. In the paper, however, they state that an authority on excitation informed them that the degree of symmetry in the vicinity of the photoreceptor molecules would have to be much higher than what would be expected in vivo for the magnetic vector to be dominant in the excitation. In fact, Jaffe himself wrote a paper entitled "Evidence that the Electric Vector Governs Light Absorption in Vision, Phototropism and Phototaxis"(1962) in which he discounts the possibility that the magnetic vector could play a role in excitation in these systems. They state that they are, therefore, driven to consider a more complex interpretation of the high intensity results. The system they end up with has radially oriented, electrically excited dichroic photoreceptors which lie within flat highly absorbent organelles which are tangentially oriented and located within the periferal cytoplasm. At the end of their paper they review the phototropic and polarotropic systems of spores and zygotes and classify them in Table I.

It has also been shown that the rate of germination of *Funaria* spores is greatly affected by the intensity of monochromatic light of a wide range of wavelengths, (Krupa 1967). In this paper it was reported that the short-wave light range (362 - 500 mμ) induces marked germination only in high intensities. Long wave radiation (500 - 700 mμ), however, promotes germination at lower intensities. This paper indicates the process of germination of *Funaria* to be highly complex and involving such systems as phytochrome and photosynthesis.

OTHER TROPISMS

Botrytis spores also exhibit other tropic responses besides phototropic ones. Muller and Jaffe (1965) showed that *Botrytis* spores also exhibit rheotropism. Rheotropism can be defined as the tropic response to flowing water. They present arguments to show that this response is mediated by the convection across each cell of a diffusible stimulator emitted by the cell. They state that this substance is a macromolecule with a half life of about ten seconds and in a stationary medium has a radius of action of about one spore diameter. They also note that there is a much lower sensitivity of the rate of germination than of the localization of growth, to this stimulator concentration although the rate of germination falls very slowly with increasing flow rates.

Autotropism of spores indicates the tropic effects that the presence of one spore can have on another spore. In 1966, Jaffe showed that if two Botrytis spores were located within 10 of each other they would affect each others direction of germination, (Jaffe, 1966a). If they were sown on a medium which was equilibrated with air they showed a strong tendency to germinate towards each other on the same side of a line joining their centers. If, however, the medium was equilibrated with an atmosphere containing a high CO_2 content they tended to germinate away from each other. In both cases their mutual orientation was strongly dependent on the distance separating the spores and whether or not the spores were in contact.

A relevant paper was published by Jaffe in 1966b. In this paper he shows that electrical fields are set up within a normally developing Fucus egg as it begins to germinate, which points in the direction of germination. He further states that this potential is sufficient to significantly concentrate some relatively large and negatively charged particles at the expansion pole, and it also may produce substantial ion gradients throughout the cells. To illustrate the significance of this he refers to evidence which shows that ion gradients may be the immediate control of gene activity. He also states the Fucus eggs subjected to electrical gradients tend to germinate to the high K end.

MATERIALS AND METHODS

MATERIALS AND METHODS

The strain of *Botrytis cinerea* was obtained from the stock culture collection of the Botany Department at the University of Manitoba. The stock culture was stored on potato carrot agar medium which has the following composition:

Potato Carrot Agar Medium

Potato	20 g.
Carrot	20 g.
Agar	15 g.
H_2O	1000 ml.

The fungus was transferred from this medium and maintained by serial culture on sucrose peptone agar medium at 20°C in glass petri dishes.

Sucrose Peptone Agar Medium

Sucrose	10 g.
Peptone	5 g.
KH_2PO_4	2 g.
$MgSO_4$	.5 g.
Agar	15 g.
H_2O	1000 g.

Occasionally, to revitalize the culture, it was transferred to an ill defined medium consisting of sucrose, soil,

potato, dextrose, agar and water.

In the germination studies a Sykes-Moore tissue culture chamber was used. This consists of two round glass cover slips separated by a silicone rubber o-ring and clamped together in a metal case with holes in it to allow for injections through the rubber ring to the interior of the chamber. In studies presented one of the cover slips was replaced by a round piece of polaroid type H film.

A glass cover slip was placed into the metal case and a rubber ring was placed over it and clamped into position. Four or five drops of liquified sucrose peptone agar were deposited with a Pasteur pipete on the slip, and allowed to harden, forming a thin sheet on the surface of the glass slip. The spores were washed from the surface of a mature culture in approximately 15 ml. of water and then this suspension was agitated and filtered through two layers of cheesecloth. Twenty 1 of this suspension were then deposited over the agar surface with a micropipette. The culture chamber was then reopened and polaroid sheet, previously cut to the appropriate size, was placed over the o-ring and the chamber was clamped shut. A hollow needle was inserted through the rubber ring into the chamber to allow an equalization of pressure inside and outside the chamber, and then the needle was removed. The chamber was then placed under the light source or into darkness.

All studies were run with a complete control, i.e. two chambers were always prepared together and whenever one was illuminated the other was in darkness. In order to keep their temperatures the same at all times they were each placed in water baths which were supplied from the same source.

The primary requirement which the light source had to satisfy, was that it would illicit a significant polarotropic response. The optical components of the light source used were arranged in the following order: (i) a variable intensity Cooke, 48W projection lamp, (ii) two infrared absorbing glass filters to remove heat, (iii) a collection of blue glass filters for wavelength control, (iv) 9 cm. of water, (v) a sheet of type H polaroid film and (vi) the spores.

The spectral transmission curve of the filters and the polaroid sheet together was obtained with a Unicam S.P.800 spectrophotometer and is shown in fig. 1. The spectrum obtained when these filters and the polaroid sheet were illuminated with the projection lamp set in the intensity used in the studies were measured with a Zeiss PM Q II spectrophotometer in conjunction with a Zeiss M 4 Q II monochromatometer is given in figure 2.

The intensity of the light reaching the spores was measured at approx. 100±50 watts/CM^2/sec. with an Eppley model D thermopile in an air case without the quartz window.

The voltage given off by the thermopile was measured with a Phillips CM60-20 millivoltmeter and the conversion to intensity was made according to the Eppley manual.

At the completion of an experiment, before measurements were made, the spores were killed by injecting acrolein vapour into both chambers after which no further growth or germination was observed.

In order to measure orientation or to count numbers of germ tubes, the chamber was placed under a Swift polarizing microscope which was tilted horizontally and fitted with a 30 X objective and a 15 X eyepiece. A strong beam of light which had passed through a heat absorbing filter projected the enlarged image through a 45° mirror onto a translucent horizontal screen. The analyser of the microscope was used to align the cross hair of the built-in goniometer of the microscope along and perpendicular to the polarization axis of the polaroid sheet in the chamber. The angles between the basal part of the germ tubes and the polarization axis of the polaroid sheet, i.e. the direction of the electric vector, of the light impinging on the spores was then measured in 10° intervals with a protractor and reduced to first quadrant equivalent angles. This was done because any meaningful parameter measuring a bipolar response must, in effect, reduce any angle to its first quadrant equivalent. As in Jaffe and Etzold (1962) the bipolar statistic which

was calculated to measure the orientation of outgrowths due to the polarotropic response was $V_2 = \sum P(\theta)\ \mathrm{Cos}\ 2\theta$ where is the angle between the direction of germination and the polarization axis of the polaroid sheet. Since angles were measured in 10° intervals θ was taken as the midpoint of each 10° interval, i.e. $\theta = 5^{o}, 15^{o}, 25^{o}...$ $P(\theta)$ is the percentage of outgrowths in the corresponding interval.

An important aspect of this statistic is that for a uniform distribution $V_2 = 0$. (Jaffe 1958) i.e. V_2 is significantly different from 0 only if we have a nonuniform distribution. We can, therefore, determine whether our results indicate a significant response by performing a X^2 test on uniformity.

$$X^2 calc = \sum \frac{(Oi - Ei)^2}{Ei}$$

where Oi is the observed result in the i'th class and Ei is the result expected in the i'th class for a uniform distribution. If some observed expected values are less than 5 then adjacent classes must be pooled in order to get meaningful results. If X^2calc. > X^2crit. then the distribution is nonuniform and hence indicates a significant response. $X^2crit = X^2{.95, n-1}$ where n is the number of classes.

Also as in Jaffe and Etzold (1962) the only spores counted were those which were lying a distance of at least

one long spore axis from any other spore. In the cases where the spore had emitted two germ tubes the longer one was used for measurement. Measurements were made on a random sample of 100 spores from each chamber.

In a preliminary study to establish how well the experimental set up would induce and measure the polarotropic responses, two chambers were prepared in the manner described above and one was placed under the light source and the other in the dark for eight hours. The spores were then killed and the orientation of the germ tubes was measured for a sample of 100 spores as described above. As a statistical check the experiment was then repeated.

Having a light source which illicited a definite polarotropic response it appeared desirable to determine whether spores subjected to this light source germinate more quickly than they would in the dark. Four experiments were performed which were identical to the preliminary study except that the spores were killed 3, $3\frac{1}{4}$, 5 and 6 hours after being wetted, and the number of spores which had germinated in a random sample of 100 were counted. For those spores which had germinated, the angle θ between the direction of germination and the polarization axis of the polaroid sheet in the chamber was measured.

It was found that the time of germination of spores varies greatly according to the age and possibly the environ-

ment of the parent culture. Therefore, in the analysis of this experiment comparisons were made only between the spores in the two chambers which had been washed from the same parent culture and little significance was placed upon the actual time when the spores were killed as compared to the number which had germinated at that time.

Another result, which it was thought might be helpful in elucidating the role of the photoreceptors in the process of germination, was to determine the time period after wetting that the light is effective. The program used to give some information about this question was similar to the preliminary study except that the chambers were shuttled between the light and the dark for different time periods. The times that the chambers spent in the light and in the dark are given in their proper time sequence in Table II.

The orientation of germ tubes for samples of 100 germinated spores were recorded. It should be noted that in both the preliminary study and this one over 90% of the spores had germinated before they were killed and only germinated spores were considered in the results.

It was also felt important to examine the spores under the electron microscope in order to see if any structures could be found on the inner half of the spore wall or around the boundary of the cell wall and the cytoplasm which might

be photoreceptors. It was also thought that it would prove interesting to see if there were any morphological changes inside the spore which might help to explain, or at least give some indication of what was occuring to promote the emission of a germ tube in any particular area. The work of Hawker and Hendry (1962) on the ultrastructure of ungerminated and germinated spores of Botrytis cinerea did not appear to provide any solutions to the above problems. However, it was felt that possibly with a different fixation and staining method and a more extensive study, some information could be gained by examining the ultrastructure of the spore and the following study was initiated.

The conidia from a mature culture were washed from the surface of the culture in about 15 ml of distilled water. This suspension was then filtered through cheesecloth to remove debris and the resulting spore suspension was centifuged, using a small table centrifuge and the supernatant was discarded. Some plain agar medium (P.A.) made up of 15 g. of agar in 1000 ml of water, which was kept just warm enough to prevent it from solidifying, was then poured into the tube containing the spores and the tube was placed into the centifuge. The agar solidified after a few minutes and the agar cylinder containing the spores concentrated in the bottom area was removed from the tube. An identical procedure, except that S.P.A. replaced the P.A., was carried out simultan-

eously.

The study was carried out in S.P.A. and P.A. because spores from a mature culture germinate if sown upon S.P.A. whereas they do not germinate if sown upon P.A. However, if nutrients are added to the P.A. at a later time germination will take place. It, therefore, appeared that the spores embedded in P.A. would develop to a certain point and remain there until nutrient was made available for development to proceed. It was felt it might prove informative to determine at what point external nutrient was necessary for development to proceed to germination.

In order to prepare the blocks for fixation and staining a hollow needle attached to a syringe was inserted into a part at the agar where the spores were concentrated. The syringe was then used to eject the tiny clinder of agar onto a flat surface where short lengths of the cylinder were cut off.

The blocks were fixed in acrolein and gluteraldehyde and then in osmium tetroxide and stained with uranyl acetate, with a method developed by Hess (1966). The technique is given as follows:

1) Fix in 3% acrolein and 3% gluteraldehyde for 1½ hours at room temperature. This is made up as follows:

   0.3 ml acrolein

   1.2 ml 25% gluteraldehyde

3.5 ml dist. H_2O

5.0 ml o.2M cacodylate buffer

The Cacodylate buffer is

A .2M Na Cacodylate

B .2M HCl

Mix 50 ml A + 2.7 ml B and dilute to 200 ml, with distilled water.

2) Wash 4 times in buffer mixed (1:1) with H_2O for 15 min.
3) Fix in 1% O_SO_4 [2% O_SO_4 (1:1) with buffer] for two hours in an ice bath.
4) Rinse 4 times in buffer (1:1) with H_2O for 15 min.
5) .5% aquacous uranyl acetate overnight at room temp.
6) Wash in distilled H_2O four times for 15 minutes.
7) Dehydrate in steps with ethyl alcohol.

ETOH 50%, 70%, 90% for 15 minutes each.

ETOH 100% twice for thirty minutes.

X-linked methacrylate was then prepared as in the method developed by Kushida (1961).

X-linked Methacrylate

1 - Methyl Methacrylate

6 - Butyl Methacrylate

.8% Benzoyl Peroxide

5% Divinyl Benzine

The dehydrated blocks were then washed in X-linked methacrylate four times for ½ hour and then placed in gela-

tin capsules which were filled with X-linked methacrylate and kept overnight at 50°C in a vacuum of 15 in. of Hg.

The blocks were sectioned on a L.K.B. Ultratone using glass knives made on a L.K.B. knifemaker. The sections were laid on 150 mesh copper grids covered by a carbon film. Some of the sections were stained with lead citrate in a variation of a procedure developed by Reynolds (1963). A grid with sections was inverted over a drop of .2N NaOH for 10 seconds and placed on three drops of water for two minutes each. The grid was then placed on a drop of lead citrate, face down, for 7 minutes, washed on 10 drops of water for one minute on each drop and then placed face up on a piece of filter paper and allowed to dry.

Since it was desired to see if any morphological change was taking place after wetting, but prior to germination, blocks of spores were fixed at intervals of ½, 1, 2, 4, and 6 hours after being introduced to the agar medium. The same program was initiated for both spores in P.A. and in S.P.A.

After the sections were prepared they were viewed and photographed with an AEI EM6B electron microscope. A Phillips EM75 electron microscope was used in preliminary work and for scanning.

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

The results of Experiment 1 are given in Table III and Table IV. By calculating the X^2 statistic at the .95 level of significance it is found that in both studies the distribution of angular frequencies is non-uniform in the light and uniform in the dark, i.e. in the light $V_2 \neq 0$; in the dark $V_2 = 0$. Therefore, the light source being used in the experiments, illicits the polarotropic response to a degree which is statistically significant.

The results of Experiment II are given in Tables V, VI, VII, and VIII. In each of these studies a test was run to see if there was any difference at the 95% confidence level between the number of spores out of 100 which germinated in the light and the number of spores out of 100 which germinated in the dark after a given time from wetting. The test statistic calculated was

$$Z = \frac{\left| P_L - P_D \right|}{\sqrt{\hat{P}\hat{Q}\left(\frac{1}{100} + \frac{1}{100}\right)}}$$

where P_L is the % which germinated in the light and P_D is the % which germinated in the dark, in a given study. $\hat{P} = P_L + P_D$ in this case because the sample sizes were the same, and $\hat{Q} = \hat{1} - \hat{P}$.

The test to determine if P_L and P_D differ significantly from each other at the .95 level of significance is to compare the calculated value of Z against 1.96. If $Z > 1.96$ there is a significant difference, if $Z < 1.96$ then the data is consistent with the hypothesis that $P_L = P_D$. This data is illustrated graphically in Fig. III. The rectangles about the plotted points have the dimension of the confidence interval about the points. Basically what it means is that if a point on the 45° degree line is inside the rectangle then there is no significant difference between P_L and P_D in that study.

X^2 tests were calculated for the distribution of angular freouencies for those spores in each group which germinated, except in expt. II (i) where the sample size was too small to yield any meaningful information. In the other three studies a significant response was illicited in those groups which were subject to the light source; while, as expected, no response was found for those spores which germinated in the dark.

The results for experiment III are given in tables IX, X, XI, XII, and XIII. These results indicate that in the initial period after wetting (about 2 hours) the direction of polarization of light does not affect the direction of germination. It appears that the light becomes effective, however, following this time but prior to the emergence

of a germ tube.

In figs. 4 through 18 are sections from each of the 10 groups of spores prepared for study with the electron microscope. The ones which are stained with lead citrate are figs. 7 - 10 and 15 - 18. In some cases (e.g. fig. 15), this stain greatly enhanced the contrast especially of membranes, but in most cases the overstain had little effect (e.g. fig. 10). Unfortunately the study did not yield any germinated spores even though the spores in the nutrient agar did eventually germinate as did those in plain agar after the addition of some nutrients.

The results of this study were essentially negative in spite of the fact that the fixation and staining methods appeared to be quite adequate. No structures were found inside the spores which would be interpreted as photoreceptive structures. Also no morphological changes took place inside the spores which would indicate that germination was to take place in a given area. In fact the only apparent morphological change was that in early killed spores, organized membranes were often found (e.g. figs. 5, 12) whereas in those killed at a later stage this degree of organization was not observed.

DISCUSSION

DISCUSSION

As shown in the literature review, the phototropic response of Botrytis cinerea related to blue light is that germination takes place in the region of the spore where maximum absorption of the light occurs. That partial illumination and polarized light effect the same result is explained through the existence of dichroic photoreceptors which are oriented radially with respect to the surface of the spore and located in the region of the interface of the cell wall and the cytoplasm.

Let us now consider other aspects of the interaction of light and germination of Botrytis conidia. It is important to note that germination takes place in the absence of light. Therefore, the germination process is not dependent on external light stimuli. In fact, as is shown in this study the rate of germination of conidia subjected to a light source is not higher than the rate of germination of conidia in the darkness. We can infer from these results that the light is not absorbed by any system which is directly involved with the process of germination, i.e. if there was no light the system which absorbs the light would play no part in the germination process. Let us consider then the following models.

1. The Weak-Spot Model

Let us assume that germination in Botrytis takes place in a spherically symmetrical manner and that any region of the cell wall has the potential to initiate germination. Since, according to Jaffe, we have an oriented set of photoreceptors lying in the region of the cell wall, let us assume that excitation of this receptor causes some deformation of the cell wall in that area, which enhances the probability of germination in that region. The observed result would be that germ tubes tend to emerge from that area of the spore wall which absorbs the most light. Once the germ tubes'direction is determined germination from other areas is then repressed, though occasionally two germ tubes are emitted. A possible mechanism for this might be the build up of interval pressure which eventually forces the emission of a germ tube though the "weak spot" which may pass through light damage of the wall in an area. This weak spot must not be permanent since we found that light does not affect germination direction in the first period of time after wetting, i.e. the "weak spot" occurs on excitation of the photoreceptor but on decay of the molecule it reverts to its natural form.

Let us consider this model in view of certain known results. The probability of a dichroic molecule being excited by a photon will be a function of $\cos^2\theta$ where θ is

the angle between the polarization axis of the photon and the optical axis of molecule. In view of this it is at least difficult to accept, with this model of independently acting photoreceptor, that a degree of orientation near what Jaffe found would be possible, even if we assume a great number of receptor molecules and a very short excitation half life.

In Jaffe and Etzold's paper they described an effect which they term "centering" which they claim "arises not from a change in the photoreceptors but a shift in the mechanism subsequent to light absorption." Thus their model does not in any way explain this effect.

2. Self-generated Electric Field Model for Germination

In order to build a model for germination let us consider the results of Jaffe's work (1966) which showed that electrical fields are set up within the zygotes of Fucus which point in the direction of germination. Jaffe concludes in this paper that the electric field which is found has a sufficient potential gradient across the cytoplasm to concentrate some relatively large charged particles at the expansion pole and may produce substantial ion gradients through the embryo.

Let us consider how it might be possible to create such a field spontaneously within one of these zygotes. It is rea-

sonable to assume that there exist many molecules in the cytoplasm which have large dipole moments associated with them. Let us make the further assumption that after wetting they become free to move and possess a tendency through their mutual interaction, to align their dipoles in the same direction. Some materials do possess characteristics which allow them to do this in crystals. These materials are termed ferroelectric. It is not completely understood what properties these materials have which allows them to exhibit this property but from the other properties of known ferroelectrics it is reasonable to assume that large organic molecules might also have this property. The degree of symmetry of crystals and the small distances between molecules in these crystals would not be expected to exist in vivo, although the idea of liquid crystals occuring in living materials is not new. It is, however, conceivable that through the properties of these molecules and other constituents of the cytoplasm the effect of aligning dipoles might manifest itself. Let us assume that polar molecules exist within the Botrytis spore and that they possess this ferroelectric property, i.e. they spontaneously align their dipoles in the same direction.

With these assumptions let us now construct the following model of the germination process in Botrytis spores. Upon wetting, water and nutrients are absorbed and ferroelectric-type molecules are either produced as the metabolism of the

spore increases or are given freedom to rotate through the dissolution of membranes as indicated by the E.M. study. Because of their ferroelectric property they then orient themselves through mutual interactions of their dipole moments, in some direction, which, if there exist no external stimuli will depend only on the random distribution which happens to exist at the time when they became free to interact. This alignment of the dipoles then induces an electric field in the area which causes the concentration of relatively large charged molecules which act as auxins in a region of the spore. This concentration then causes emission of a germ tube from the now determined pole of the spore.

Since Botrytis spores are known to emit more than one germ tube, one must conclude that these electric fields may be isolated in a region of the spore and that more than one field can arise within a spore. Evidence which supports this is that in Fucus zygotes, (the organism where these fields are found to exist), polarized light being a bipolar stimulus induces a greater number of zygotes to emit more than one rhizoid than would be expected. This "twinning" effect of polarized light correlates very highly with the effectiveness of various sources of polarized light to orient the emission of germ tubes in Fucus. That these germ tubes are usually in opposite directions implies the existence of oppositely directed fields in each half of the zygotes. It

was previously noted that when Botrytis spores emit more than one germ the two germ tubes are usually oriented in opposite directions. We, therefore, allow that two differently directed electric fields may be set up in different regions of the spore, though usually one will dominate and determine the direction of germination.

Let us now consider how light could interact with this model in such a way as to affect the direction of the electric field. We assume the existence of a set of oriented dichroic photoreceptors as in Jaffe's model. We further assume that effect of excitation of one of these photoreceptor molecules is to induce a strong dipole moment along the axis of maximum absorption of the molecule. Since these molecules are oriented in such a way that their optical axis is perpendicular to the spore wall, the resultant induced dipole is also in this direction. Assuming a half life of the order of seconds for the excited state, the effective dipole moment of all the excited molecules where the probability of excitation is a function of $\cos^2\theta$, will be a very strongly oriented in the direction of maximum absorption of the spore. The effect, therefore, of the interaction of the dipoles of the photoreceptors and the dipoles of the ferroelectric-type molecules in the cytoplasm could conceivably affect the direction of germination to the degree found by Jaffe in 1962.

Let us now consider whether this model can explain the appearance of "centering" when spores are illuminated horizontally with high intensity unpolarized light. Let us consider a two dimentional model of a spore being illuminated horizontally with unpolarized light and the probability of excitation of a molecule as a function of position. Even though the middle peak of absorption is small compared to the other two peaks, it is conceivable that after a certain degree of overlap, i.e. a high enough intensity, the dipoles may start to interact to create one region affected by both dipoles. The resultant direction of the two dipoles would be a large dipole pointing midway between them and the centering effect would result.

Let us now attempt to apply this model to the rheotropic response. It is interesting to note that Jaffe himself states that these hypothetical stimulator molecules do not appear to promote the germination but only to monitor the direction of it. As before, we can therefore argue that this implies the existence of a system which is not directly involved in the germination process but only affects the direction.

The flow of water across any semiconductor will result in a net electric potential across the object. Thus the flow of water around the surface of a spore will effect a net elec-

tric potential across the spore. Even though this potential would be insufficient to cause a migration of charged particles inside the cell it could be sufficient to ineract with the electric field being set up within the spore by the alignment of the ferroelectric-type molecules and thus determine the direction of germination.

Let us now consider the autotropic response of Botrytis spores. The effect was observed to be strongly dependent on the distance between the spores and was not observed at all for pairs of spores whose distances from each other exceeded 10 μ. The effect was in fact much stronger between touching spores than spores which were not touching. All that can be said at this point is that there seems to exist some interaction of the fields set up within a spore which germinate in the same vicinity. This would be expected if our model is correct since any spore would set up a field around itself and this field would affect any other spore in the same vicinity. The different autotropic response caused by a high presence of CO_2 cannot be explained by this model but in no way does it contradict the model. CO_2 has long known to be a developmental control in many systems and so may affect basic metabolic processes which may influence the autotropic response.

Because the basic processes of biology usually occur

in a variety of systems, the general aspects of this model, i.e. an alignment of dipoles which create an electric field in order to effect transportation of auxins, should be found in many systems and especially in other germinating structures. Therefore, this model should help to explain observations in other systems. For example in Fucus zygotes, in which electric fields are known to arise during germination, the phototropic and polarotropic responses can be explained by determining the proper orientation of the dichroic photoreceptors and the necessary directions of the dipole that an excitation will induce so as to align the electric field, which transports a stimulator or repressor in the proper area of a cell to induce germination in the observed direction.

The explanation of germinating structures will not always be so simple since other photosensitive and metabolic systems may complicate the response to light. In Funaria spores, for example, the rate of germination is affected by the intensity and wavelength of incident light and it is thought that photosynthesis and the phytochrome system play a part in germination (Krupa 1967). The observed complex phototropic responses which result from these interactions cannot therefore be so easily explained.

There are systems other than germination which appear to be directed by electric fields. Zurzycki (1967) showed

that the photoreceptors for the orientation of chloroplasts in Funaria hygrometrica do not exist in the chloroplasts but in the cytoplasm of the cell. Let us assume that there exist molecules which, when excited by light, exhibit strong dipole moments which will spontaneously align themselves. The molecules which become excited will tend to be oriented with their optical axis lying perpendicular to the direction of propagation of the incident light. If chloroplasts have a directed electric dipole associated with them they will tend to orient themselves with respect to this induced electric field. Thus the light will induce an electric field which is oriented by the direction of the propagation of the light and which in turn orients the chloroplasts. Therefore, the orientation of the chloroplasts is determined by the direction of propagation of the light.

It is believed by this author that on the basis of the evidence discussed above that it would be profitable to:

1) Consider the effect of a directed electric field on the germination of Botrytis conidia.

2) Consider whether some large organic molecules, especially flavins and other pigments, are ferroelectric.

3) Determine whether other growth responses can be explained in terms of directed internal electric fields.

TABLES AND FIGURES

TABLE I. Possible Classes of the Phototropic Responses of Spherically Symmetrical Cells

Light is blue at all effective intensities unless otherwise noted. Cells are spores or zygotes.

Growth from	Electric excitation			Magnetic excitation		
	Receptors...Tangential (T)	Radial (R)	Disoriented (D)	T	R	D
Brightest part	*Funaria* (medium, red)	*Botrytis* (2)	*Funaria* (low, red)			
Darkest part	*Fucus* (3, 15) *Equisetum* (15, 16) *Osmunda* (2)	[*Funaria* (high, red)] ?	[*Codium* (17)] ?	[*Funaria* (high, red)] ?		

TABLE II. The Sequence of times that the Chambers were under the influence of Polarized Light and in the dark in the third experiment.

Chambers EXPT.	A			B		
	Lt.	Dk.	Lt.	Dk.	Lt.	Dk.
III (i)	$5\frac{1}{2}$	$4\frac{1}{2}$	-	$4\frac{1}{2}$	$5\frac{1}{2}$	-
III (ii)	3	3	-	3	3	-
III (iii)	2	3	2	2	3	2
III (iv)	$2\frac{1}{4}$	2	1	$2\frac{1}{4}$	2	1
III (v)	2	$1\frac{1}{4}$	1	2	$1\frac{1}{4}$	1

TABLE III. Angular distribution frequencies of germ tubes of 100 spores of *Botrytis cinerea* which germinated under the influence of polarized light and of germ tubes of 100 spores of *Botrytis cinerea* which germinated in the dark.

	LIGHT	DARK
CLASS	Angular Dist'n Freq.	Angular Dist'sn Freq.
$(0 - 10)^\circ$	31	12
$(10 - 20)^\circ$	15	9
$(20 - 30)^\circ$	12	10
$(30 - 40)^\circ$	12	13
$(40 - 50)^\circ$	11	7
$(50 - 60)^\circ$	5	12
$(60 - 70)^\circ$	2	13
$(70 - 80)^\circ$	2	11
$(80 - 90)^\circ$	7	13
V_2(calc).	46.320	-4.304
X^2calc	55.455	3.143
X^2crit	12.542	15.507
	Non-Uniform $-V_2 \neq 0$	Uniform $-V_2 = 0$

TABLE IV. Angular distribution frequencies of germ tubes of 100 spores of Botrytis cinerea which germinated under the influence of polarized light and of germ tubes of 100 spores of Botrytis cinerea which germinated in the dark.

	LIGHT	DARK
CLASS	Angular Dist'n Freq.	Angular Dist'n Freq.
(0 - 10)°	27	12
(10 - 20)°	26	7
(20 - 30)°	16	11
(30 - 40)°	15	14
(40 - 50)°	5	11
(50 - 60)°	4	9
(60 - 70)°	1	9
(70 - 80)°	4	12
(80 - 90)°	8	15
V_2	46.078	-5.284
X^2calc	55.487	4.183
X^2crit	12.592	15.507
	Non-Uniform $-V_2 \neq 0$	Uniform - $V_2 = 0$

TABLE V. Number of spores of Botrytis cinerea from a sample of 100 which germinated under the influence of polarized light and the number of spores of Botrytis cinerea from a sample of 100 which germinated in the dark within 2½ hours of wetting and their angular distribution frequencies.

No. of Spores	LIGHT	DARK
which germ.	8	8
CLASS	Angular Dist'n Freq. of Germ Spores	Angular Dist'n Freq. of Germ Spores
$(0 - 10)^{\circ}$	1	2
$(10 - 20)^{\circ}$	1	0
$(20 - 30)^{\circ}$	2	1
$(30 - 40)^{\circ}$	1	2
$(40 - 50)^{\circ}$	2	2
$(50 - 60)^{\circ}$	1	0
$(60 - 70)^{\circ}$	0	0
$(70 - 80)^{\circ}$	0	0
$(80 - 90)^{\circ}$	0	1
V_2	39.213	28.750
X^2calc	-	-
X^2crit	-	-

TABLE VI. Number of spores of *Botrytis cinerea* from a sample of 100 which germinated under the influence of polarized light and the number of spores of *Botrytis cinerea* from a sample of 100 which germinated in the dark within 3 hours of wetting and their angular distribution frequencies.

No. of Spores which germ.	LIGHT 32	DARK 50
CLASS	Angular Dist'n Freq. of Germ Spores	Angular Dist'n Freq. of Germ Spores
$(0 - 10)^{\circ}$	10	4
$(10 - 20)^{\circ}$	6	7
$(20 - 30)^{\circ}$	3	10
$(30 - 40)^{\circ}$	4	4
$(40 - 50)^{\circ}$	1	4
$(50 - 60)^{\circ}$	3	6
$(60 - 70)^{\circ}$	1	5
$(70 - 80)^{\circ}$	1	4
$(80 - 90)^{\circ}$	3	6
V_2	40.166	6.318
X^2calc	17.718	1.354
X^2crit	$X_3^2 = 7.815$	$X_4^2 = 4.488$
	Non-Uniform-V_2=0	Uniform - V_2=0

TABLE VII. Number of spores of *Botrytis cinerea* from a sample of 100 which germinated under the influence of polarized light and the numbers of spores of *Botrytis cinerea* from a sample of 100 which germinated in the dark within 5 hours of wetting and their angular distribution frequencies.

No. of Spores	LIGHT	DARK
which germ.	57	63
CLASS	Angular Dist'n Freq. of Germ Spores	Angular Dist'n Freq. of Germ Spores
$(0 - 10)^{\circ}$	17	9
$(10 - 20)^{\circ}$	11	8
$(20 - 30)^{\circ}$	11	9
$(30 - 40)^{\circ}$	3	10
$(40 - 50)^{\circ}$	2	3
$(50 - 60)^{\circ}$	3	7
$(60 - 70)^{\circ}$	1	4
$(70 - 80)^{\circ}$	4	6
$(80 - 90)^{\circ}$	5	7
V_2	42.653	12.608
X^2calc	33.123	4.999
X^2crit	$X_5^2 = 11.0$	$X_4^2 = 12.592$
	Non-Uniform-V_2=0	Uniform-V_2=0

TABLE VIII. Number of spores of *Botrytis cinerea* from a sample of 100 which germinated under the influence of polarized light and the number of spores of *Botrytis cinerea* from a sample of 100 which germinated in the dark within 6 hours of wetting and their angular distribution frequencies.

No. of Spores which germ.	LIGHT 66	DARK 75
CLASS	Angular Dist'n Freq. of Germ Spores	Angular Dist'n Freq. of Germ Spores
$(0 - 10)^{\circ}$	13	8
$(10 - 20)^{\circ}$	10	7
$(20 - 30)^{\circ}$	13	7
$(30 - 40)^{\circ}$	9	12
$(40 - 50)^{\circ}$	5	9
$(50 - 60)^{\circ}$	2	8
$(60 - 70)^{\circ}$	4	7
$(70 - 80)^{\circ}$	6	8
$(80 - 90)^{\circ}$	4	8
V_2	31.076	.502
X^2calc	17.450	2.354
X^2crit	$X^2_6 = 12.542$	$X^2_8 = 15.507$
	Non-Uniform - $V_2 \neq 0$	Uniform - $V_2 = 0$

TABLE IX. Angular distribution frequencies of 100 germinated spores of *Botrytis cinerea* which were subject to 5¼ hours of polarized light followed by 4½ hours of darkness and of 100 germinated spores of *Botrytis cinerea* which were subject to 5¼ hours of darkness followed by 4½ hours of polarized light.

CHAMBER A			CHAMBER B	
TIME SEQUENCE	ANGULAR DIST'N FREQ.	CLASS	ANGULAR DIST'N FREQ.	TIME SEQUENCE
5¼ hrs lt.	32	$(0\text{-}10)^\circ$	12	5¼ hrs.dk.
4½ hrs.dk.	19	$(10\text{-}20)^\circ$	6	4½ hrs lt.
	11	$(20\text{-}30)^\circ$	14	
	4	$(30\text{-}40)^\circ$	11	
	6	$(40\text{-}50)^\circ$	11	
	9	$(50\text{-}60)^\circ$	9	
	5	$(60\text{-}70)^\circ$	11	
	7	$(70\text{-}80)^\circ$	12	
	7	$(80\text{-}90)^\circ$	14	
	37.265	V_2	4.553	
	58.638	X^2calc	3.652	
	15.507	X^2crit	15.507	
	Non-Uniform $V_2 \neq 0$		Uniform $V_2 = 0$	

TABLE X. Angular distribution frequencies of 100 germinated spores of *Botrytis cinerea* which were subject to 3 hours of polarized light followed by 3 hours of darkness and of 100 germinated spores of *Botrytis cinerea* which were subject to 3 hours of darkness followed by 3 hours of polarized light.

CHAMBER A			CHAMBER B	
TIME SEQUENCE	ANGULAR DIST'N FREQ.	CLASS	ANGULAR DIST'N FREQ.	TIME SEQUENCE
3 hrs lt.	25	$(0\text{-}10)^\circ$	21	3 hrs drk.
3 hrs drk.	20	$(10\text{-}20)^\circ$	13	3 hrs lt.
	13	$(20\text{-}30)^\circ$	17	
	13	$(30\text{-}40)^\circ$	8	
	6	$(40\text{-}50)^\circ$	10	
	4	$(50\text{-}60)^\circ$	7	
	2	$(60\text{-}70)^\circ$	5	
	6	$(70\text{-}80)^\circ$	12	
	10	$(80\text{-}90)^\circ$	7	
	36.065	V_2	22.714	
	41.091	X^2calc	19.898	
	12.592	X^2crit	15.507	
	Non-Uniform $V_2 \neq 0$		Uniform $V_2 = 0$	

TABLE XI. Angular distribution frequencies of 100 germinated spores of Botrytis cinerea which were subject to 2 hours of polarized light followed by 3 hours of darkness followed by 2 hours of polarized light and of 100 germinated spores of Botrytis cinerea which were subject to 2 hours of darkness followed by 3 hours of polarized light followed by 2 hours of darkness.

CHAMBER A			CHAMBER B	
TIME SEQUENCE	ANGULAR DIST'N FREQ.	CLASS	ANGULAR DIST'N FREQ.	TIME SEQUENCE
2 hrs lt.	17	$(0-10)^{\circ}$	21	2 hrs drk.
3 hrs drk.	16	$(10-20)^{\circ}$	17	3 hrs lt.
2 hrs lt.	9	$(20-30)^{\circ}$	9	2 hrs drk.
	14	$(30-40)^{\circ}$	15	
	10	$(40-50)^{\circ}$	9	
	6	$(50-60)^{\circ}$	8	
	10	$(60-70)^{\circ}$	9	
	9	$(70-80)^{\circ}$	6	
	9	$(80-90)^{\circ}$	7	
	16.553	V_2	25.890	
	9.840	X^2calc	19.249	
	15.507	X^2crit	15.507	
	Uniform $V_2 = 0$		Non-Uniform $V_2 \neq 0$	

TABLE XII. Angular distribution frequencies of 100 germinated spores of Botrytis cinerea which were subject to $2\frac{1}{4}$ hours of polarized light followed by 2 hours of darkness followed by 1 hour of polarized light and of 100 germinated spores of Botrytis cinerea which were subject to $2\frac{1}{4}$ hours of darkness followed by 2 hours of polarized light followed by 1 hour of darkness.

CHAMBER A			CHAMBER B	
TIME SEQUENCE	ANGULAR DIST'N FREQ.	CLASS	ANGULAR DIST'N FREQ.	TIME SEQUENCE
$2\frac{1}{4}$ hrs lt.	16	$(0\text{-}10)^\circ$	25	$2\frac{1}{4}$ hrs drk.
2 hrs drk.	15	$(10\text{-}20)^\circ$	22	2 hrs lt.
1 hr lt.	15	$(20\text{-}30)^\circ$	10	1 hr drk.
	8	$(30\text{-}40)^\circ$	9	
	7	$(40\text{-}50)^\circ$	4	
	7	$(50\text{-}60)^\circ$	7	
	7	$(60\text{-}70)^\circ$	5	
	10	$(70\text{-}80)^\circ$	7	
	15	$(80\text{-}90)^\circ$	11	
	10.801	V_2	30.859	
	11.792	X^2calc	39.134	
	15.507	X^2crit	14.067	
	Uniform $V_2 = 0$		Non-Uniform $V_2 \neq 0$	

TABLE XIII. Angular distribution frequencies of 100 germinated spores of *Botrytis cinerea* which were subject to 2 hours of polarized light followed by 1¼ hours of darkness followed by 1 hours of polarized light and of 100 germinated spores of *Botrytis cinerea* which were subject to 2 hours of darkness followed by 1¼ hours of polarized light followed by 1 hours of darkness.

CHAMBER A			CHAMBER B	
TIME SEQUENCE	ANGULAR DIST'N FREQ.	CLASS	ANGULAR DIST'N FREQ.	TIME SEQUENCE
2 hrs lt.	15	$(0\text{-}10)^{\circ}$	22	2 hrs drk.
1¼ hrs drk.	11	$(10\text{-}20)^{\circ}$	21	1¼ hrs lt.
1 hrs lt.	10	$(20\text{-}30)^{\circ}$	11	1 hrs drk.
	11	$(30\text{-}40)^{\circ}$	7	
	10	$(40\text{-}50)^{\circ}$	11	
	7	$(50\text{-}60)^{\circ}$	5	
	13	$(60\text{-}70)^{\circ}$	5	
	12	$(70\text{-}80)^{\circ}$	6	
	11	$(80\text{-}90)^{\circ}$	12	
	2.503	V_2	24.114	
	3.50	X^2calc	26.806	
	13.507	X^2crit	15.507	
	Uniform $V_2 = 0$		Non-Uniform $V_2 \neq 0$	

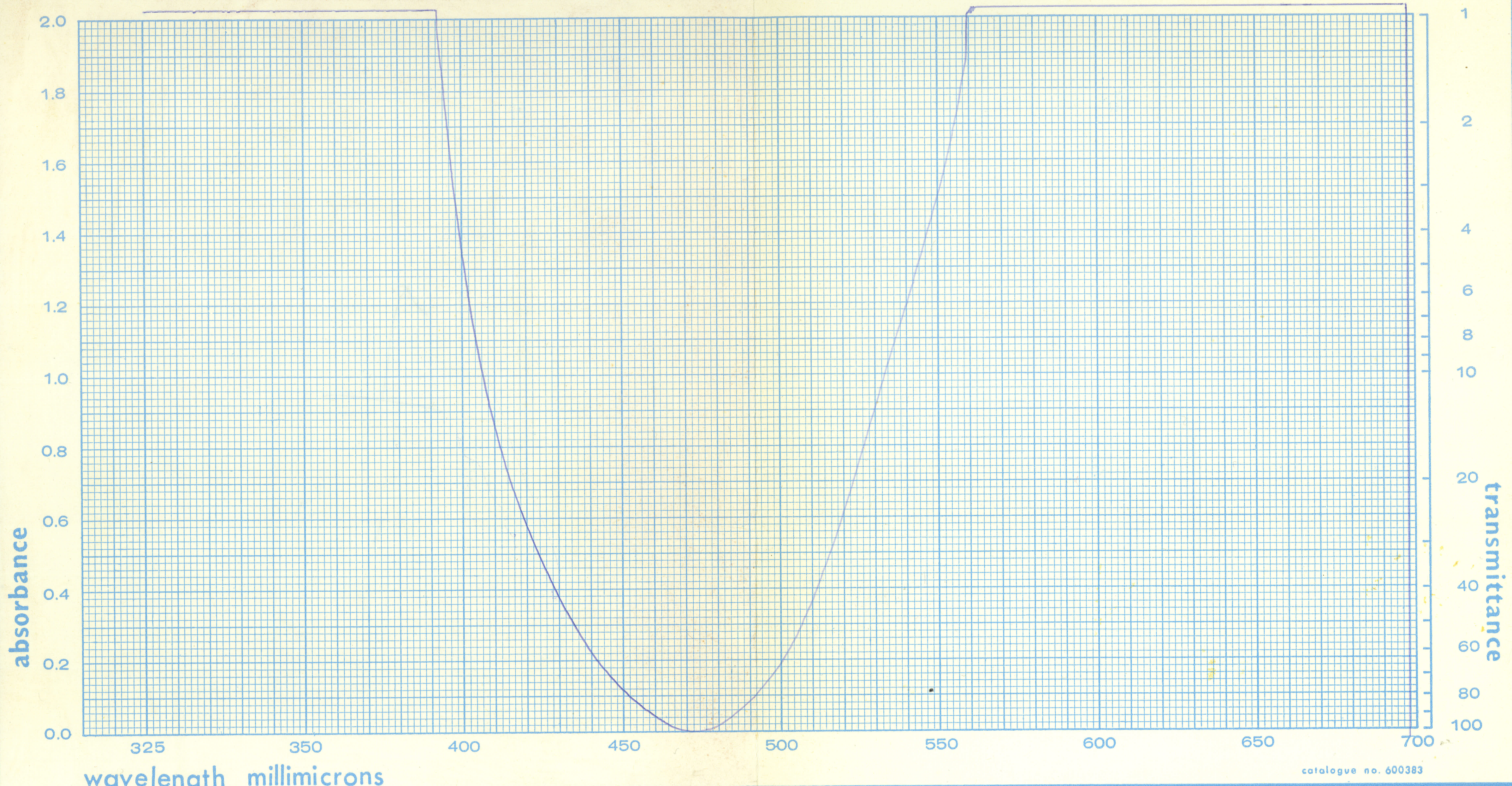


FIGURE 1. Spectral transmission curve of the polaroid sheet and light filters as obtained with Unicam S.P.800 spectrophotometer.

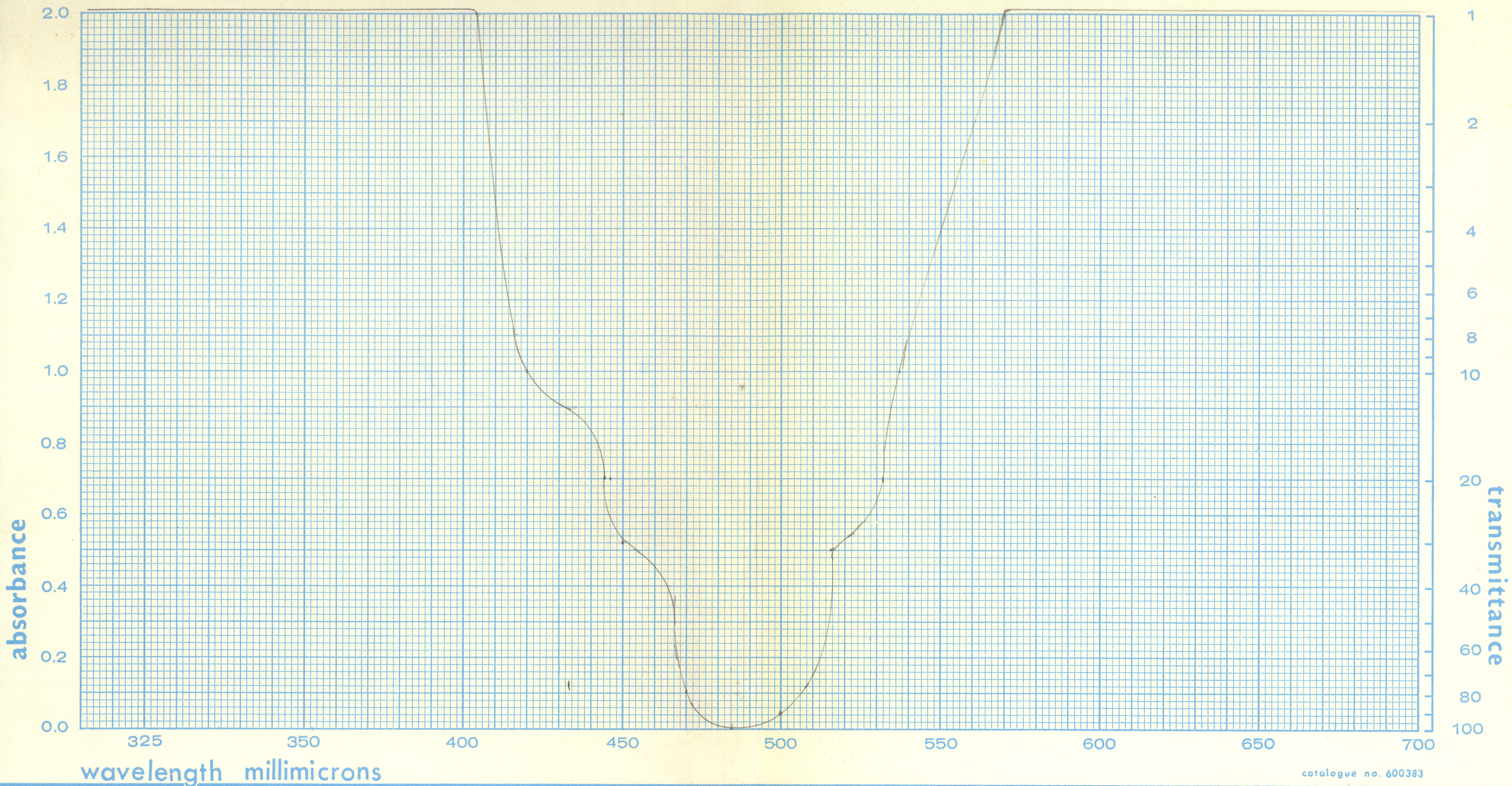


FIGURE 2. Spectral transmission curve of the polaroid sheet and light filters as obtained with the Zeiss PM. Q II spectrophotometer in conjunction with the Zeiss M4QII monochromatometer when illuminated with the projection lamp used in the experimental studies.

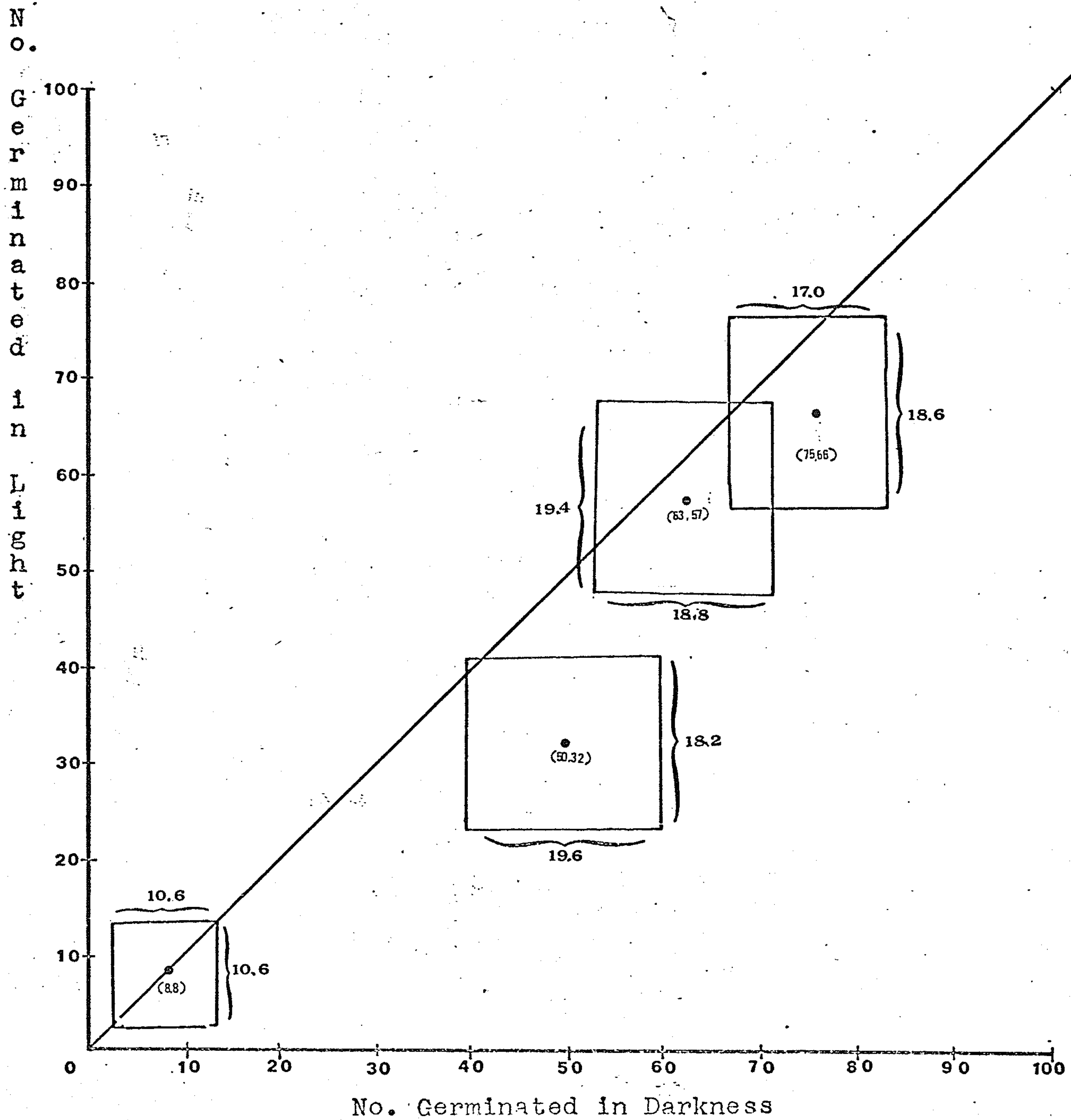


FIGURE 3. Plot of results of Experiment II with confidence rectangles.

FIGURES 4. - 10.

Section of spores which were embedded in plain agar for different lengths of time prior to fixation.

Spores in figures 4 and 5 were fined after half an hour, the spore in figure 6 after 1 hour, the spore in figure 7 after 2 hours, the spore in figure 8 after 4 hours, and spores in figures 9 and 10 after 6 hours.

All spores were fixed in acrolein gluteraldehyde, and asmium tetroxide. Figures 7 - 10 were also stained with lead citrate.

FIGURE 4. Note the mitochondria (m) and the endoplasmic reticulum (E.R.), the nucleus (N), the nucleolus (Nu), the cell wall (CW), the vacuole (V) (X16,000).

FIGURE 5. Note the organization in the endoplasmic reticulum (ER) (X15,000).

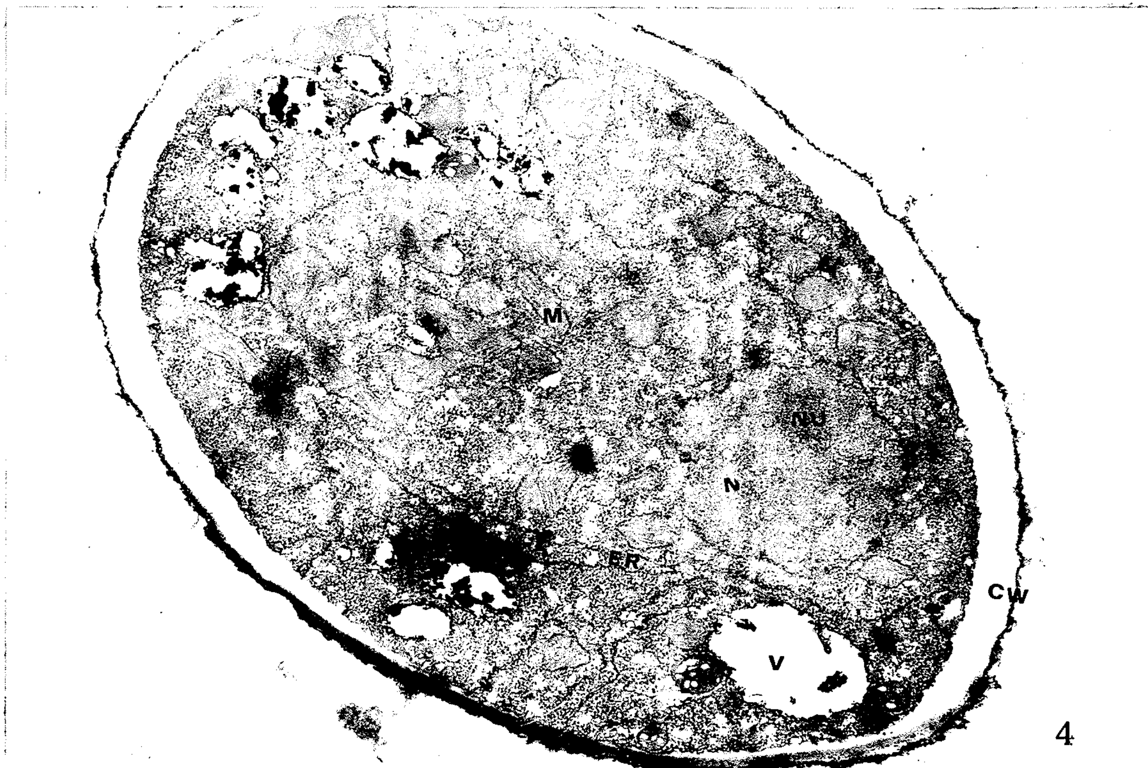
FIGURE 6. Note the four nuclei (N) and the profusion of mitochondria (M) (X15,000).

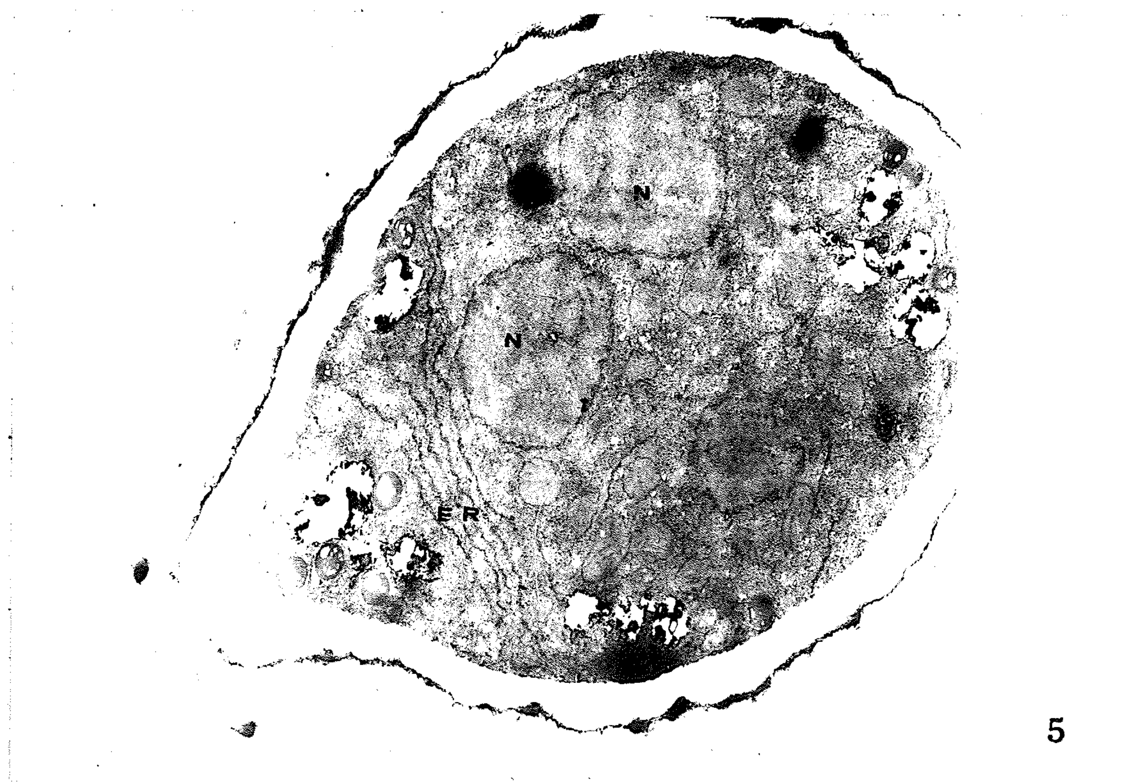
FIGURE 7. Notice the disorganized endoplasmic reticulum (ER), the plasmolemma (P) and the cell wall (CW) (X19,000).

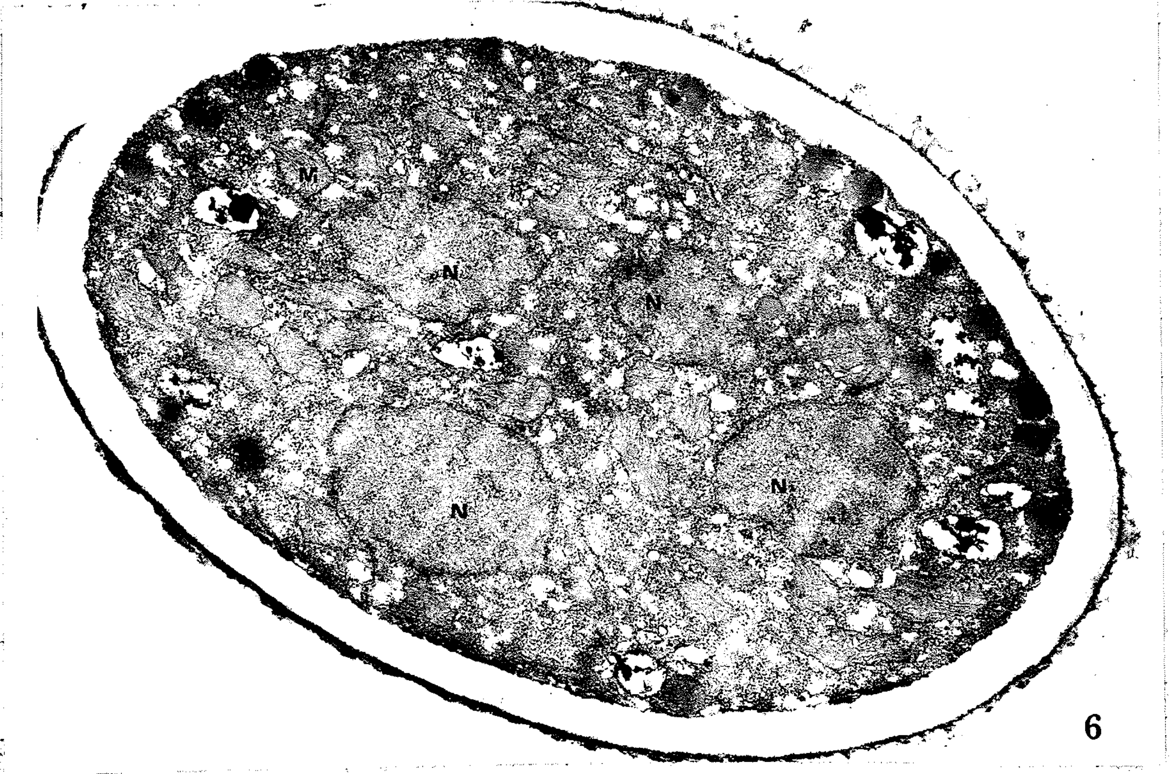
FIGURE 8. Note the profusion of riliosomes(R) (X22,000).

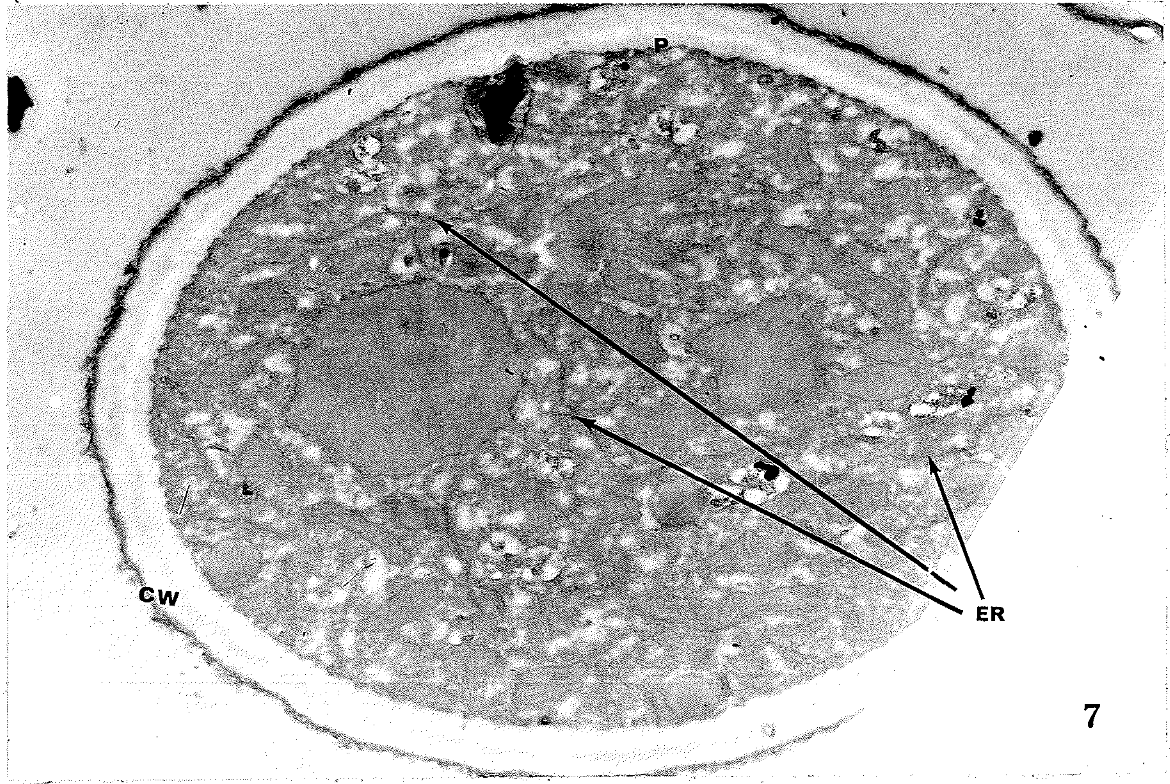
FIGURE 9. Note again the proliferation of reliosomes (R) and the extended mitochondria in particular M_1 and M_2. M_3 appears to be in a state of division (X20,000).

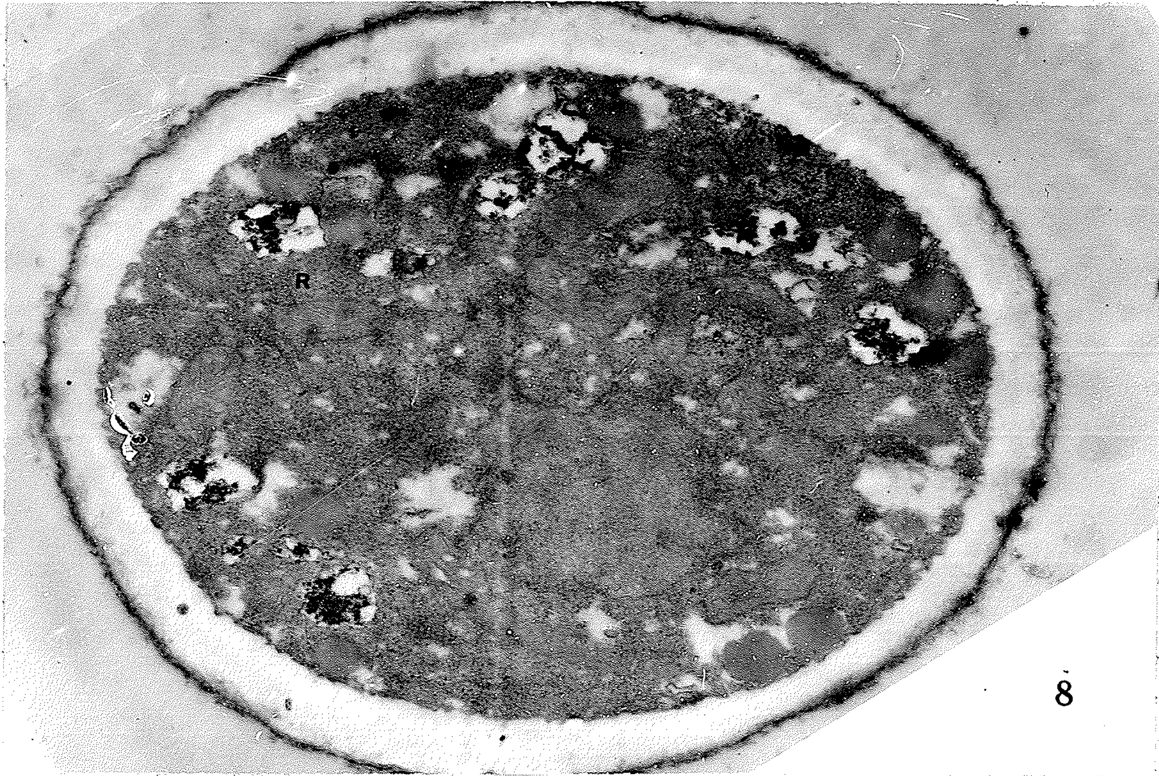
FIGURE 10. Note the disorganized endoplasmic reticulum (X20,000).

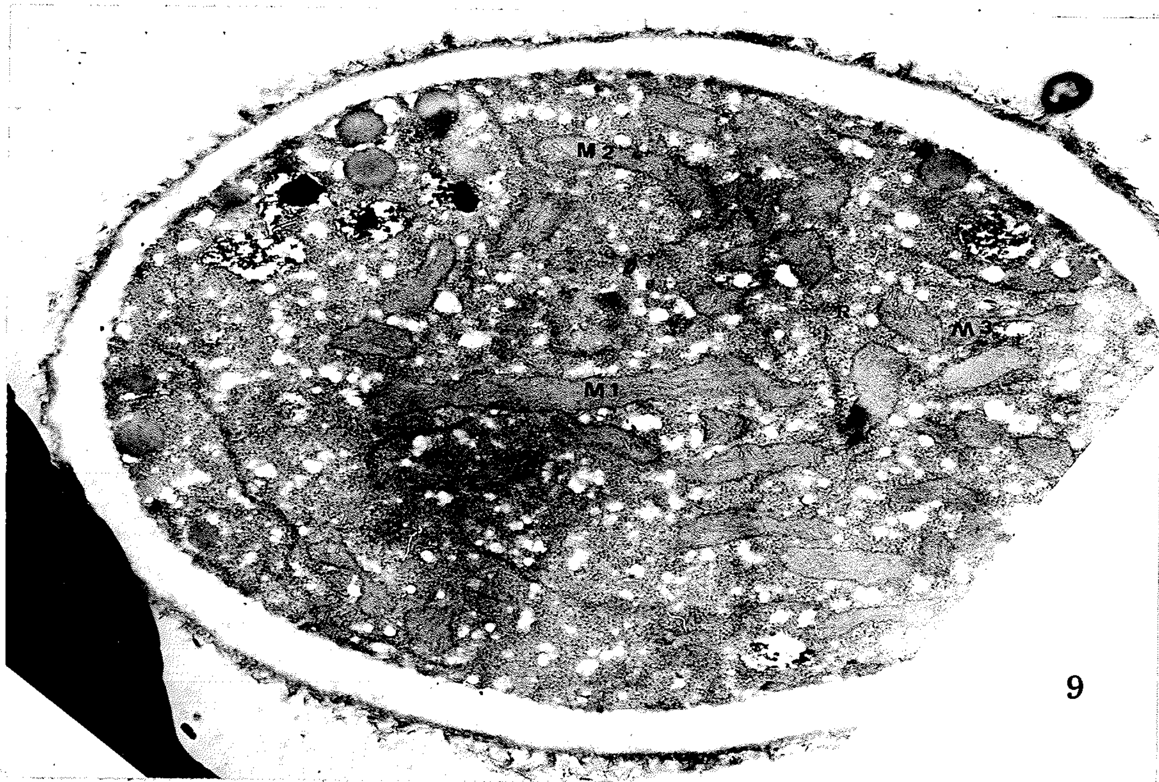

M
NU
N
ER
CW
V
4

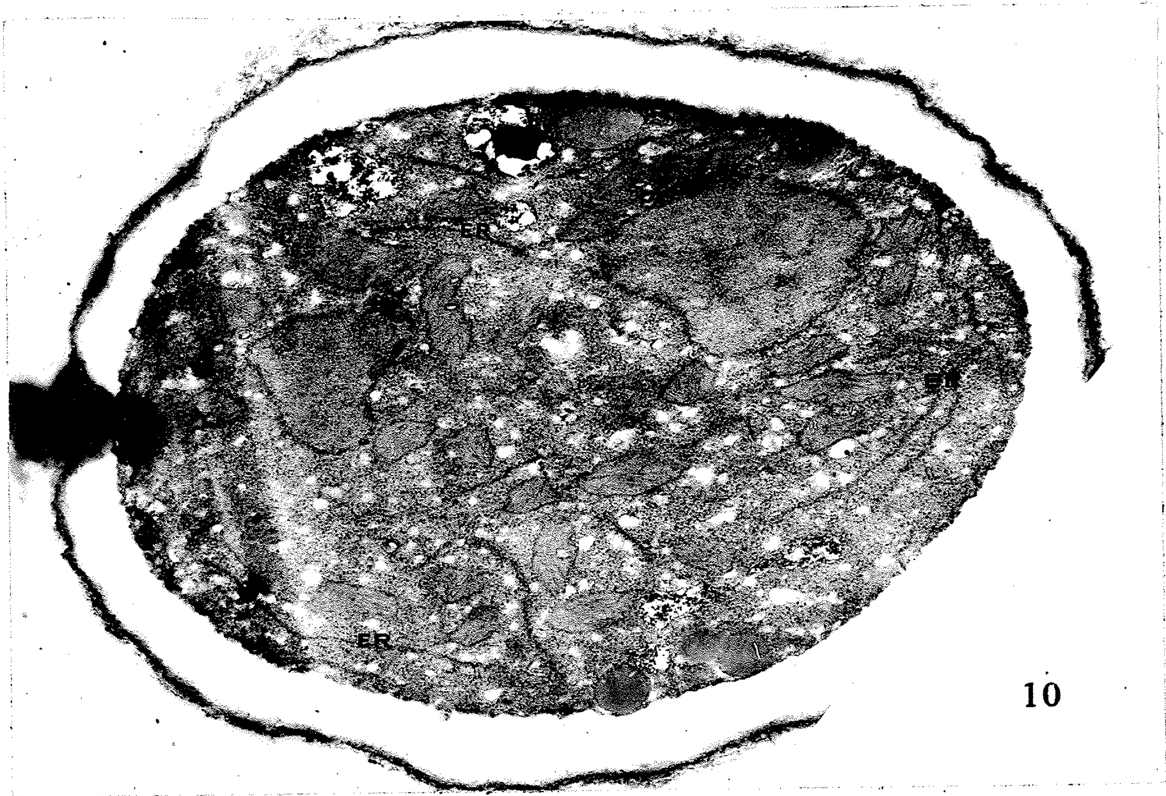

N
N
ER
5


M
N
N
N
N
6


P
CW
ER
7


R
8


M 2
M 3
M 1
9


ER
ER
ER
10

FIGURES 11. - 18.

Sections of spores which were embedded in sucrose peptone nutrient agar for different lengths of time prior to fixation.

The spore in figure 11 was fined after half an hour, the spores in figures 12 and 13 after 1 hour, the spore in figure 14 after 2 hours, the spores in figures 15 and 16 after 4 hours, and the spores in figures 17 and 18 after 6 hours.

All spores were fixed with acrolein, gluteraldehyde and asmium tetroxide. The spores in figures 15 - 18 were also stained with lead citrate.

FIGURE 11. Note the 5 nuclei (N) (X17,500).

FIGURE 12. Note the organized endoplasmic reticulum (ER) and the density of riliosomes (R) (X15,000).

FIGURE 13. Note the disorganized pieces of endoplasmic reticulum (ER) (X20,000).

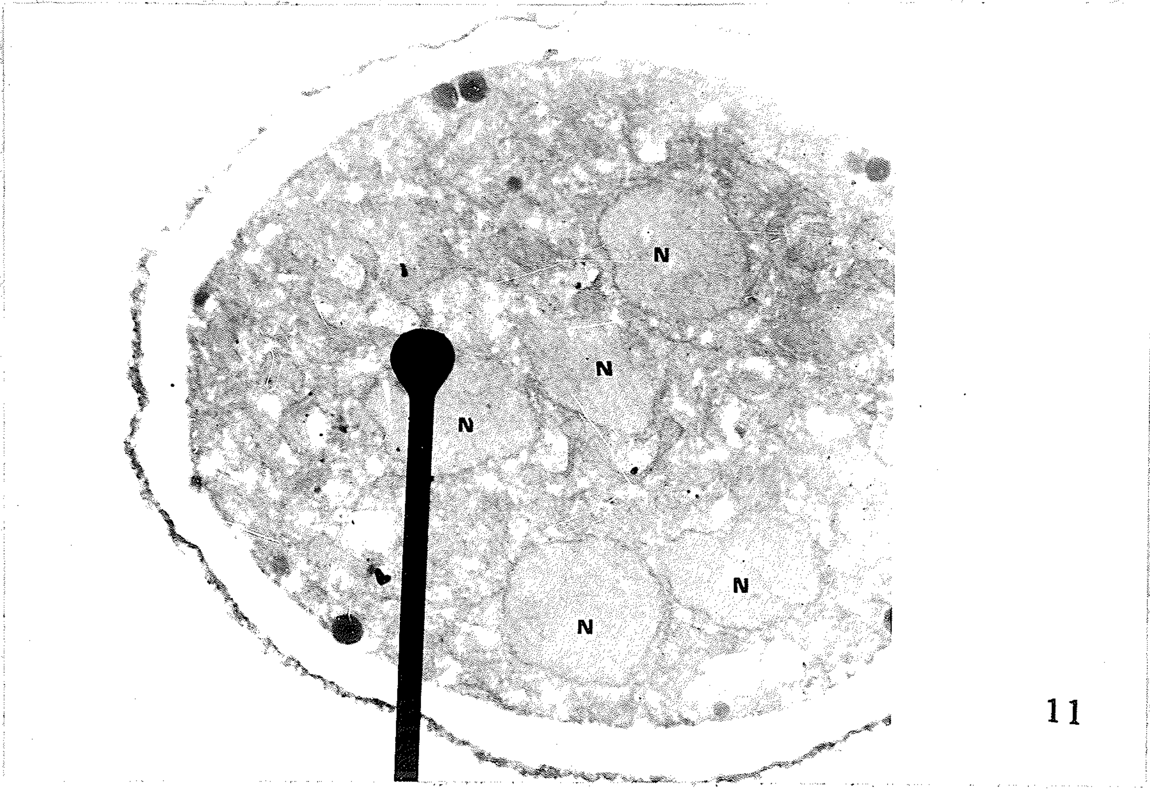
FIGURE 14. Note mitochondria (M), which appears to be dividing (X16,500).

FIGURE 15. Note the well defined cristae in the mitochondria (M), also note vacuoles (V), cell Walls (CW), plasmolemma (P), and nuclei (N). Note also the effectiveness of the lead citrate stain on this micrograph (X16,000).

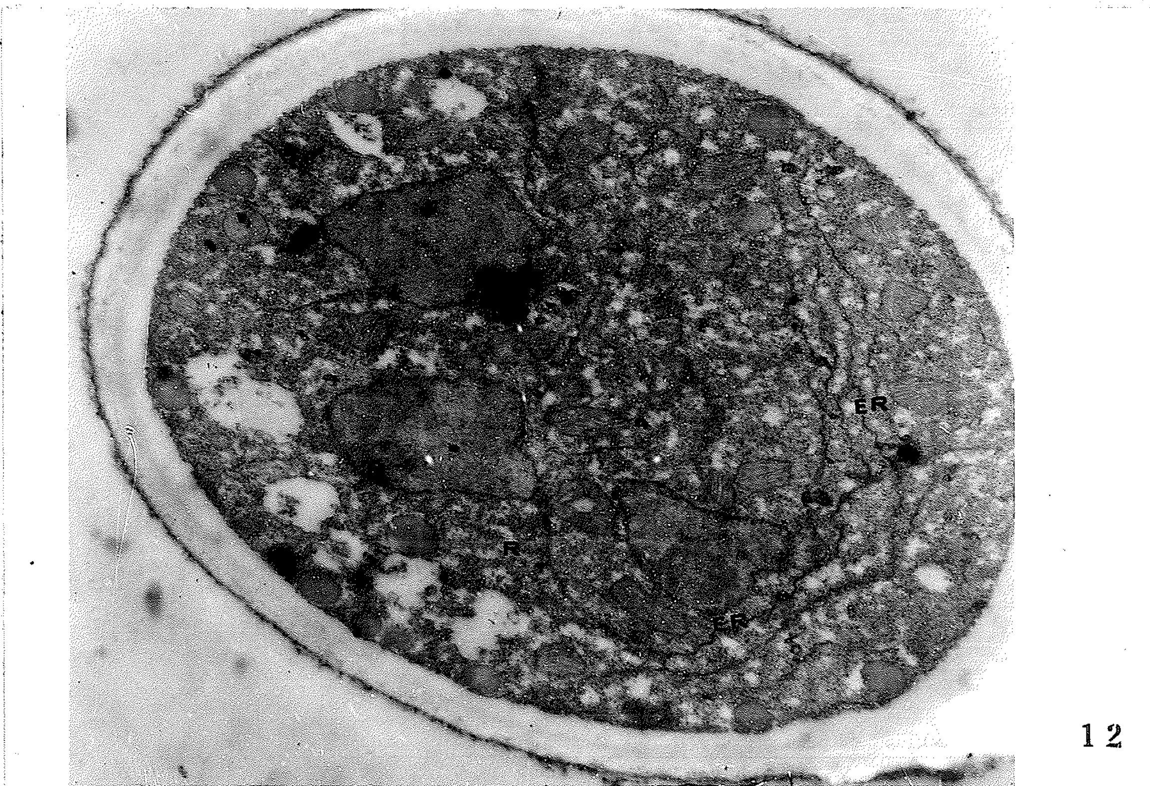
FIGURE 16. Note mitochondrium (M), which appears to be in a state of division and the tubules (T) X16,000).

FIGURE 17. Note the vacuoles (V) along the plasmolemma (P) and mitochondrium (M) which appears to be dividing (X18,000).

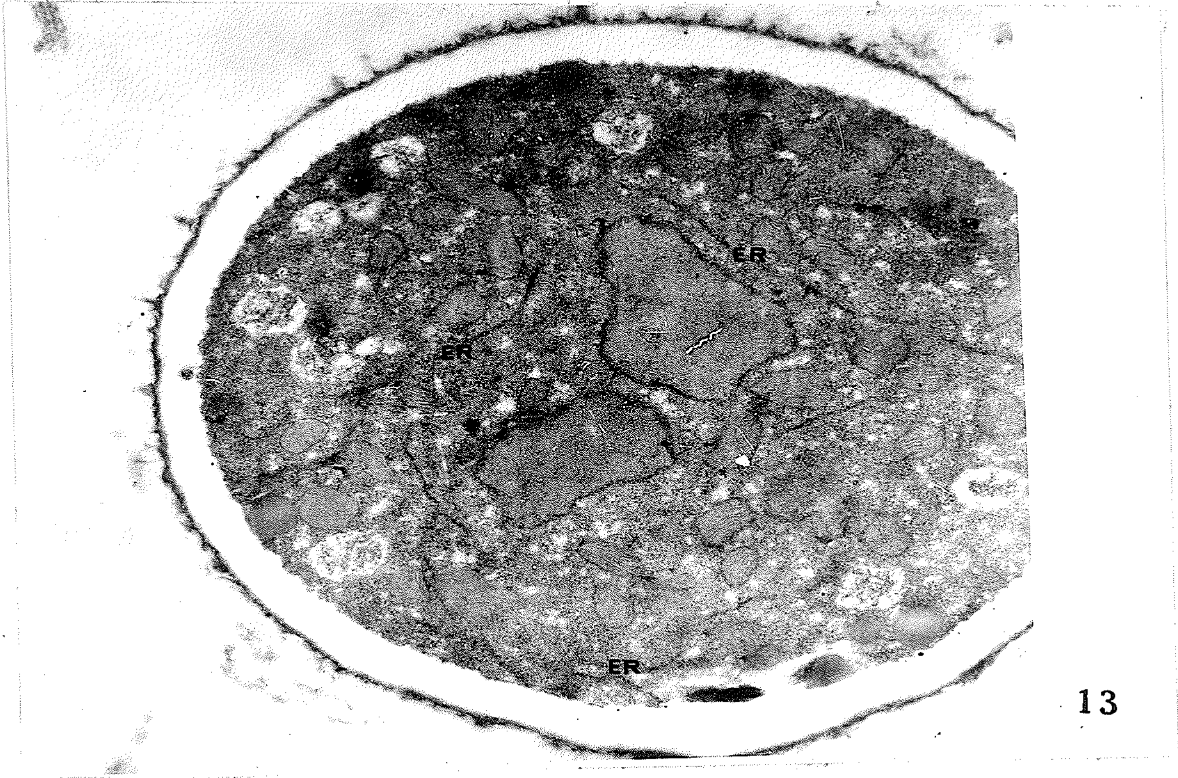
FIGURE 18. A polar section - note width of cell wall and odd organization of the endoplasmic reticulum (ER) (X25,000).



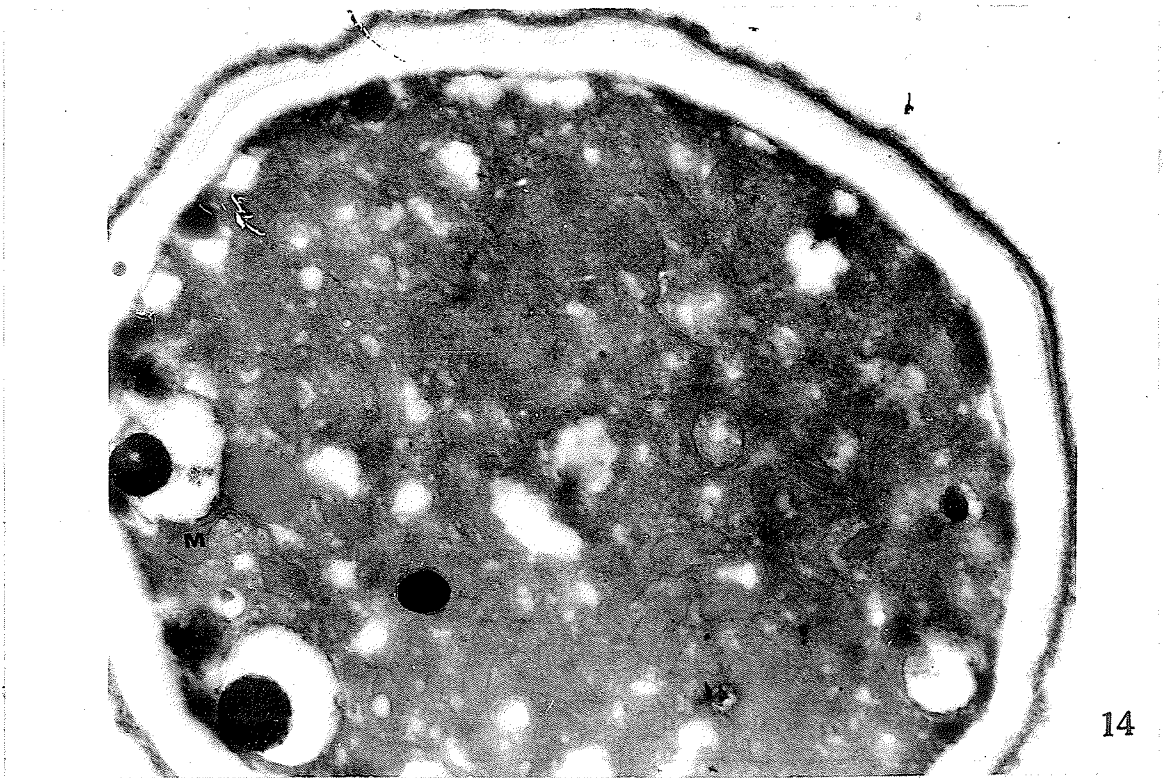
11



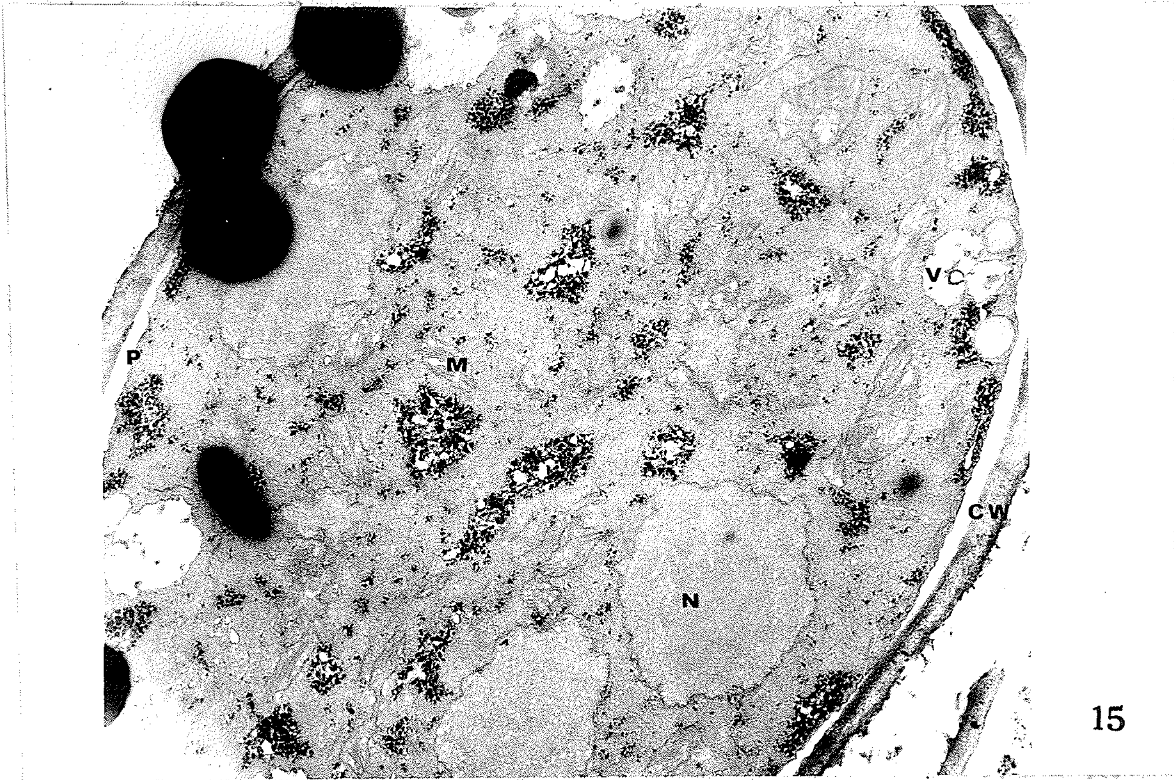
12

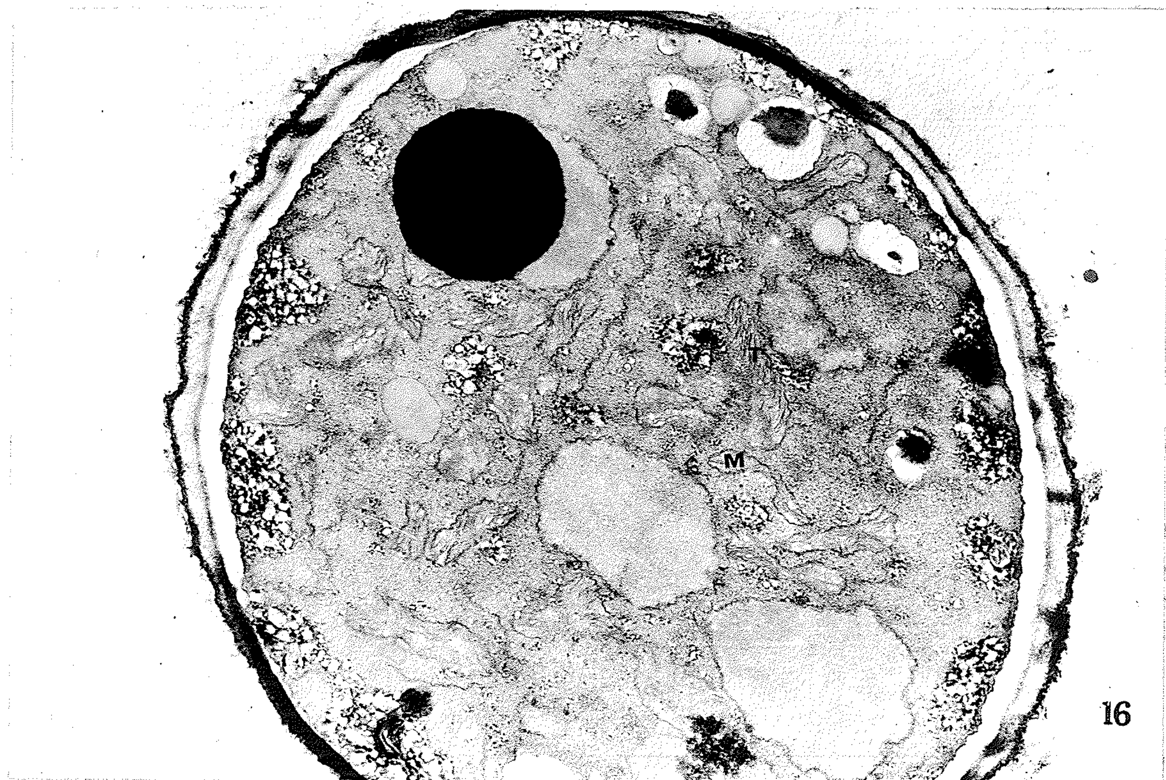


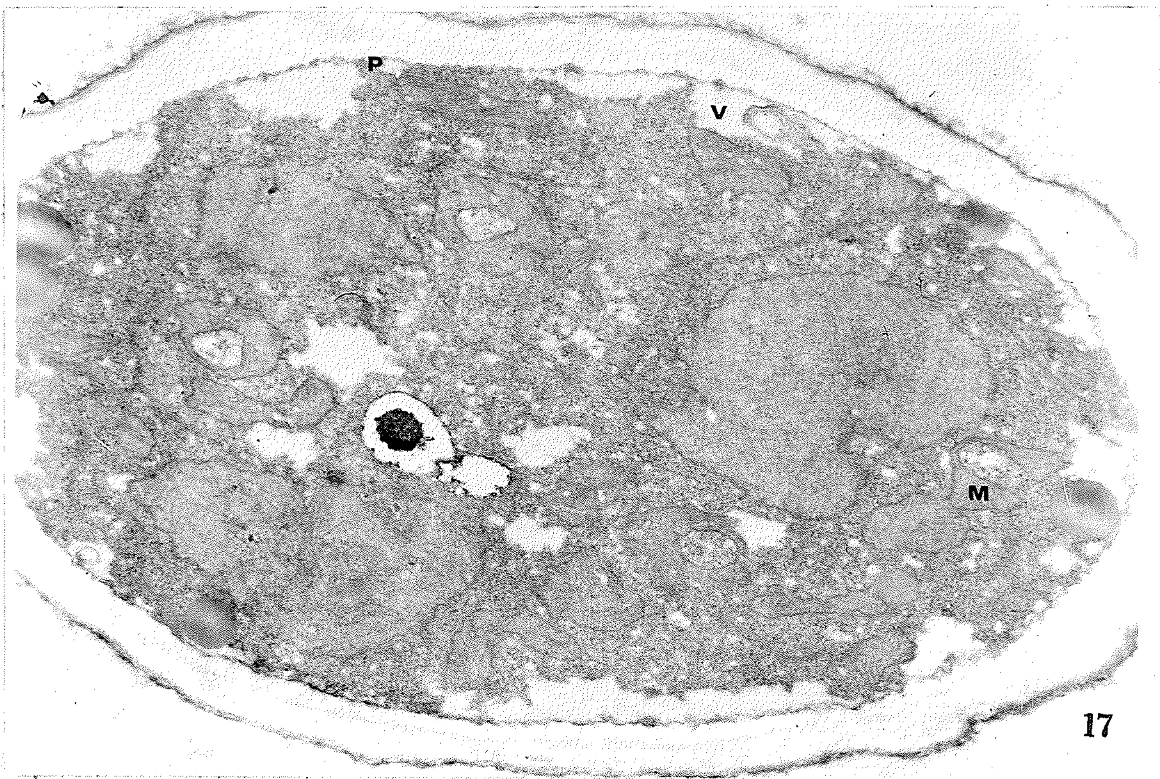
13

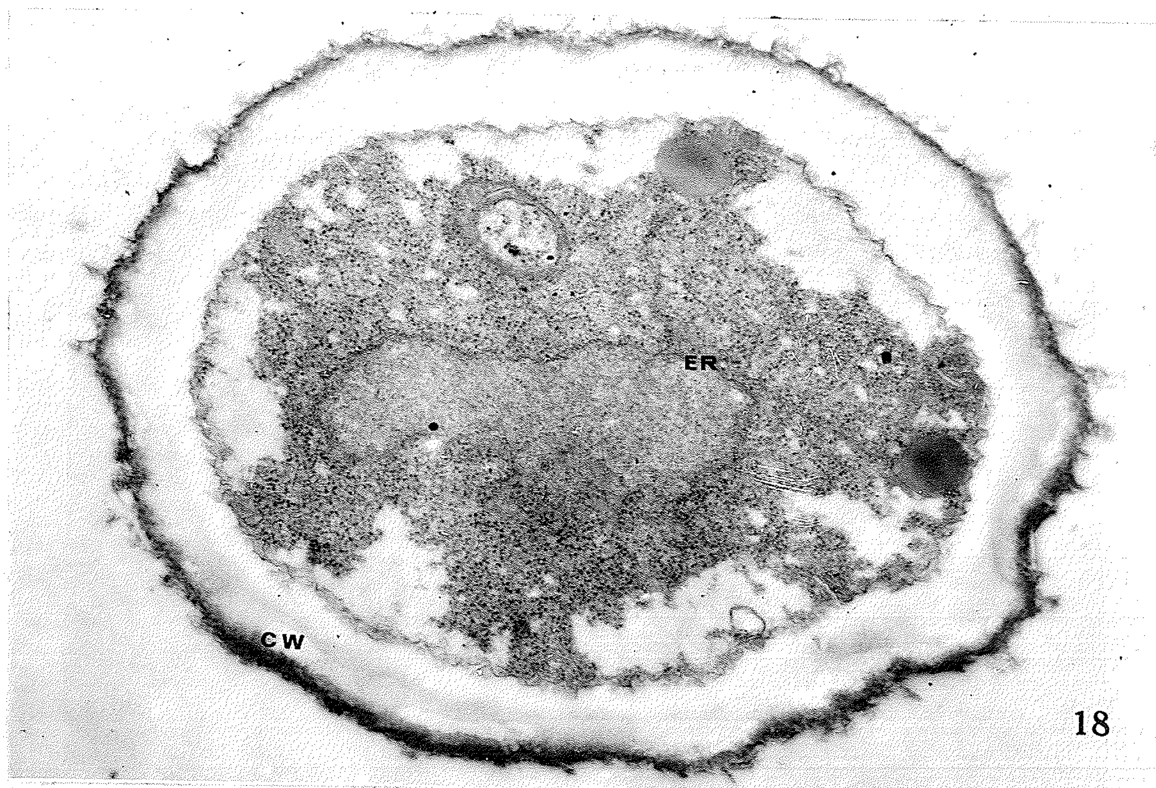


14


P
M
V
CW
N
15


M
16


P
V
M
17


ER
CW
18

SUMMARY

SUMMARY

1. Spores of *Botrytis cinerea* which are subject to polarized blue light which is capable of eliciting the polarotropic response do not germinate at a faster rate than do spores germinating in the dark.

2. Polarized light appears to be effective in inducing a polarotropic response only in the time immediately prior to germination.

3. It appears that the number of highly organized membranes in *Botrytis* conidia diminishes with time after wetting.

4. A model of the physical aspects of germination can be constructed which explains many of the observed phonemena of germinating structures.

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APPENDIX

APPENDIX

THE PHYSICAL ASPECTS OF THE INTERACTION OF LIGHT AND BIOLOGICAL SYSTEMS[1]

Light, according to the quantum theory, can be characterized in terms of energy particles or photons, and the energy content E of a radiation quantum amounts to $E = h\nu$ where ν is the frequency of radiation and h is Plank's constant. The absorption of light by matter consequently results in a transition from one energy state of a material particle to another. The effect of light on living systems involves the absorption of quanta of radiation of appropriate wavelengths and the promotion of photochemical reactions.

Since a material particle can only exist in certain discrete energy states, radiation absorption is possible only if the energy absorbed equals the energy difference of two physical states which can occur. The probability of a photon of a particular energy being absorbed and thus inducing a transition of a molecular system to a different energy state depends upon the initial and final states of the system. In some cases this probability is so low that the transitions

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[1]The material in this Appendix is condensed from Thomas (1965), Geise (1962b)(1957), McElroy and Glass (1961), Seigler and McElroy (1965), Geise (1964), Setlow and Pollard (1965).

are termed very weak or forbidden.

The energy of a molecule is divisible into its electron orbital, and electron spin components; as well as its translational, vibrational, rotational and nuclear spin motions. To treat these separately, a first order approximation, is to ignore important crossterms, between the electric orbital and spin motion and the molecular vibration, which give rise to important second order effects which are particularly important in the appearance of weak or low probability transitions. In large complex molecules these second order effects may enhance the probability of absorption of a photon, with specified energy, of a particular polarization. Molecules of this type are called dichroic since the absorption of a given wavelength of polarized light changes with the orientation of the molecule.

The energy per photon of the absorbed light need not be exactly the same for each single electronic transition. It may in fact, vary stepwise, as far as permitted, by additional energy of vibration or rotation. Therefore, the absorption spectra of molecules occur in bands instead of in lines as with atoms.

In polyatomic molecules the vibration and rotation levels are quite closely packed and quite a number of electronic levels occur with relatively small energy differences between them. Under certain conditions these properties may

allow radiation less transitions between the excited and ground states.

The increase in the energy content of matter upon the absorption of a photon is coupled with a change in the nuclear magnetic equilibrium and a redistribution of electronic charge. Since the chemical properties of a molecule depend not only on its energy content but also on the charge densities at various sites, electronic excitation may result in the formation of an essentially novel molecule which is often more chemically active than the ground state molecule.

Two important types of molecular orbitals are called σ and π orbitals. Electrons in the σ orbitals are more tightly bound to the nucleus than are those in π orbitals and are excited by radiation in the far u.v., while the π electrons are excited by photons whose energies are in the near u.v. and visible regions of the spectrum. As we are dealing with visible radiation σ orbitals will be omitted from further discussion.

The energy of π electrons, i.e. electrons in a π-type orbital, can be either of lower or higher energy than corresponding electrons in the disassociated atoms. These orbitals are termed bonding and antibonding respectively, and the transition from a bonding π orbital to an antibonding π^* orbital is called a $\pi \rightarrow \pi^*$ transition.

Electrons in molecules often occur in orbits which are

not engaged in actual bonding, i.e. they interact with only one nucleus, and are called non bonding or lone pair electrons and designated "n" electrons. These electrons can be excited by low energy stimulation and raised into the lowest empty type orbital. Such a transition is termed an $n \rightarrow \pi^*$ transition, and the energy involved in this type of transition is of the same order as that involved in $\pi \rightarrow \pi^*$ transitions. Although $n \rightarrow \pi^*$ transitions should be forbidden due to symmetry considerations; vibrational interactions make them partially allowed with the probability of absorption dependent on the polarization of the photon.

Only two electrons can occupy an spatial orbital at any one time and according to the Pauli exclusion principle these two electrons must have opposite spins. If one of the electrons making up such a pair occupying the same orbital is excited with a prerequisite amount of energy to a higher energy state, the spins may be either opposite, as before, or alike. The former is known as the singlet state and the latter as the triplet state. Transitions to triplet states usually have very low probabilities and are thus weaker transitions than transitions to singlet states, in spite of the fact that the triplet state energy is closer to the ground state than is the singlet state energy. Therefore, the transition to a triplet state is usually achieved through decay

of the singlet state of same electronic configuration.

That the transition to a triplet state is much weaker than the transition to a singlet state indicates a longer life-time for the triplet state. This is understood if it is appreciated that in order for the triplet state to return to the ground state it must undergo a spin inversion. Doing this by a radiation process is of very low probability. Therefore, these triplet states empty slowly and the lifetime of the excited state can be long enough to provide a short-lived storage of useful energy. Because of the above information and the fact that non-binding electrons, principally from nitrogen, oxygen and sulphur are present in most hetrocycltic compounds occuring in living cells, the $n \rightarrow \pi^*$ singlet-triplet transition is probably of primary biological importance.