INDUCTION OF CHANGE IN THE FUNGUS CHAETOMIUM BY IRRADIATION WITH MONOCHROMATIC ULTRA- VIOLET AND THE MECHANISM OF THE REACTION.

Thesis presented to the University of Tasmania for the degree of Master of Science.

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SUMMARY

1. Change induced in the fungus Chaetomium by irradiation of the spore with monochromatic ultra-violet has been investigated from a quantitative viewpoint.

2. The methods used in irradiation and growth of the material and the measurement of irradiation dose are described. Samples of spores were irradiated monochromatically at 265, 313 and 334 mu. Colonies of single spore origin were obtained by single-spore and dilution plating and each grown in single petrie dishes.

3. Variation was induced by irradiation in the short, middle and long ultra-violet, and included genetic effects, lethal effects and "growth-damage". The nature of variation is discussed.

4. The relative quantities of the genetic effects and "growth-damage" differed at each wavelength, the short ultra-violet being much more effective in inducing "growth-damage" than genetic effects, and the long wavelengths less effective. The lethal effects are shown to involve genetic change.

5. Evidence is presented associating the genetic effects with qualitative gene change, which is considered to involve reaction by the protein component of nucleoprotein.

6. "Growth-damage" is considered to involve an effect upon nucleic acid whereby the normal functioning of the nucleus is prevented, resulting in aberrant cell growth.

7. Nucleoprotein is visualised as providing the mechanism of heredity, the protein component being concerned with qualitative gene action and nucleic acid with the reproduction of the genetic protein. Protein and nucleic acid react independently.

INTRODUCTION

These investigations into the association of gene change with a photochemical-type reaction were begun by Professor McAulay in the Physics Department of the University of Tasmania, and in 1940, when the author undertook the investigation, some work had already been carried out on the induction of change in the ascomycetous fungus Chaetomium globosum Kunze with monochromatic ultra-violet. Evidence for a chemical[1] mechanism of gene action has been accumulated from a wide variety of investigations. Few of them, however, have been designed to determine whether photochemical change could be induced in the system. Evidence for this would not only provide additional proof of chemical mechanism, but might, if thresholds of action could be shown, enable the mechanism to be analysed. Such analysis would seek, firstly, to resolve the functions of nucleic acid and gene protein, and finally, if genetic change could be induced selectively, to study gene change at specific loci. In this paper variation induced in C. globosum by ultra-violet radiation will be considered in relation to the genetic mechanism and, in particular, to the functions of nucleic acid and gene protein.

The literature on the genetic effects of ultra-violet radiation is not extensive, chiefly because of the technical difficulties associated with its use, and until 1939 few quantitative results had been published. This literature often shows a lack of appreciation of the fundamental differences in action of ultra-violet and the ionising radiations. A controlled and specific photochemical reaction is induced by ultra-violet, while the latter produce their effects by ionisations which occur haphazardly

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Footnote 1 : The word "chemical is used to express reaction of atomic or molecular dimension.

in so far as the type of problem which can be solved by it is concerned.

In the fungi Dickson (1932, 1933) investigated effects induced in various species of Chaetomium by X-rays and ultra-violet radiation. No essential difference was noted between character change induced by X-raying spores or mycelium, or between changes induced by X-rays or ultra-violet. Any one variant character appeared to be produced independently of any other; the different variant characters, of which there were a large number, could occur singly or several could be associated in the same variant. Variants induced in irradiation of other variants differed from their parents in much the same way as these parents differed from the original species. In so far as some characters were concerned, a reverse change was possible. McAulay (1939), irradiating spores of Chaetomium globosum with ultra-violet at wavelengtns from 254 mu to 365 mu, found evidence for the selective production of a variant st 2 by long wavelengths. The order of magnitude of the dose required to produce change at 313 and 334 mu was 100 times and at 365 mu 1000 times the effective dose in the 230-265 range. Emmons and Hollaender (1939) reported on mutations induced in dermatophyte fungi by irradiation of the spores with monochromatic ultra-violet over the range 238 mu - 295 mu. They found the rate of mutation to reach a maximum and then to decrease rapidly with increasing dose. Most of the mutants (which were classified by inspection) showed an increase in pigment production and a decrease in growth rate. A few were indistinguishable from other species or other varieties of dermatophytes. Reversions were recorded. The percentage of mutation ranged between 1.3 and 2.9. Lindegren and Lindegren (1941) found in Neurospora that diminished fertility, which was due to chromosomal rearrangement, was induced by X-ray treatment but not by ultra-violet irradiation at 254 mu. The changes induced by the latter included single gene mutations as well as "degenerate phenotypes", whose numbers significantly exceeded those of the single gene group.

In Drosphila Mackenzie and Muller (1940) showed that ultra-violet irradiation resulted in gene mutation as distinct from gene rearrangement, but this finding has since been contradicted by Slizynski (quoted in Cold Spring Habor Symposium, "Genes and Chromosomes").

In maize, Stadler (1941) found that numerous endosperm deficiencies and embryo abortions resulted from ultra-violet irradiation. Chromosome rearrangements were not produced, whereas they were common in X-ray material. Unlike the X-ray results also, the frequencies of the endosperm deficiencies and embryo abortions induced by ultra-violet irradiation were different. The ultra-violet dosage was the same at all wavelengths and under these conditions the ultra-violet effect did not occur at wavelengths longer than about 310 mu, and 254 mu was the most effective wavelength for the production of endosperm deficiencies, "the effect diminishing with both shorter and longer wavelengths and reaching a negligible value at 302".

In the liverwort Sphaerocarpus, Knapp and Schreiber (1941) found that the frequency of mutation induced by ultra-violet radiation was a maximum at 265 mu, falling off on either side and reaching zero at 313 mu. Equal doses of radiation were applied at all wavelengths. Genetic effects and an effect on sporogonium attachment were found, the latter having a high frequency of occurrence at short wavelengths.

In general, the literature presents an incomplete picture of the mechanism of the changes induced by ultra-violet radiation. The range of ultra-violet wavelengths has been explored incompletely, either because only a single wavelength was investigated (Mackenzie and Muller, 1940), or because the dose applied at long wavelengths was too small (Emmons and Hollaender, 1939; Knapp and Schreiber, 1941), or because method of test or cultural technique could not show up differences in effect (Emmons and Hollaender, 1939). Moreover because nucleic acid was known to be a universal constituent of nucleoprotein and to absorb strongly in the short ultra-violet, it was concluded invariably that the results obtained were to be interpreted in terms of nucleic acid absorption, and even that gene change depended upon initial absorption by nucleic acid (Mackenzie and Muller, 1940). In many cases the similarity found between the freqneucy of the induced effect and the curve of nucleic acid absorption is fallacious. However, the data of Knapp and Schreiber (1941), Lindegren and Lindegren (1941), and Stadler (1941) are indicative rather of differential effects of ultra-violet radiation, associated on the one hand with nucleic acid absorption and on the other hand with absorption in the gene protein.

Study of the effects of ultra-violet radiation upon C.globosum has had to take into account a number of features of its biology (Appendix 1).The colony is fundamentally a cell population and considerable variation was observed in it. The term "variation as used in this work on C. globosum denotes heritable change as distinct from environmental modification. The nature of this heritable change is concluded to be qualitative gene change at least in those variants used for the assessment of radiation effect. However, variants of a group all resembling the parent type more or less closely, were not used om these experiments as a measure of radiation effect because they could either appear spontaneously or be induced by the irradiation. Variants of this residual group are described by the term "strain", which is used in the same sense as "variety",

"race", "subspecies" and so on are used in other organisms; all such terms describe forms more or less genetically similar to the parent.

In elaborating an experimental procedure for this work the composition of the colony and in particular the functional individualism of its cell units, had a profound effect. Factors which had to be taken into account were:-

(1) Character change was not distinct but showed great intergradation, and there was lack of constancy in response to the irradiation between successive experiments. Results were therefore assessed over a series of experiments rather than single ones, which in turn made it necessary to select the characters in which change was to be observed so that variation could be determined by inspection rather than by detailed examination. Because a large number of colonies had to be examined, the use of characters requiring detailed examination was not practicable.

(2) The occurrence of strains in both control and irradiated series made it desirable to disregard them in assessing the effect of the irradiation. (Even so, it was most difficult to set limits in practice to their range, particularly in respect to those involving characters of the mycelium and perithecium, which made up a majority).

(3) Irradiation had different effects on different spores, influencing early growth as well as causing variation. The spores had therefore to be considered individually and the colonies obtained from them grown singly. Single spore and dilution plating methods were therefore employed and the colonies isolated in single petri dishes. Such methods were also required to obtain quantitative data.

(4) Spores were irradiated, not only because they were uninucleate while the mycelium was multinucleate, but because any sample of mycelium would consist of several cell units each of which could be affected and then reproduce separately and independently.

(5) Variation in response by the material necessitated the use of a biological dose indicator to measure the mean radiation effect on the sample, the physical measure of dose only providing information concerning the amount of radiant energy reaching the nucleus, in itself subject to considerable error because of technical difficulties and the lack of absorption data for the biological material. The biological indicator was, moreover, the only basis for comparison between wavelengths.

Of the range of ultra-violet wavelengths available in the mercury vapour spectrum, (which was the source used), it was decided to work with three only in these experiments, namely 265, 313 and 334 mu. These three wavelengths were selected not only because data from a long series of wavelengths might obscure a general mechanism in a preliminary examination, the mechanism becoming evident if data were compared from a few wavelengths covering the ultra-violet range, that is, short, middle and long ultra-violet;

but also because they lie, respectively, within, on the boundary of, and outside that region of the ultra-violet for which induced change has been reported. Apart from the work of McAulay (1939), ultra-violet wavelengths longer than about 300 mu have been considered generally ineffective in inducing heritable change.

METHODS

(a) THE GROWING OF THE COLONY.

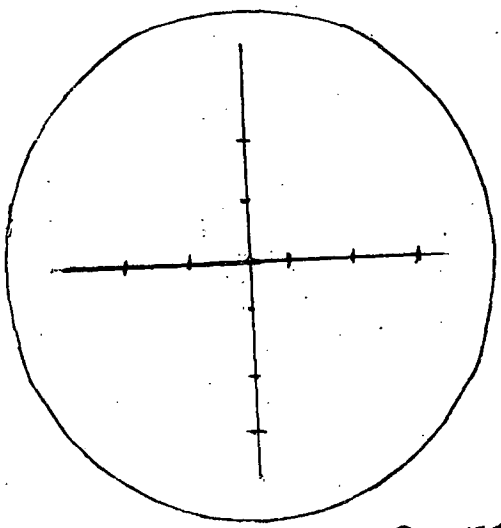
Colonies of single spore origin were obtained from the irradiated and control spore samples by dilution and single spore plating; the colonies so obtained were then grown in separate petri dishes. Usual aseptic methods were used at all stages.

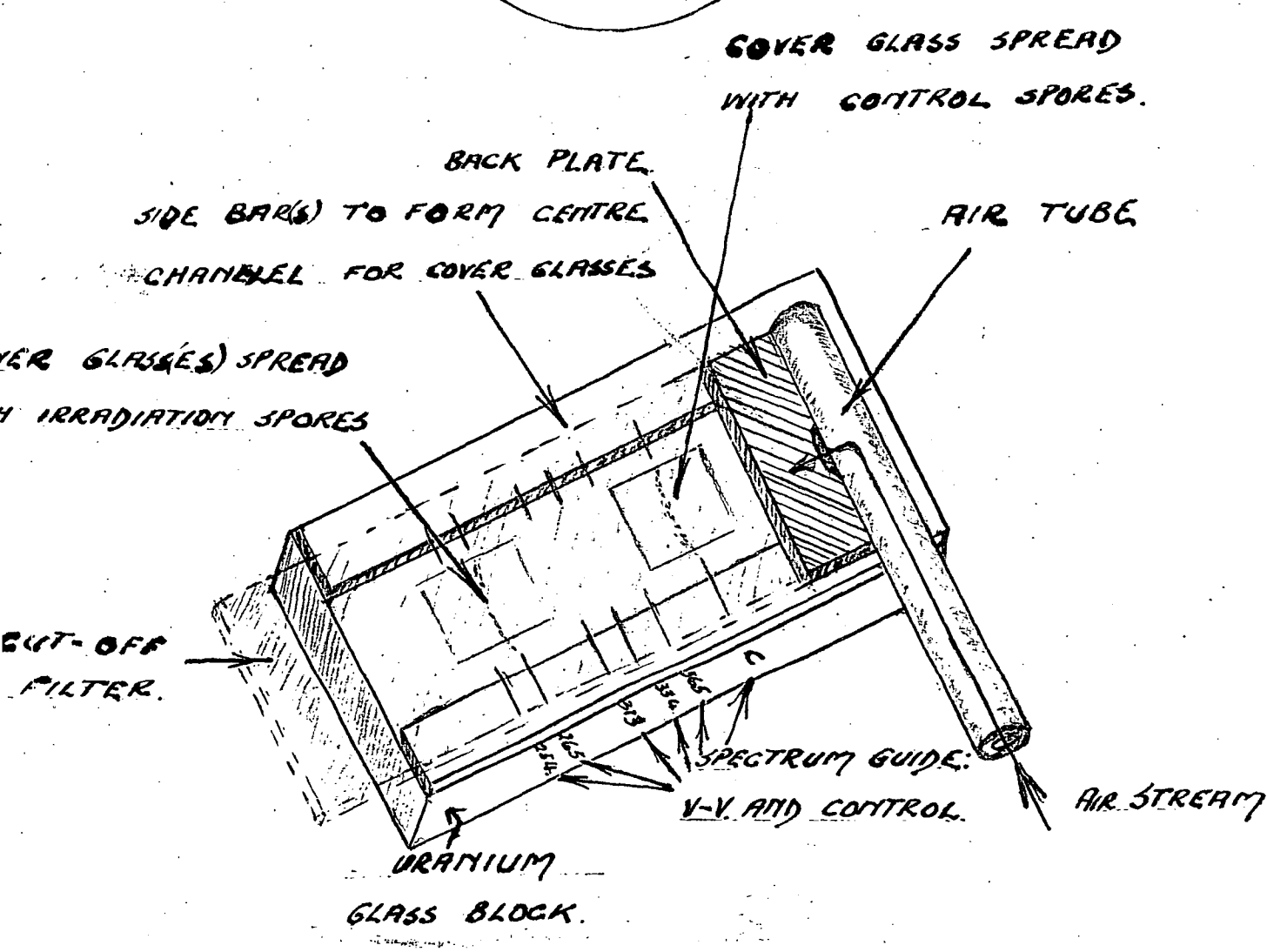
Single spore platings were first made from each sample to determine their germination and early growth characteristics. A hard cleared Agar agar was poured into a petri dish in a thin layer and allowed to solidify. Two intersecting lines were drawn on the underside of the dish with a glass pencil and small regularly spaced scratches made in the agar to cross them. (Text-fig. 1A). A small dab of spores, as few as possible, was lifted from the spore sample on a coverslip (see below) with a rounded platinum needle tip and placed on the agar to prevent overgrowth from it. Under a binocular microscope single spores were picked up with a platinum point and one placed at an end of each small scratch in the agar. The spores were spaced about 1 cm from each other.

This method of single spore plating was found to be reliable with C. globosum spores and was developed as a fairly quick routine method. The spores, which measure about 9 μ x 7 μ, could be seen and handled readily under the binocular microscope using the high power objective and 10 X eyepiece (total magnification about 60 X). A fine point was ground on the needle which was about an inch long and of fairly stout platinum wire so that it did not bend readily. Before using it each day the needle was usually re-ground to a clean point of such a size that an individual spore could be touched. Much of the success of the method depended upon getting a satisfactory needle point. The needle was mounted firmly in a metal holder selected to balance comfortable in the hand; in this way it could be held loosely enough to minimise shaking and vibration. Except for slight movements, chiefly the vertical movement of picking up and putting down the spore, all manipulation was done by moving the petri dish with

TEXT FIGURE 1.

A.





B.

SCALE — ABOUT NATURAL SIZE.

A. Diagram of typical plating of single spores ~~(see p. 4)~~. Cross lines marked on bottom of dish. Scratches in surface agar. Spores transferred singly from central mass and plated close by end of each scratch.

B. Holder for cover glasses spread with spore sample. Control and irradiation samples mounted in constant relation to spectrum by guide lines, screened from scattered short wavelength radiation with cut-off filter, and constant environment maintained by air flow.

with the other hand. It was found that with practice the movements of needle and dish were co-ordinated and smooth, with a minimum of shaking and irregular movement in the microscope field. The routine platings in this work were 100 irradiated and 50 control spores; they would take about 4 hours to plate.

The single spore plates were examined from time to time and each spore recorded as having either (a) failed to germinate, when there was no sign of any germination of the spore; or (b) formed a visible colony or failed to do so, when a germinated spore did or did not reach such a size that it was clearly visible to the naked eye (arbitrarily about 5 mm diameter; if a colony reached this size it would continue to grow in nearly every case).

Dilution plates were not prepared until an approximate germination count had been determined from the single spore platings. Using these counts as a basis, dilutions were calculated to give a plate in which the colonies would not be crowded. Dilution plates were then prepared in the usual way. The spores left on the coverslips were suspended in water; the cover glass was dropped into a conical flask containing the required volume of sterile water and shaken thoroughly. 1 ml of suspension was pipetted into each sterile petri dish. Melted agar was then mixed well with the suspension and allowed to solidify. The plates were incubated and all the colonies in one or more of the dishes picked off as they appeared and plated out each in a single petri dish.

The control and irradiated series were made up partly of colonies appearing in the dilution plates and partly of colonies of the single spore plates. Usually all the visible colonies were taken from the single spore plates in the irradiated series and a total of up to 200 colonies obtained with the addition of colonies appearing in one or more of the dilution plates. The colonies from the single spore plates comprised about 36% of all the experimental colonies. In each experiment 50 control colonies

were plated usually, comprising colonies from single spore and dilution plates. The colonies were incubated at constant temperature until mature, when they were examined and counted. The incubation temperature was about 28°c. In the earlier experiments temperature could not be controlled accurately and differed sufficiently through the incubator space to cause appreciable effects. Such environmental modifications could, however, be discounted by maintaining the positions of the plates by stacking them in sequence in frames. In later experiments on the other hand, incubator temperature was controlled within small limits and heat layering minimised by mechanical stirring of the air. By this means environmental modification was so reduced as to be negligible.

By means described above, series of irradiated and control colonies were obtained, each colony in its petri dish being of single spore origin. This was definite with those colonies taken from the single spore plates because each spore had been plated individually, and was probably the case with the dilution plate material also.

(b) SAMPLING OF SPORES FOR IRRADIATION.

Spores were gathered from a single spore colony with a sterile brush and thoroughly mixed with a few drops of sterile water on a microscope slide. This suspension was then smeared over the slide and allowed to dry off. This sample of spores was drawn upon for a series of experiments.

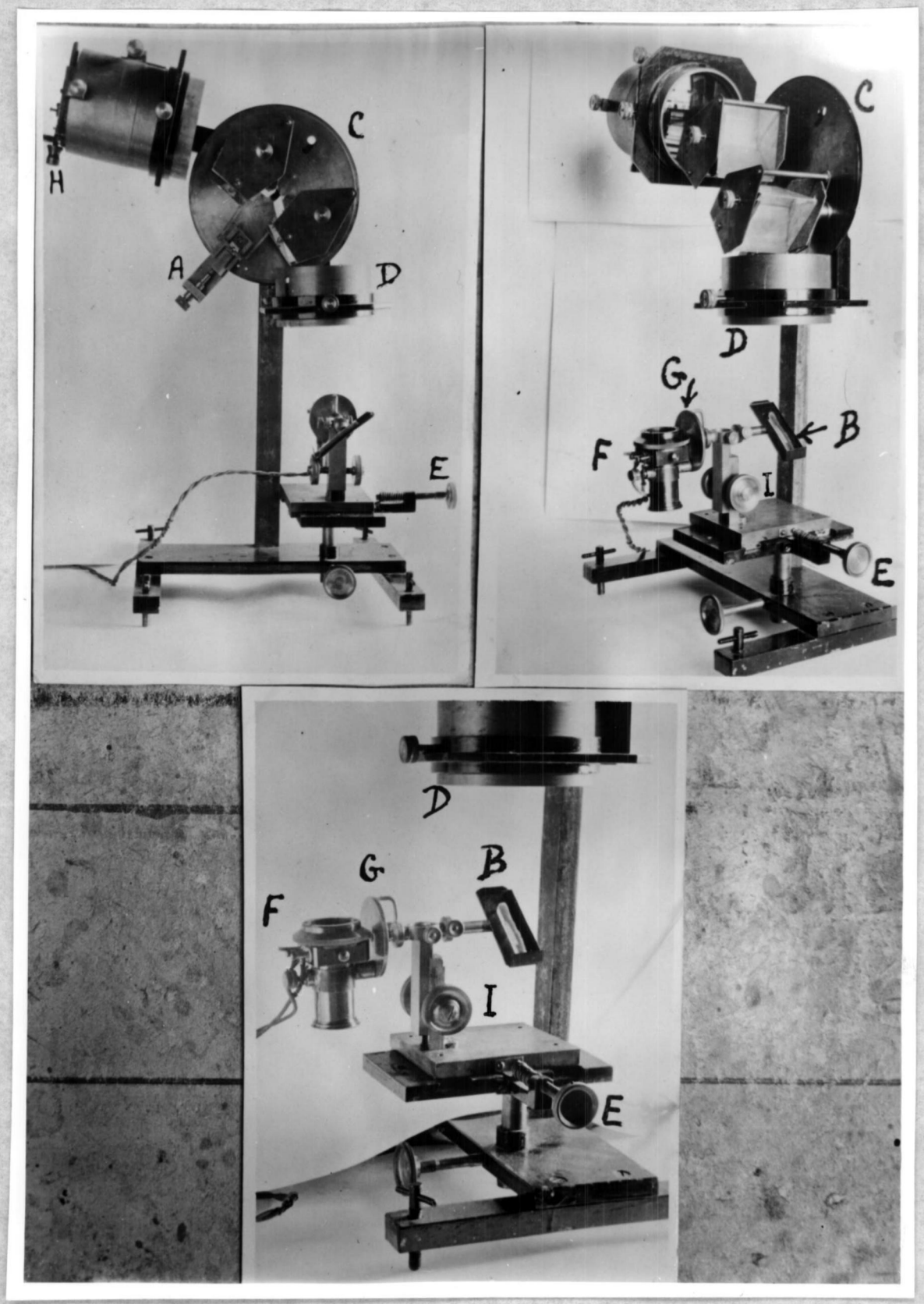
(c) PREPARATION OF THE SPORE SAMPLE FOR IRRADIATION.

The wavelength beam focussed by the monochromator was a slightly curved band about 25 mm long x 1.5 mm wide within which the spore sample had to be confined. A narrow band of spores was spread on a microscope cover glass in the following way. A good quality camel-hair brush was trimmed to a long point consisting of 2-3 hairs only, and sterilised in the

autoclave. A guide plate had already been prepared by cementing to a glass plate a 2" x 1" microscope cover glass, marked on its under surface in indian ink with two lines spaced about 1 mm apart, i.e. less widely than the wavelength beam, and with the same curvature as the beam. The guide plate was sterilised by flaming and a sterile $\frac{5}{8}$" x $\frac{5}{8}$" microscope cover glass mounted on it across the guide lines; a simple and effective way of fixing the cover glass to the plate was to touch each of its corners with a molten bead of paraffin wax. Moistening a brush in sterile water, spores were picked up on its tip from the spore sample and transferred to the coverglass. As with single spore plating, the mounted cover glass was observed with the high power binocular, the brush being held in the focus while the other hand moved the guide plate. First, heavy dabs of spores were set within the guide lines, then they were spread into a narrow dense line, and finally the band was widened out and thinned within the boundaries of the guide lines with a clean damp brush. The chief difficulty was to thin the spore band so that spores were not clumped together or over-lying one another; this was desirable so that spores were not shielded from the irradiation. In this and other techniques spores were in contact with water for minimum time as it appears that growth begins within 2-3 hours (Appendix 1).

Two cover glasses were spread with spores for each experiment one was irradiated and the other used as a control. The glasses were mounted for irradiation in a holder (Text-fig. 1B) with a uranium glass base. Guide lines with the same spacings as the spectral lines were marked on this base so that the control and irradiation spore bands were always mounted in the same positions relative to the spectrum projected by the monochromator, the control being in the visible spectrum and the experimental spores in the selected wavelength beam. Fluorescence in the uranium glass enabled the selected spectral line to be focussed accurately on the spore band; at short wavelengths where the cover glass acted as a cut-off filter shielding the uranium glass, the line could be focussed on the spore band by means of the guide lines and

TEXT FIGURE 2.



MONOCHROMATOR

Top left: side view of monochromator, Top right: half front view of monochromator, Bottom: enlarged view of irradiation table.

Explanation: A: adjustment for moving prisms, B: uranium glass sample holder (Text-fig. 1B) fits here, C: mounting holding fused quartz prisms, D: tube holding telescope lens of fused quartz, E: screw for adjusting horizontal position of table and thermopile, F: thermopile and slit, G: adjustment for rotating thermopile, H: collimator slit, I: screw for adjusting vertical position of table and thermopile.

by the bluish fluorescence of the spores themselves.

During irradiation a stream of dry air was passed over the spores to remove any ozone formed and to maintain control and irradiation spores at the same temperature. A constant air flow was maintained from a filter pump - aspirator bottle setup, the air being dried by passing through calcium chloride.

The experiments were usually arranged so that there was an interval of about two days between spreading the spore band and its irradiation, and another two days from the end of irradiation to plating.

(d) IRRADIATION OF THE SPORES

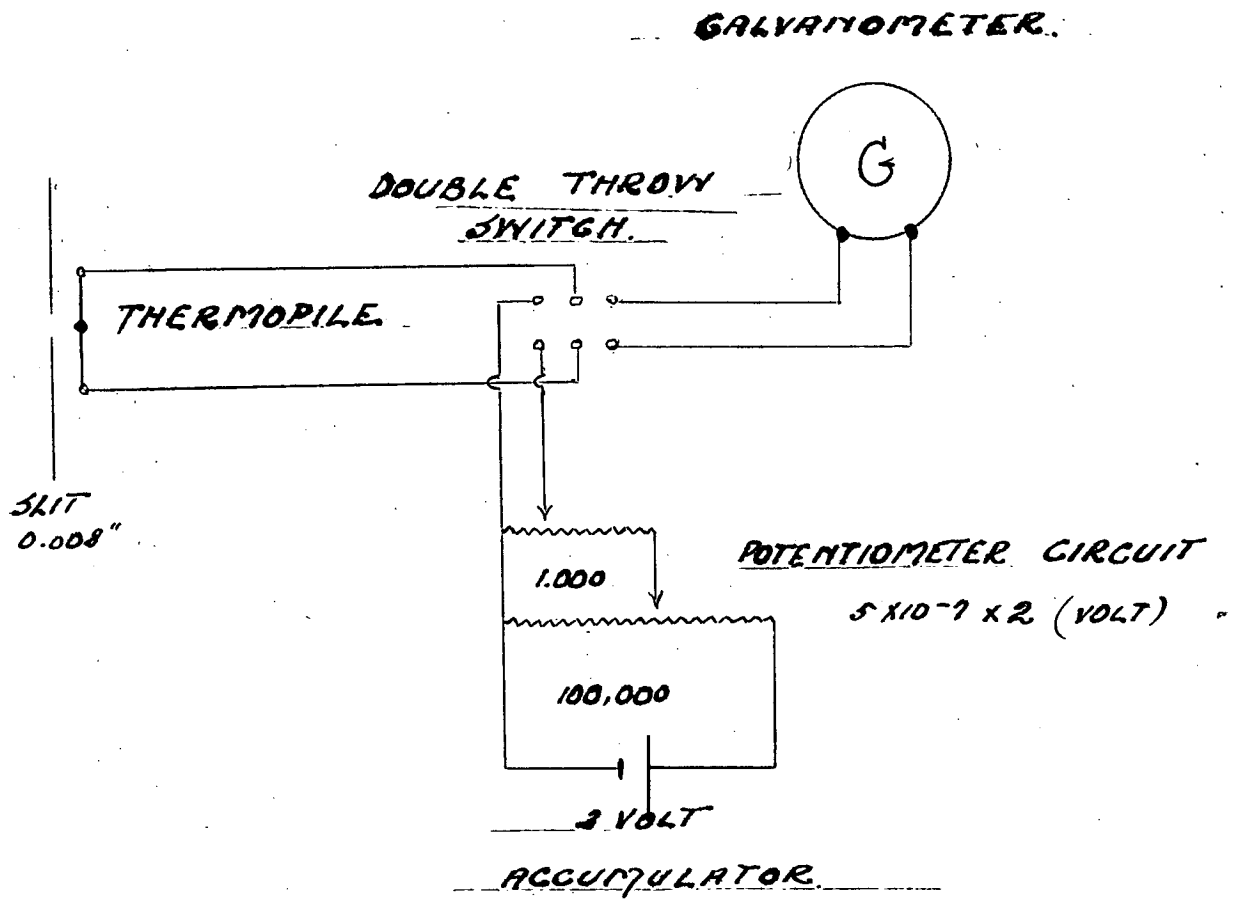
The monochromator used in the present work was that described by McAulay (1939), details of which are shown in Text-fig.2. The irradiation table was fixed to a vertical column which could be moved by a rack and pinion to bring any part of the spectrum into focus. The table could also be moved horizontally so that any spectral line could be brought to a definite position on the table.

The ultra-violet source used in these experiments was a "Mercra" 125 watt mercury vapour lamp (a commercial street-lighting unit with the outer envelope removed). In a few of the earlier experiments the source was an "Hanovia" UVS 500 lamp, but this type was found unsatisfactory. Because only ultra-violet entering the collimator slit was used, intensity per unit area was of importance rather than the total lamp output. In this respect the "Hanovia" lamp compared unfavourably with the "Mercra". Moreover, the "Mercra" would function continuously for several weeks at a time, while the "Hanovia" type was unreliable, breaking down continually during long irradiations. The long life of the "Mercra" was another good feature of this type of lamp.

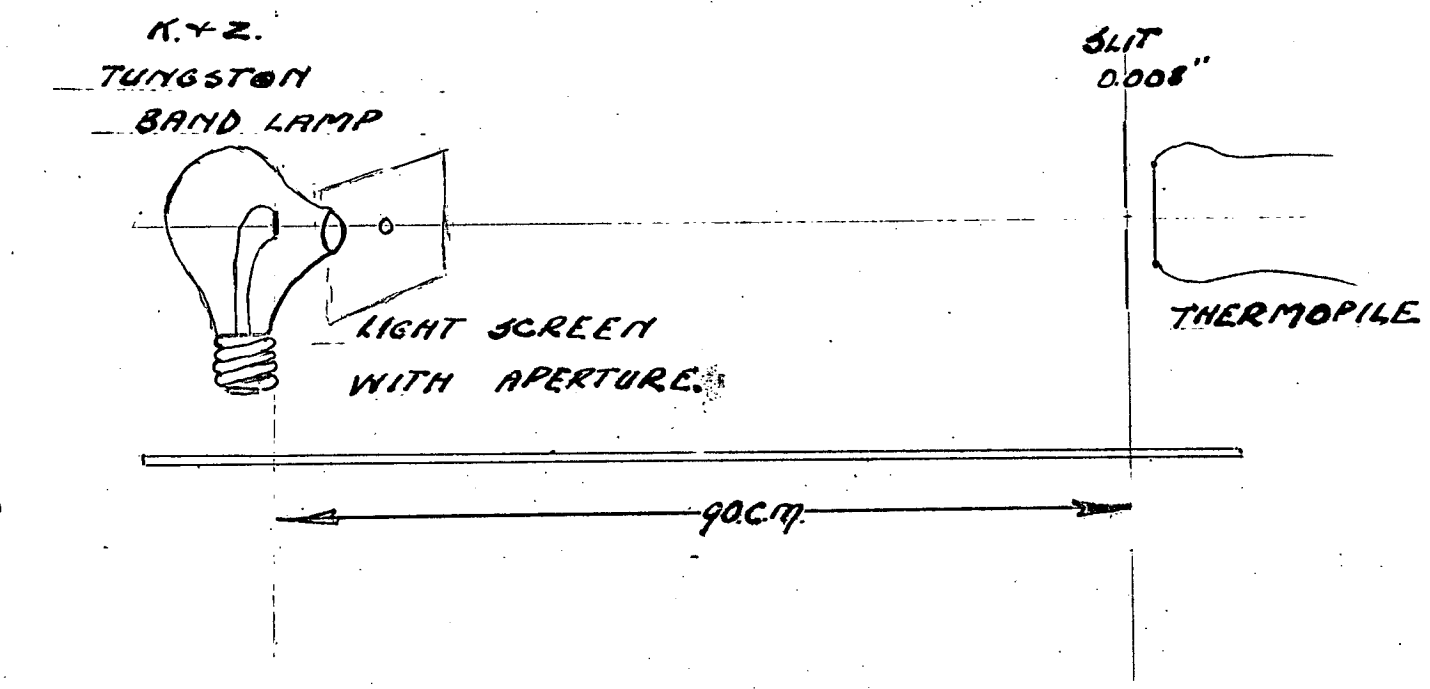
To shield the spores from scattered radiation, particularly from the highly effective short wavelengths during long wavelength irradiations, monochromator and lamp were enclosed and the spores

TEXT FIGURE 3.

THERMOPILE CIRCUIT:



THERMOPILE CALIBRATION BENCH.



screened with cut-off filters. These filters were selected so that wavelengths shorter than that being used in the irradiation were not transmitted. The filter was "Corex" glass in 265 mu irradiations, and various thicknesses of microscope slides in 313 and 334 mu irradiations. That these methods were very effective was shown by the absence of induced effects in controls and short wavelength effects in long wavelength irradiations even though the duration of long wavelength irradiations was much greater; thus, at 334 mu spores were irradiated for about 4000 times as long as at 265 mu. The filters used for 313 mu irradiations were not effective in screening off all short wavelengths and transmitted a little 297-302 mu light; this probably explains the greater variability in results at this wavelength,

(e) MEASUREMENT OF RADIATION DOSE

The dose of incident radiation was determined from measurements of the intensity of the spectral beam, using a calibrated thermopile and sensitive galvanometer. The apparatus consisted essentially of a thermopile-galvanometer circuit, with a subsidiary circuit for routine checking of galvanometer sensitivity (Text-fig. 3).

Thermopile. Various thermopiles were used during the work, usually either a Hilger F8o or a locally made vacuum thermopile. The thermopiles were mounted behind a slit; in all work the slit width was o.oo8 inch, adjusted to a feeler gauge. Continual trouble was experienced with the thermopiles, due mainly to variability in response (sensitivity), creep and fluctuations caused by temperature change. The thermopile and slit were mounted on the vertical column carrying the irradiation table in such a way that table and thermopile could be interchanged (Text-fig. 2). The spectral beam whose intensity was being measured was focussed on the slit by its fluorescence of uranium glass.

Galvanometer. A Cambridge "Broca" galvanometer was used throughout the work. In spite of heavy shielding to minimise the effect of varying magnetic fields and wall mounting to reduce mechanical vibration, the instrument was subject to variability, particularly

as regards sensitivity. Galvanometer sensitivity was always checked before using against a standard voltage, approx. 1 X 10-6 volt, before making routine intensity measurements.

Calibration of Thermopile and Galvanometer. The thermopile-galvanometer circuit was calibrated against the emission of a standard lamp, the type used being a Kipp and Zonen Tungsten band lamp. Lamp and thermopile were mounted on an optical bench (Text-fig. 3) and galvanometer deflection measured for standard distance between slit (thermopile) and lamp filament.

Procedure. The following general procedure was adopted. At frequent intervals, usually before each measurement, the thermopile was calibrated against the standard lamp, and the sensitivity of the galvanometer determined by means of the voltage applied through the potentiometer circuit. A figure was thus obtained for thermopile sensitivity in terms of units of galvanometer deflection equivalent to a known lamp output, and was the standard for the series of intensity measurements. In measuring spectral beam intensity, slit and thermopile were adjusted so that the beam was focussed sharply on the slit and galvanometer deflection recorded; a mean value was taken for at least six swings to and from rest. In all work the thermopile was adjusted, to ultra-violet beam and to standard lamp emission, for maximum galvanometer deflection. Such adjustment was most important, because (a) slight skewness to the plane of the beam would mean that the thermocouple junctions would be radiated incompletely, giving low values, and (b) the narrowness of the slit necessitated accurate centering of the thermocouple junctions behind the slit. Altogether, in any measurement of a spectral line, attention had to be paid to the following adjustments:- (1) the alignment of the spectral band to the slit, (2) its sharp focus (horizontal and vertical) on the slit, (3) the centering of the thermopile below the slit, and (4) the tilt of the thermopile (skewness to beam). After these adjustments were made, the cut-off filter suitable to the chosen wavelength was placed above the slit, i.e. the intensity measurement represented the incident energy falling on

on the spore.

Some Calibration and Intensity Measurements

(a) Calibration of Thermopile:

Deflection of galvanometer for emission of standard lamp	130 mm.
Intensity of lamp at slit (90 cm), from tables	4.06×10^3 ergs/cm^2/sec
Galvanometer sensitivity (potentiometer)	132 mm.

i.e. energy of lamp emission 4.06×10^3 ergs/cm^2/sec produces a galvanometer deflection of 130 mm.

thermopile constant is:

$$4.06 \times 10^3 / 130 = 31.2$$

(b) Intensity Measurements:

All intensities were measured on filtered radiations i.e. the measurements represented incident energies at the spore. Dose was calculated as: intensity x irradiation time.

Two measurements of 313 mu were:-

(1) Galvanometer deflection for spectral beam 210 mm.
Galvanometer sensitivity 132 mm

Intensity 313 mu

= Thermopile constant x galvanometer deflection x galvanometer sensitivity correction x time (hour).

= 31.2 x 210 x 132/132 x 3600

= 2.36×10^7 ergs/cm^2/hour

(2) Galvanometer deflection for spectral beam 162 mm
Galvanometer sensitivity 108 mm

Intensity 313 mu

= 31.2 x 162 x 132/108 x 360

= 2.22×10^7 ergs/cm^2/ hour

(c) Miscellaneous Values:-

Wavelength	intensity
334 mu	4.0×10^6 ergs/cm^2/hour
do	4.4×10^6 do do
313 mu	2.4×10^7 do do
do	3.2×10^7 do do
265	8.7×10^6 do do
do	9.8×10^6 do do

(f) Biological Dose Indicator

The biological effectiveness of the dose of irradiation was measured by the extent to which colony formation was inhibited. The data for these measurements were obtained from the single spore plates, counts being made of the numbers of germinating spores which were successful in forming visible colonies. The ratio of this quantity to the total quantity of germinating spores indicated the mean effect of the radiation on the sample. These visible colony counts, as they were called, were made as a routine in each experiment rather than physical measurements. On the basis of equal biological effect at each wavelength, about 46% visible colonies(mean), the following is a summary of the dosage data:-

	265 mu	313 mu	334 mu
Spectral intensity (ergs/cm^2/sec)	1.0×10^7	2.5×10^7	4.5×10^6
Irradiation time	3 mins.	10 hours	9 days.
Incident dose ergs/cm^2	0.05×10^7	25×10^7	100×10^7
Wall transmission	28%	26%	42%
Dose transmitted through wall	0.014×10^7	6.5×10^7	42×10^7
Contents transmission	20%	42%	39%
Dose incident to nucleus	0.0028×10^7	2.7×10^7	16.4×10^7
Factor of 265 mu dose	x1	x1000	x6000

The above figures should be taken as representing an order of magnitude rather than the actual mean dose, since no account could be taken of variables arising in the physical measurements, and in biological variation; while for the correction for absorption in the spore wall and cytoplasm Uber's (1939 and Appendix 3) figures for maize pollen were used.

Footnote 1: McAulay (1939) gives the order of magnitude of the optimum dose for production of change in C.globosum as 2×10^7 ergs/cm^2/ at 265 mu and 200×10^7 ergs/cm^2 at both 313 and 334 mu, while McAulay and Taylor (1939; fig. 3) give the doses for 50% germination of C. globosum spores as 1.3×10^7 ergs/cm^2 at 265 mu, 15×10^7 ergs/cm^2 at 313 mu and 150×10^7 ergs/cm^2 at 334 mu. McAulay's figures are based on a multispore plating technique.

RESULTS

(a) VARIATION IN C. GLOBOSUM

1. Characters selected for observation

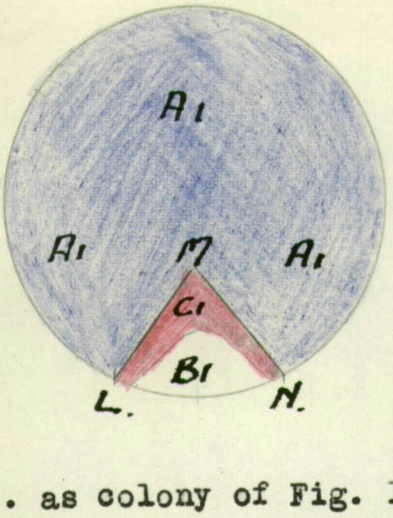
The variants selected for observation in C. globosum were those in which change had occurred in the character of mycelium, perithecium or some feature of growth in the colony; variation was determined by inspection. Distinctions between the variants were not clear-cut, intergradation being extreme. Variation in one character was frequently associated with variation in another. For example, a mycelial variant would often have atypical perithecia; and variation of growth form covered a wide variety of individuals showing not only a considerable variation in this character but differing greatly in other respects, some having perithecia others none, some pigmentation others none, and so on. Description of the variants would, in fact, involve description of individuals in most cases, which for the purpose of this investigation of irradiation effect would be meaningless. A brief account will therefore be given of the way in which each character was found to vary, descriptions being supplemented by photographs in suitable instances (Figs. 1-43):

Mycelial Variants (Figs. 34,35). Variation in mycelial characters could involve increase or reduction in the amount of aerial mycelium. The hyphae could form a dense mat over the surface of the colony or aerial mycelium could be almost lacking or the surface mycelium could form a tough layer. The colour of the mycelium ranged from white to brown and reddish.

Variation involving mycelial characters was usually associated with variation involving a diminution in the number of perithecia, or their absence. In fact with many forms it was the smaller numbers or lack of perithecia rather than the character of the mycelium which was the distinguishing feature of the variant.

Perithecial Variants. The perithecia were subject to a great deal of variation, involving their number and distribution as well as their size and colour. The perithecia could be larger than in the normal or tiny, sparse or occur in zones, and could be

TEXT FIGURE 4.



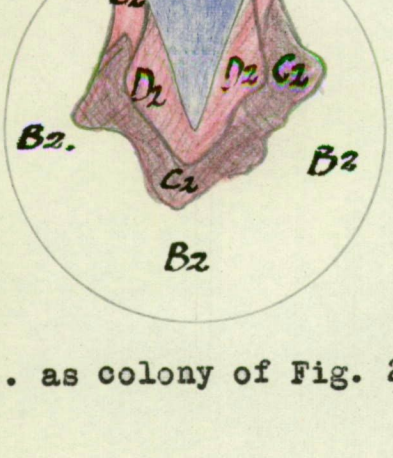
X ... as colony of Fig. 19.

A_1 ... normal mycelium.

LMN... part of colony showing "damage".

B_1 ... empty space in agar medium into which mycelium has failed to grow.

C_1 ... fringe of mycelium showing "growth-damage". It is devoid of perithecia.

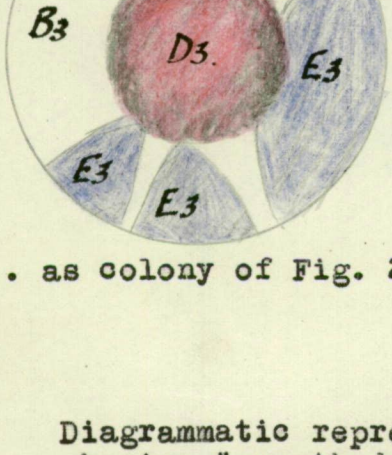


Y ... as colony of Fig. 24.

A_2 ... normal mycelium. The rest of the colony shows "growth-damage".

B_2 ... empty space into which mycelium has failed to grow.

C_2, D_2 ... damage mycelium, consisting of an outer fringe devoid of perithecia and with heavy brown pigment formed within the medium (cross hatching); and an inner part D_2 more or less free from pigment and containing a few scattered perithecia.



Z ... as colony of Fig. 28.

D_3 ... colony in which growth has ceased early leaving central mass of "damage" mycelium, densely matted and knotted; with heavy pigmentation.

E_3 ... Secondary growth of normal mycelium into space B_3 surrounding central mass.

Diagrammatic representation of three colonies showing "growth-damage" of various degrees.
X.. light "damage"; Y,Z..heavy"damage".

coloured brown, yellow, black and so on. The variant Fld which was used as parent in many of these experiments and had been induced originally by ultra-violet irradiation of C. globosum, differed principally from C. globosum in its peritnecia being smaller, brown in colour and not so numerous.

Variation in Colony Development .

(a) Variation involving growth rate (Figs. 36, 37). These variants were distinguishable from the parent only in their growth rate. The slow growing types usually occurred as sectors, the rest of the colony being normal. This normal mycelium would eventually enclose the slower growing mycelium. Usually the slow growing mycelium had its origin at the centre of the colony, but a form was found in one experiment in which slow growing sectors, (which bred true in subculture) developed around the edge of the colony (Fig..37).

(b) Variation involving growth form (Figs. 38-43). The characteristic feature of these variants was that growth of the mycelium appeared to be directed along axes, like the spokes of a wheel. There were two main types between which were many intergradations. The "seaweed" type superficially resembled one of the Red Algae such as Rhizoglossum; aerial hyphae were or were not developed and the subsurface mycelium could be so sparse that the colony was nearly invisible. In the "scollop" type the mycelium appeared to have been laid down in successive small semicircular segments; it resembled Chaetomium elatum in this respect.

(c) "Growth-damage" (Figs. 16-29, 33; Text-fig. 4). "Growth-damage" was an easily recognisable and characteristic condition. It had the same features whether it was found in C. globosum , in its variants or in other species of Chaetomium ; the condition was similar in C. globosum; in its variant Fld, and in its variant st 2 which closely resembles Chaetomium murorum; as well as in Chaetomium elatum . The plate colony showed spaces empty of mycelium, and when the condition was not pronounced (Figs. 16-20; Text-fig. 4 X.) the rest of the colony was normal

and the "damage" consisted of a fringe of mycelium devoid of perithecia, which bounded the empty medium. However, when the condition was more marked (Figs. 21-29; Text-fig. 4. Y,Z), all the mycelium of the colony or "damage" sector was more or less abnormal, heavy brown pigment being formed within the agar medium especially at the outer limits of growth; a few perithecia occurred scattered here and there over the surface. The surface mycelium in the most extreme condition (Figs. 26-29; Text-fig. 4 Z) consisted of a central mass of heavily matted and knotted hyphae. Mycelium and perithecia could eventually appear in the empty spaces, as in the colony of Fig. 28 (Text-fig-4 Z.), but they probably originated secondarily in normal mycelium and not in "damaged" mycelium. Only very incomplete information is at present available concerning the spores from such perithecia and from those scattered in the mycelium of areas of "damage"; those that grew nearly always developed into normal colonies, so that it is probable that most of the peritnecia had been formed in "undamaged" normal mycelium. Mycelial subcultures from "damage" areas either did not grow, or produced colonies showing normal and "damage" sectors, or produced normal colonies.

(Certain other forms were tentatively classified as "growth-damage" types, although their true nature was far from certain. (Tables 1-3, Group 8). They were much less common than the true "growth-damage" form, their frequency being of the same order as that of the other variant types. In them early growth of the colony was abnormal, patches of sparse and abnormal mycelium occurring at the centre of the colony. Surrounding this abnormal tissue was more or less typical mycelium. The mycelium of the "damage" areas was frequently brownish).

2. Mode of occurrence of the variants

The variants were found either as whole colonies or as sectors in the colonies. It was most usual for them to occur as sectors (see Figures generally). Many of the mixed colonies were known to have originated in single spores: all those from the single spore plates did so and it was likely that colonies

from the dilution plates had single spore origin in most cases. All variant types, and the "growth-damage" forms in particular, could appear as sectors. In size these sectors ranged from very small ones arising near the edge of the colony to those occupying almost the whole of the colony, their shape and the area of the colony occupied being determined by the relative growth rates of variant and normal mycelium in the mature and first formed colony (Pontecorvo and Gemmell, 1944; Plomley, Appendix 2). Although it was usual for only one variant type to occur in a colony (either with or without the normal tissue) colonies were found occasionally in which two or more variant sectors occurred (Figs. 30-33). Most of these mixed colonies were found in dilution plates, but some came from the single spore plates so that there was no doubt that they could have single spore origin.

A number of variants, particularly those in which change had occurred in mycelium or perithecium, were found quite commonly in both control and experimental series. They resembled the typical form more or less closely and could be traced through a series of gradations to it. In accordance with usual biological practice, the term "strain" is used to describe these variants closely resembling the parent organism. The strains most usually found were those in which size, colour, numbers and distribution of the peritnecia, and colour, density and characters of the aerial mycelium were affected (Figs. 1-15). Perithecia ranged in size from small to large, in colour from the usual dark green to blue-green or to yellow-green and white (sterile), while spores were extruded abundantly or not at all. There were more perithecia than typically or few to none, and they were distributed evenly over the plate, or in zones, or irregularly. Aerial mycelium was more sparse than in the typical form or more dense; in extreme cases there was little development of aerial mycelium or it was so densely matted as to hide the perithecia and form a thick mass filling much of the space above the agar surface. In colour the aerial mycelium ranged from white to pink and brown. It was quite usual for

for variants to appear in or to be isolated from old colonies. Only one or two types were found as a rule, most of which did not produce fertile spores; in a common form there were no peritnecia and the aerial mycelium was dense and fluffy, while in another form perithecia were few or absent and the aerial mycelium sparse.

In practice the strain was delimited from the measured change induced by irradiation by the regular and normal appearance of the former in the control series; the latter, of which "seaweeds" and "scollops" and "growth-damage" are typical, were found rarely or not at all in controls. As the variant character diverged from the normal it became increasingly difficult to decide whether to classify the form as induced variant or strain; the colony illustrated in Fig. 9 was just such a case, representing in extreme the reduction in number of the perithecia which was found in many induced variants (Figs. 34,35.).

(c) QUANTITATIVE ANALYSIS OF INDUCED VARIATION.

Spores of C.globosum were irradiated monochromatically at 265, 313 and 334 mu. Colonies growing from these spores and from the control spores were grouped by inspection into (a) induced variants and (b) the rest of the colonies comprising normal colonies (including those showing environmental modification) and the strains. In each experiment as many of the variants as possible were subcultured; all types of induced variant (except "growth-damage") were found to breed true.

The induced variants were classified into eleven groups according to the dominant character change exhibited (Tables 1-3):-

Mycelial variants

1. Aerial mycelium longer and more dense than in normal.
2. Aerial mycelium sparse or lacking.
3. Aerial mycelium leathery.

Peritnecial variants

4. Perithecia same size as normal or larger but colour differen
5. Perithecia smaller than normal and colour different.

"Growth-damage"

6. Light and heavy "damage"; no formation of brown pigment.
7. Light and heavy "damage", with brown pigmentation,
8. "Damage" other than Groups 6 and 7: ranging from light "damage" at colony centre to heavy "damage" with knots of white mycelium.

Variation involving growth rate

9. Variant growth rate slower than normal rate.

Variation involving growth form

10. "Seaweed" forms
11. "Scollop" forms.

Other than "growth-damage" (Groups 6-8), the maximum frequency of occurrence of any variant type is only about 1%, with a mean value of about 0.4%. However, because the variants intergrade so much, the individual groups are indefinite and any separate analysis of them is meaningless.

The experiments listed in the tables cover two strains of C. globosum, LJ and KE, as well as one variant Flo of C. globosum. The variants produced by irradiation of these different materials are of the same type so that they may be grouped together in a general way, such general groups representing the type of effect produced by the irradiation. Table 2 for 313 mu irradiation includes one experiment, 035, which is atypical of the series. The high percentage of "damage" in this experiment is quite unlike its occurrence in all other experiments at 313 mu. The % visible colonies is low but, against this, heavier doses in other experiments have not resulted in the production of more "damage"; the highest production of 14.3% "damage" in Expt. 071 for nil% visible colonies is much less than the 40.5% "damage" for 29.4% visible colonies in Expt. 035. There are grounds, therefore, for rejecting this experiment as not being representative of irradiation at 313 mu. It is possible that leakage of short wavelength light (e.g. 297 or 302 mu) affected the spores during irradiation; the result is typical of short wavelength irradiation.

Two groups of variants may be distinguished quantitatively in Tables 1-3. The first includes those in which there has been change

in the character of mycelium, perithecium and colony development and in which genetic change will be shown to have been involved; in the second group "growth-damage" has occurred, which will be shown to be due to reaction involving nucleic acid:-

	Wavelength of Irradiation		
	265 mu	313 mu	334 mu
Lethal effect (% visible colonies)	44.1%	39.2%	45.9%
Genetic effects (Groups 1-5,9-11)	3.9%	4.1%	4.4%
"Growth-damage" (Groups 6-8)	31.3%	3.0%	1.3%
Factor of 265 mu dose	x1	x1000	x6000

These results may be summarised as follows:-

1. Heritable variation is induced by short, middle and long ultra-violet irradiation.

2. The dose of irradiation producing equal total genetic effect differs considerably at the three wavelengths tested, at 313 mu being about 1000 times and at 334 mu about 6000 times the 265 mu dose.

3. Induction of genetic effects bears the same ratio to inhibition of colony formation at each wavelength, so that similar reactions are involved in each.

4. "Growth-damage" is quantitatively quite unlike the genetic effect. It occurs much more often at 265 mu, and less often at long wavelengths, being negligible at 334 mu.

DISCUSSION

Before examining the results of the experiments reported here in relation to the mechanism of gene action and in particular to the functions of nucleic acid and and gene protein, those aspects of variation in C. globosum will be considered which concern, firstly, the high frequency of variability observed and, secondly, the nature of the change involved in variation.

(a) VARIATION IN C. GLOBOSUM

1. Frequency and characteristics of variability

Although organisms show considerable differences in the frequency with which variants are found in their populations, these differences are observed rather than real, and arise primarily from differences in reproductive rate. The fundamental identity of mutation rates is shown by the data of Gowen (1941) on the induction of heritable variation by X-rays:-

Tobacco mosaic virus mutations per roentgen per particle	1.2×10^{-6}
Tobacco aucuba virus mutations	5.1×10^{-5}
Phytomonas stewartii mutations	3.7×10^{-5}
Drosophila wild type genes mutations	1.0×10^{-5}
Drosophila mutant genes mutations	1.2×10^{-3}

To some extent mutation frequencies are a feature of the particular population and gene, but differences are small and all values are of the same order. In Drosophila, for example, not only were different strains found to have different mutation frequencies, but different loci in the chromosomes also showed differences (Plough, 1941).

Reproductive rate has two effects upon the observed variability of a population: as it increases mutation appears to occur more often and evolutionary processes in the population are speeded up, so that the observer of the bacterial or virus culture examines a changing population as compared with the relatively stable populations observed in the higher animals and plants. These conditions have often led to doubt whether variation or environmental modifications is involved in groups having high reproductive rates, so that such workers in bacteriology as Topley and Wilson have declared (1936) that "the significance of many of the observations

that have been recorded is at the moment difficult to assess"; a remark which may be applied equally to the fungi. High reproductive rates associated with a mutation frequency of the usual low order can lead to a high observed variability. Many variants can appear potentially during cultural life and the culture will change if the variant is more successful in the environment than the original parent, selection operating within the population. This variability will be enhanced by the changes in the environment in which the population is living, the system being a closed one. Thus, in cultures of Bacterium lactis aerogenes the proportion of snake-forms changes with glucose concentration in the medium (Hinshelwood and Lodge, 1944); and it is well known that old bacterial cultures show more variation than young cultures of the same organism. In virus, high reproductive rate has similar effects and variants are often found, particularly under experimental conditions where the selection factor is prevented from operating.

In its high variability C.globosum resembles the bacteria and viruses. Of fundamental significance in the study of this variability is the composition of the colony, which has been shown (Appendix 1) to function as though composed of isolated units each growing independently. A regular pattern of cells is built up in the colony by holding the cells fixed in space by the agar medium. The occurrence of sectors is further evidence of this cell individuality; in the sectoring colony normal and variant mycelium grow independently, and the shape of the sector depends upon their relative growth rates. The C. globosum colony, consisting of a mass of functionally individual cells, therefore has the properties of a cell population and in it variation will normally occur from time to time. Whether the variants will be apparent or masked will depend on their position in the colony; if they arise at the outer growing edge a sector of some sort will develop, but if they arise within the colony their presence will be shown only when spores or mycelium from the site are subcultured.

These properties of the colony have determined both the experimental method and the way in which the radiation effect had to be assessed:-

(a) Variants could be isolated by spore or mycelial sub-culture from the colonies, especially from old cultures, as might be expected with changing cultural environment.

(b) A wide range of strains were of normal occurrence.

(c) It was impossible to obtain a genetically uniform spore sample even from a parent colony of single spore origin.

(d) It was impossible to obtain a uniform effect in any one experiment or to repeat, except in a general way, the results of any experiment in further experiments made under apparently the same conditions.

(e) A considerable range of effect (intergradation) was observed in respect to any one type of variation, as might be expected if the spore sample treated were not genetically uniform.

All these conditions have limited the usefulness of the material for critical genetical work and have made it necessary to adopt many of the experimental procedures employed:-

(a) Single spore plating was necessary so that colonies would originate in single spores (and also to obviate overgrowth from adjacent spores in multispore platings, the spores being affected differently by the irradiation)

(b) Results had to be assessed over as many experiments as possible and in a general way, the characters selected for observation being those that could be recognised witnout detailed examination, and types of change being noted rather than specific variations recorded

(c) Dose measurements were given more accurately in terms of the biological indicator which recorded the mean effect of the radiation on the particular sample, than by physical measurement giving a figure for the radiant energy falling on the spore.

2. The nature of the change involved in variation

Induced variation in C.globosum cannot be shown absolutely to involve nuclear change because cytogenetic studies cannot be made and cross-breeding cannot be carried out. All other sources of evidence, however, show the change to be a nuclear one. Such evidence rests, firstly in the fact that these induced variants breed true in subculture, and although it is always possible that

the change is a cytoplasmic effect or environmental modification when mycelial subculture is the criterion, the maintenance of the variant through single spore suoculture is indicative of genetic change. Secondly, the occurrence of variants as sectors shows that environmental modification is not involved. Such colonies act virtually as their own controls with variant and normal mycelium growing side by side, so that there can be no differential effect of the environment. Thirdly, a cytoplasmic effect, unless it involves some body with similar properties to the plasmogene, cannot be involved when the variant appears as a sector of a colony of single spore origin, and all variant types can appear so. Cytoplasmic processes are under genetic control so that their perpetuation and segregation through one group of cells is impossible unless there has been genetic change also.

The action of the irradiation on the nucleus has the characteristics of an activation of the photochemical type. The variant may comprise the whole colony, the activation being stablished immediately; or stabilisation may be delayed until the original spore nucleus has divided a number of times. In most of the latter cases stability is attained quickly, the sectors originating at or near the centre of the colony, but it may be delayed, or never attained as in cyclic variation in the so-called "eversaltating strain". These "eversaltating strains"are colonies in which variation occurs spontaneously in each cultural life. The two cases of "eversaltation" recorded in these experiments were induced by irradiation of spores of C.gloeosum Fld, variant and C. gloeosum Fld parent occurring as sectors of the colony. In one form the variant was of the slow growing mycelial type and in the other it was of the dense aerial mycelial type with similar growth rate to the parent. In the first the parent bred true in subculture while the variant was unstable, subcultures from it throwing sectors of the parent; in the second case the variant was stable and the parent unstable, throwing sectors of the variant in subculture. Cyclic variation has also been reported in bacteria.

While it holds true generally that nuclear change is

involved in induced variation, three classes of effect may be distinguished:

(a) a genetic effect (Groups 1-5, 9-11) probably qualitative gene change,

(b) a lethal effect, and

(c) "growth-damage" (Groups 6-8).

Genetic effect:- It is not possible to determine whether the genetic effect represents qualitative gene change or a mechanical alteration such as deletion, translocation, terminal deficiency or gross or minute rearrangement, but there is evidence against mechanical alteration[1] : firstly, Lindegren and Lindegren (1941) found in Neurospora that diminished fertility, which is due to chromosomal rearrangement, was not induced by ultra-violet irradiation at 254 mu; secondly, Stadler (1941) has reported that gene rearrangement is induced seldom if ever in Zea mays by ultra-violet irradiation; and lastly, the occurrence of the cyclic change of unstable variant back to parent recorded above is contrary to any idea of gene deletion or destruction and indicative of qualitative gene change.

Lethal effects :- Irradiation of C.gloeosum spores can prevent their germination or so affect early growth that the mycelium soon dies, the so-called visible colony effect. The ratio of these lethal effects[2] to genetic effect is the same at 265, 313 and 334 mu, and it is therefore concluded that the lethal effect has involved qualitative gene change. However, because the lethal effect occurs much more often than the genetic effect, the reaction must involve any one of a very large number of genes, and in fact is so common that some general reaction involving the gene protein is

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Footnote 1. Results are contradictory in Drosophila.

Footnote 2. The killing of cells and organisms by radiations, drugs and other treatments has been widely studied in biology. However, for the interpretation of results the effect studied should be appropriate to the problem and defined precisely. The word "kill" can cover a wide range of effect from immediate death, to death sometime in the future after there have been a number of cell divisions. Not only is there a great difference between the doses required to kill at the extremes but the treatment may act in different ways, so that while the dominant effect in the short ultra-violet is a genetic one, in the long ultra-violet a physiological effect may become apparent or even exceed ~~exceed~~ the genetic one, the very large doses applied at 334, 365 mu and longer wavelengths being sufficient to affect other cell components, such as an enzyme system, more readily. (Scott, 1937).

indicated rather than specific gene reactions. This conclusion that lethal action can involve genetic change has also been arrived at by Plough (1941) who noted that the reversibility of lethal genes to normal "makes it probable that a considerable proportion of the lethals are gene mutations rather than chromosome breaks or major deficiencies".

(c) "Growth-damage". Three features distinguish "growth-damage" from the qualitative genetic effects:-

1. "Growth-damage" is a condition, not a qualitative change in some feature. All colonies exhibiting the condition have the same general appearance although "damage" may differ considerably in degree and brown pigment may or may not be formed. It cannot be said that "growth-damage" involves change in any character in the sense that variant characters differ from those of their parent.

2. "Growth-damage" may affect the mycelium of a variant induced by the same irradiation (Figure 33), although it is usually found in mycelium of the parent type.

3. The condition itself cannot be transmitted, except perhaps to a limited extent. It appears to involve some sort of nuclear derangement which ultimately prevents normal growth, rather than qualitative gene change. Mycelial subcultures from "damage" areas usually failed to grow, or produced "damaged" mycelium; when normal mycelium was formed it could have originated in normal mycelium included in the inoculum. The same may be said of growth from spores from "damaged" colonies; when normal colonies developed, as nearly always happened when the spores were viable, it was likely that the spores originated in normal mycelium.

Analogous forms appear to have been reported in the literature in connection with ultra-violet treatments. The "degenerate phenotypes" described by Lindegren and Lindegren (1941) in Neurospora crassa appear to resemble Chaetomium "growth-damage" very closely. In fact, the written descriptions of the "degenerate phenotype" could be applied equally to Chaetomium and to N.crassa e.g. N. crassa U.4 : "a typical degenerate phenotype with poorly growing, straggly browning mycelium, mostly beneath the surface, and a dark-brown substrate colour". Generally speaking, mycelial subculture (hyphal tip) produced cultures of the degenerate type or were not viable; matings of degenerate to control mycelium yielded asci which gave normal colonies or were inviable, degenerates being produced occasionally and only from old asci. These results are in conformity with the work so far carried out on Chaetomium "damage"; it remains to be seen whether these Chaetomium spores and Neurospora ascospores are developed exclusively in normal tissue, that is,

whether gene transmission is impossible, as seems to be indicated. The frequencies of occurrence of mutants (4.5%) and "degenerate phenotypes" (2.7%) at 254 mu in N.crassa are quite unlike those of genetic effects and "growth-damage" at 265 mu in Chaetomium; however, in the Neurospora experiments the dose was very high, less than 1% of spermatia surviving treatment, plating technique was unfavourable for counting; and the colonies were grown in tubes which would mask the appearance of the "degenerate phenotypes". In Chaetomium when colonies were crowded in dishes "damage" was masked to a great extent by overgrowth of normal mycelium. In the experiments of Emmons and Hollaender (1939) on ultra-violet induced change in dermatophyte fungi, "damage" was not recorded, presumably because the cultures were grown in tubes in these experiments also.

In their work on Sphaerocarpus Knapp and Schreiber (1941) found genetic effects as well as an effect on sporogonium attachment in the sporphyte generation developing from the irradiated spermatozoid. The very high frequency of the latter at short wavelengths makes it unlikely that the effect on sporogonium attachment represents action of a dominant lethal, and it is tempting to consider it as being a "growth effect" analogous to Chaetomium "damage", especially in view of its much greater frequency at 265 and 200 mu than that of mutation. Stadler's (1941) work on maize is also indicative of a non-genetic effect in which growth is aberrant, the endosperm deficiency. These deficiencies consist of patches of endosperm in which cells have not developed. In experiments with ultra-violet it was found that a high proportion of the deficiencies were fractional, only portion of the endosperm showing the condition, and it should be noted that "damage" in Chaetomium is also fractional, most colonies having some normal mycelium. The endosperm deficiencies, moreover, have a different frequency distribution to that of embryo abortion, which is a genetic effect.

(b) THE MECHANISM OF GENE ACTION

Many lines of research have shown the genetic mechanism to be

chemical one. Evidence for this has come from studies of chromosome absorption spectra, from chemical analysis of virus nucleoprotein, from stain reactions; and from such findings that spontaneous gene mutation in Drosophila has a temperature coefficient, as might be expected in a chemical process (Plough, 1941); and that flower colour is due to the production of specific pigments under genetic control (Lawrence and Price, 1940); and so on.

The evidence has led to the further conclusion that the mechanism of heredity is associated with nucleoprotein metabolism. The constituents of nucleoprotein are nucleic acid in its desoxyribose or ribrose form, and protein; from their nature the proteins might be expected to be concerned with the specific reactions controlled by the gene while the non-specific nucleic acid is concerned with the functioning of the protein component, either in its reproduction or as a prosthetic group between gene protein and cell reaction. If this is so - and the cycle of attachment and detachment of nucleic acid from the protein fibre of the chromosome strongly indicates such a role - it should be possible to modify each component separately by means of a specific agent such as ultra-violet radiation. Such a differential effect would involve a predominately nucleic acid reaction in the short ultra-violet where this component absorbs strongly, and associated with it but to a much smaller degree a group of protein reactions each having the same general characteristics but differing qualitatively among themselves. As wavelength increases absorption by nucleic acid and by protein diminish greatly, nucleic acid absorption reaching a negligible value at about 313 mu and protein absorption, though small, exceeding nucleic acid absorption at longer wavelengths than this. Much greater doses of irradiation should therefore be needed to produce the same amount of each reaction and at wavelengths longer than 313 mu the reaction associated with nucleic acid absorption should become negligible. In the present experiments ultra-violet radiation has induced

two differential effects having the characteristics to be expected if changes took place separately as a result of nucleic acid and protein absorption. Quantitatively and qualitatively the genetic and lethal effects may be associated with absorption by ~~the~~ gene protein, and "growth-damage" with absorption by nucleic acid.

The genetic effects are not only qualitative changes which can concern a number of different characters and can be inherited, but the dose of irradiation needed for their production is what might be expected for absorption by protein. The dose needed to produce equal genetic effects is 6000 times greater at 334 mu than at 265 mu, a factor which is of the same order as the range of differences in absorption by protein over these wavelengths. Change in the absorption coefficient of the protein urease, for example, has a factor of 5000 times over the wavelength range 196-366 (Kubowitz and Haas, quoted by Delbrück, 1940).

"Growth-damage" is quite unlike genetic change. The condition shows great uniformity and does not seem to be heritable. At 265 mu it occurs much more often than genetic change but at 313 mu "growth-damage" is found less often than genetic change and at 334 mu is negligible.

Differential effects similar to those found in these experiments have been found by other workers, although results were nearly always interpreted as a nucleic acid effect. This, it has been pointed out, was due chiefly to limitations inherent in their experiments. The data of Emmons and Hollaender (1939) and Mackenzie and Muller (1940) appear to refer to genetic effects only, the experimental results merely expressing a general decrease in absorption with increasing wavelength rather than specific nucleic acid absorption. Re-examination of the results of Knapp and Schreiber (1941), Lindegren and Lindegren (1941) and Stadler (1941) shows, however, that differential effects were induced, growth and genetic effects being distinguished qualitatively and quantitatively. The data of Knapp and Schreiber distinguish an effect on sporogonium attachment from genetic effects, those of Lindegren and Lindegren between "degenerate phenotypes" and

and single gene mutations and those of Stadler between endosperm deficiencies and embryo abotion. In all these cases the quantitative data complete the association between the non-specific growth effects and nucleic acid absorption, and between the genetic effects and absorption by protein.

A relationship has now been deduced between nucleoprotein and the mechanism of heredity whereby the protein component is concerned with qualitative genetic change and the nucleic acid component with the functioning and reproduction of the gene. Gene protein and nucleic acid function independently to the extent that each can be activated separately; activation of nucleic acid and transfer of energy from it to the gene protein are not involved in gene mutation, as Mackenzie and Muller (1940) have suggested. Indepencence is not visualised as meaning that nucleic acid and protein are not associated intimately (of nucleoprotein virus crystals), but only that they have independence of action. In this association the protein is the specific reactant while the nucleic acid would seem to be concerned with the reproduction of that protein.

No one type of genetic change has been found to be associated particularly with any wavelength, and it is doubtful whether either the inducing agent or the material are suitable for an investigation of gene mutation. Certainly it would seem preferable either to use "pure" nucleiprotein, in the form of virus, or to compare under different treatments sharply defined genetic effects of the types found in the higher animals and plants. Moreover, method and material should be selected in such a way that the lethals can be analysed, this class including under the conditions of most techniques and particularly when sperm and pollen are treated not only killed individuals but those not able to compete with more active forms. Induction of specific genetic effects by specific reagents will be the ultimate problem of analysis of the mechanism of heredity.

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ACKNOWLEDGEMENTS

The author is indebted to Professor A.L. McAulay for advise during the work and to Miss Joan Ford who carried out much of the routine culture work. A Commonwealth Research Grant enabled this work to be undertaken.

FIGURES 1-15

Figures 1-15: Strains

Chaetomium globosum. Figs. 1-11

Ch. globosum saltant Fld. Figs. 12-15.

Figures 1-6 show typical variations of normal globosum which has dense, evenly distributed peritnecia (as Fig. 16 apart from sector light damage). The peritnecia of tnese colonies are distributed in zones or irregularly. Note that many of the colonies show a dense massing of the perithecia at the centre; this found commonly. Fig. 4 shows two strains as sectors.

Figures 7-9 show the commonly occurring reduction in the number of perithecia. These strains merge into mycelial saltants showing sparse aerial mycelium (Figs. 44,45), the colony shown in Fig. 9 probably belonging to this class. Note the sectoring.

Figures 10-11 show the character of dense aerial mycelium. Note the sectoring.

Figures 12-15 show similar types of variation in C.globosum saltant Fld. to those found in C. globosum .

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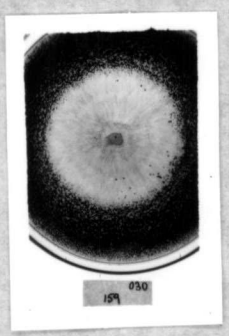

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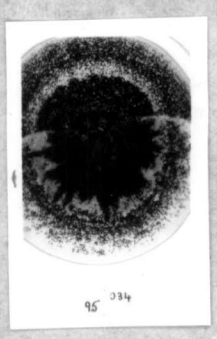

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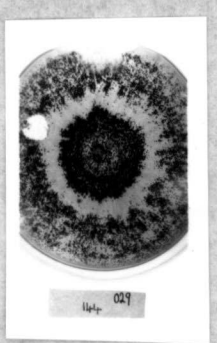

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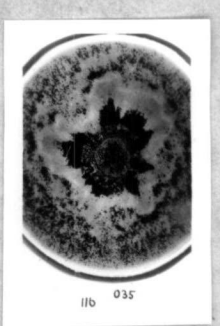

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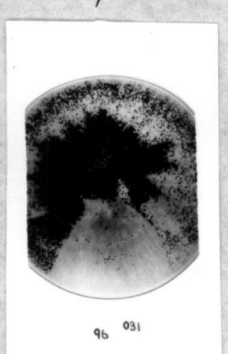

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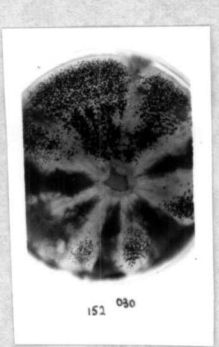

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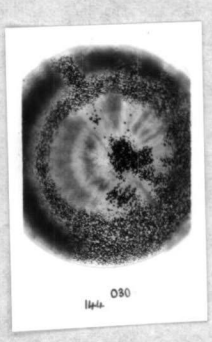

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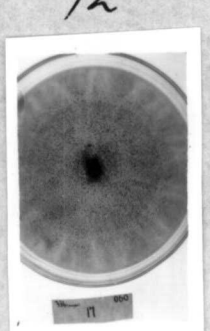

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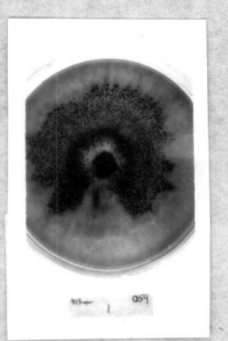

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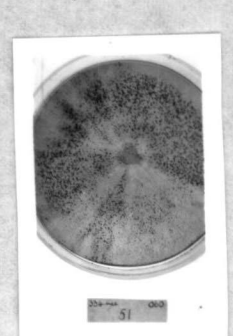

FIGURES 16-29

Figures 16-29: "Growth-damage" - Groups 7 and 8.

While these figures only show "damage" in C. globosum, "damage" in C. globosum saltant Fld. has the same characteristics.

The figures illustrate the range of "damage" found. Colonies may be normal except for a small sector of "damage" (Fig. 16) or show little or no normal mycelium (Figs. 23-29).

The characteristic brown pigmentation of heavy "damage" is well shown (Figs. 21-29); it forms at the edge where growth stops. After growth stops the free space left in the plate may become occupied by mycelium growing out into it (see particularly Figs. 26-29).

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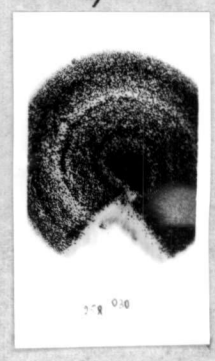

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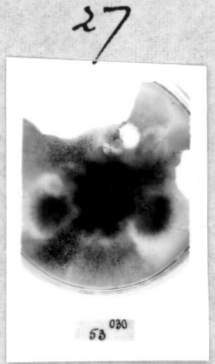

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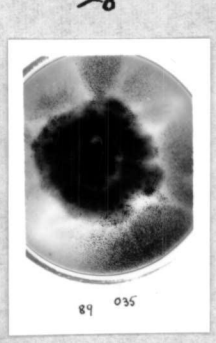

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FIGURES 30-43

Figures 30-33: Mixed Colonies

Figure 30: Normal plus strains plus slow growing sector. The slow growing sector showed in subculture as a "scollop".

Figure 31: Normal plus strain plus light damage plus "scollop".

Figure 32: Normal plus strains.

Figure 33: Mycelial variant dense aerial mycelium plus light damage.

Figures 34-35: Mycelial variants.

Figures 34-35 show the variant type of Group 2 in which perithecia are absent and the mycelium appears sparse, lying close on the surface of the agar plate. The growth rate is normal.

Figures 36-37: Variation involving growth rate

Figure 36 shows the typical characteristic of Group 9 with the slow mycelium growing from the centre of the colony and soon surrounded by the faster normal mycelium.

Figure 37: Note the sectoring into normal and slow growing saltants around the edge of the colony and the surrounding of the slow mycelium by the faster growing normal mycelium.

Figures 38-43: "Seaweeds" and "Scollops".

Figures 38-41 show "seaweed types". On the one hand there are the slow growing forms with dense mycelium of Figs. 38-40, and on the other hand the fine sparse mycelium types of Fig. 41; the latter type gave the name to the group from their resemblance to some of the Red Algae.

Figures 42-43 show "scollop" types.

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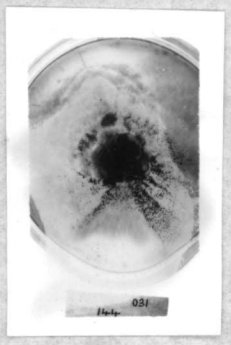

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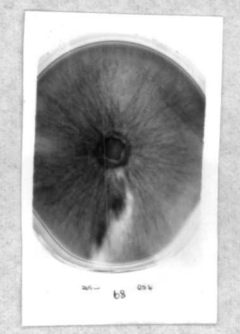

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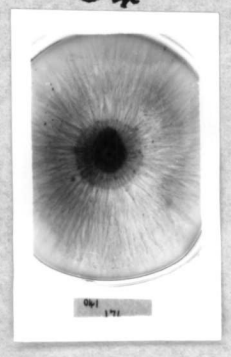

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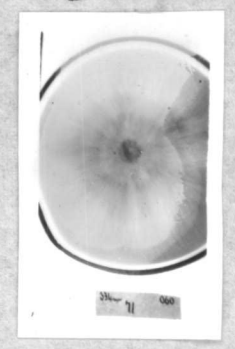

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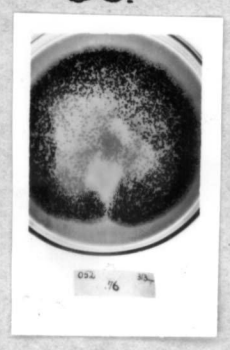

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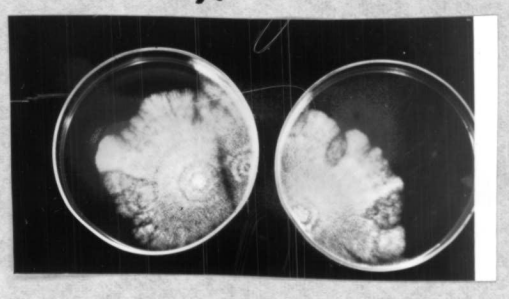

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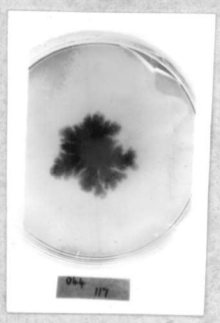

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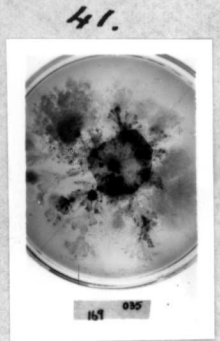

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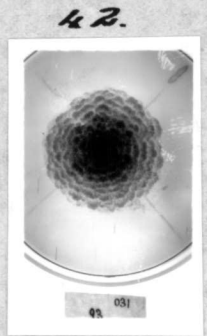

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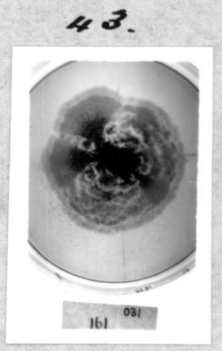

TABLE 1

QUANTITATIVE ANALYSIS OF VARIATION INDUCED BY 264 mu IRRADIATION

Material irradiated: Chaetomium globosum strains LJ and KB, C. globosum saltant Fld.

Control and Experiment Germinations: actual count shown in each case is written as, say, 50/60 meaning 50 germinating of a total of 60 plated, either control or irradiated spores.

% Visible Colonies: percentage of spores germinating that grow to colony visibility. The percentages shown refer to the irradiation series. When all controls do not grow to visibility, the % visibility in the controls is the first percentage shown.

No. of Colonies: the number of colonies in the irradiation series plated out and forming colonies. They comprise colonies from the single spore and dilution plates.

Variant Groups, Frequency of Occurrence: The variants are classified into the groups described on pages 18 and 19. Note that in a number of experiments some or all "damaged" colonies of Groups 7 and 8 were not definitely classified into one or other group but were merely described as Group 7-8: figures are shown between the two groups in the table.

Total "Damaged"Colonies: total of groups 7,8 and 9. Total "damaged" colonies found in the irradiation series is shown as percentage of all colonies grown in irradiation series.

Total Genetic Effects: total of all groups other than "damaged" colonies.(Groups 7,8,9). Total of these found in irradiation series is shown as percentage of all colonies grown in irradiation series.

TABLE 1.

265 mu.

Expt.	Strain	Control Germination	Experiment Germination.	% vis. Colonies	No. of Colonies	Variants. 1	2	3	4	5	Frequency of Occurrence in each Group. 6	7	8	9	10	11	Total damaged Colonies.	Total genetic effects.
Controls	all				About 50 controls in each Expt.				2								0	2
029	LJ	90/100	78/100	15.4	83		2				5	28	1		3	2	34	7
030	LJ	80/ 94	88/100	68.2	168		1				8	14		2		2	22	5
031	KB	88/100	77/100	2.6	121				3		17	17		1		3	58	7
038	LJ	30/37	73/90	26.0	19						16						16	
039	LJ	60/64	103/121	100.0	84			1										1
040	LJ	57/65	106/119	74.5	78			1		1	19			1		1	19	4
042	LJ	54/65	96/114	60.4	45				1		12					1	12	2
048	LJ	50/63	109/164	45.9	50			1			13	9	1				23	1
054	Fld.	38/46	99/146	76.8	160	2	1		1		10	4	2				16	4
056	Fld.	46/59	111/144	25.2	167	1	5		2		51	33	9			1	93	9
058	Fld.	37/50	108/142	69.4	160		2		1	1	21	38	3				62	4
069	Fld.	25/50	17/100	96.0:5.9	10						1	2				1	3	1
081	Fld	20/50	24/100	20.8	62			1			10	17 2			1		29	2
082	Fld.	26/49	26/99	26.9	58						3	6			1	1	9	2
					1265	3	11	4	8	2	139	94 147	16	4	5	12	396	49
																	31.3%	3.9%

TABLE 2.

QUANTITATIVE ANALYSIS OF VARIATION INDUCED BY 313 mu IRRADIATION.

Material irradiated. Chaetomium globosum strains LJ and KB, C. globosum saltant Fld.

Control and Experiment Germinations: as for Table 1.

% Visiole Colonies: as for Table 1.

No. of Colonies: as for Table 1.

Variant Groups, Frequency of Occurrence: as for Table 1.

Total "Damaged" Colonies: as for Table 1.

Total Genetic Effects: as for Table 1.

Experiment 035: as pointed out on page 19, there are grounds for rejecting this experiment as not being typical of irradiation at 313 mu.

TABLE 2.

313 mu.

Expt.	Strain	Control Germination	Experient Germination	% vis. Colonies	No. of Colonies	Variants. 1	2	3	4	5	Frequency of Occurrence in each Group. 6	7	8	9	10	11	Total damaged Colonies.	Total genetic effects
Controls	all				About 50 controls in each Expt.			1	3								0	4
034	LJ	20/68	50/140	82.0	207		1	1	1			2		1	2		2	6
035	LJ	24/68	34/140	29.4	116	2	1	..	4		5	35 7			4	1	47	12
036	LJ	51/69	1/140	1/1	2		1										-	1
043	KB	38/49	33/138	0	7												-	-
046	KB	40/47	28/153	0	9							1					1	-
047	KB	48/52	83/149	97.9:0	19			1									-	1
050	KB		sampling.		4												-	-
051	KB		sampling		100			1			1		1		1		2	2
052	KB	34/46	104/149	43.3	149	2	2							2	2	1	-	9
055	Fld	47/59	101/148	44.6	162	1	3				5	1	4		1		10	5
057	Fld	41/52	74/140	5.4	52						1	2	1		1		4	1
059	Fld	44/48	102/136	97.7:45.1	46										1		-	1
077	Fld	22/50	28/100	35.7	134		3					1	1		1		2	4
078	Fld	22/50	26/100	92.3	122	3	1		1						3		-	8
079	Fld	20/50	29/99	44.8	153		4				2 1		1	1	1	3	4	9
071	LJ	13/47	34/129	0	84	1	1				7	2	3		1	1	12	4
		All experiments			1366	9	17	3	6		21 36	16	11	4	18	6	84	63
		Expts. less 035			1250	7	16	3	2		16 1	9	11	4	14	5	37	51
																	6.2%	4.6%
											All experiments.						3.0%	4.1%
											Expts. except 035							

TABLE 3.

QUANTITATIVE ANALYSIS OF VARIATION INDUCED BY 334 mu IRRADIATION

Material irradiated: Chaetomium globosum strain KB, C. globosum saltant F1d.

Control and Experiment Germinations: as for Table 1.

% Visible Colonies : as for Table 1.

No. of Colonies: as for Table 1.

Variant Groups, Frequency of Occurrence: as for Table 1.

Total "Damaged" Colonies: as for Table 1.

Total Genetic Effects: as for Table 1.

Notes: (a) In many of the experiments, the control series does not show 100% visible colonies. This is probably due to very slight short wavelength leakage (or reflection) affecting both irradiation and control series. The amount of leakage must be negligible, even with the irradiation series which is more favourable placed in the mount to receive it, for no "damaged" colonies were found in the control series and the occurrence of these types in the irradiation series is much smaller than production of other variants. The average time of an irradiation at 334 mu is about 200 hours compared with about 3 minutes at 265 mu.

(b) Experiment 061: the three variants put in Group 7 (marked "a") are not typically "damaged". They show the feature of brown pigment characteristic of this group but belong to in no other respect.

T A B L E 3.

334 mu.

Expt.	Strain	Control Germination	Experient Germination	% vis. Colonies	No. of Colonies	Variants. 1	2	3	4	5	6	Frequency of Occurrence in each Group. 7	8	9	10	11	Total damaged Colonies.	Total genetic effects
ontrols	all				About 50 controls in each Expt.												0	0
044	KB	47/52	130/153	34.6	135		4	1						1	3	3	-	12
049	KB	43/51	55/151	97.7:5.5	62										4		-	4
053	Fld	53/60	78/138	19.2	110	1						1			1		1	2
060	Fld	37/54	52/101	44.2	154	1	3		2		1					2	1	8
061	Fld	32/53	76/106	96.9:52.6	172			2	2			3a	2	1			5	5
070	Fld	31/50	0/96	96.8:0	6	1	1										-	2
072	Fld	Sampling			87		2			1			1				1	3
074	Fld	16/50	32/100	53.1	160		1	1			1		5			1	6	3
075	Fld	18/52	26/100	94.4:76.9	77		2			2					1		-	5
076	Fld	19/50	40/100	94.7:37.5	172		3			1			3		5		3	9
083	Fld	27/48	37/95	89.2	131	1								2			-	3
					1266	4	16	4	4	4	2	4	11	4	14	6	17	56
																	1.3%	4.4%

APPENDIX 1.

COLONY FORMATION IN THE FUNGUS CHAETOMIUM GLOBOSUM KUNZE.

BY

N.J.B.PLOMLEY AND J.M.FORD

APPENDIX 1.

COLONY FORMATION IN THE FUNGUS CHAETOMIUM GLOBOSUM KUNZE

N. J. B. PLOMLEY and J. M. FORD

SUMMARY

1. The increase in size of the colony of the ascomycete Chaetomium globosum Kunze growing on agar gel has been investigated in detail. Increase in colony diameter, change in colony density, growth within the medium, and increase in total length and amount of mycelium, have all been studied, as well as growth of the hyphae themselves. Growth has been compared on complete and incomplete media. Observations on the morphology of the hyphae are reported.

2. Growth of the hyphae is logarithmic but in the formation of the colony such a growth rate is maintained only while the environment remains constant. In the young colony where there is no restriction on growth by the environment growth is logarithmic, but as the colony ages an environmental restriction operates. Because the cells are fixed in the environment, the environmental restriction acts in two ways to build up a colony pattern:
   (a) growth inside the colony falls off from logarithmic until a maximum hyphal density eventually results, and,
   (b) marginal growth settles down to a constant rate.

3. The fungal colony is found to be a cell population, so that examination of its functions should be made from this point of view.

INTRODUCTION

Most workers have studied the growth of fungi in relation to the effects upon it of some environmental factor. Growth has been measured either as increase in colony diameter or by weighing the colony. By the first method a single colony can be observed continuously; but this may have little meaning because the relationship between colony diameter and the mass of tissue in the colony is unknown. It has often been noted, for example, that one treatment will result in the growth of a dense aerial mycelium while in another treatment the aerial mycelium is relatively sparse; here comparison of growth rates by measurement of colony diameters is almost meaningless. Adair and Moore (1941) attempted to overcome the deficiencies of the method by determining from photo-electric measurements all the growing material in the colony. Their method, however, was unsatisfactory with small colonies.

Weaknesses of the method of determining growth by weighing

are,firstly, that the colony must be grown on liquid media, and, secondly, that as the determination involves destruction of the colony, the experiment must be designed on a statistical basis.

Apart from such growth measurements, undertaken chiefly as a basis for comparison of environments, there has been little detailed consideration of the factors involved in the formation of the colony in fungi. In fact it has not been clearly established whether a fungus is to be considered as an individual organism or as some type of colony. In the present paper the growth of the ascomycete Chaetomium globosum Kunze has been analysed.

MATERIAL AND METHODS

The material used in these experiments was a Dutch strain of the fungus C. globosum Kunze. Platings were made on 1% malt agar, or on plain agar when starvation effects were being examined, in petri dishes selected for uniformity of size and flatness of bottom. The amount of medium poured was always about the same and with the 10 cm. diameter plates used the agar depth was 6 to 8 mm. In certain experiments, especially those where a colony was to be stained and mounted or photographed, the depth was less, although always greater than the maximum colony radius being considered, so that there would be no question of any asymmetrical growth because the bottom of the dish had been reached. The plates were incubated at 28°C. All colonies grown from spores were of single spore origin. The age of the colony was reckoned from the time of spore plating. To obtain the age from germination about six hours should be deducted, this being the period from spore plating to spore germination. All data on colony development given in this paper are in terms of time from plating.

The following methods were used in measuring the colony:-

Colony diameter - From germination until colony diameter reached 3-5 mm, measurements were made with microscope and eye piece micrometers; when larger, a millimeter rule was used. Measurements were made along two diameters marked out at right angles and a mean value

taken. When measuring the very small colony just germinated, one diameter was taken along the most prominent hyphae and this, and a diameter at right angles, were used as axes in all subsequent measurements on the colony.

Depth of Mycelium: - Thin vertical sections were cut through the agar culture. Depth of mycelium in the section was measured with micrometer or millimeter rule.

Density of hyphae per unit area - The surface of the colony was photographed and from it an accurate sketch made of the hyphae. The depth of focus of the optical system included all hyphae in a surface layer 016 mm deep. The area of the sketch was divided into concentric rings of equal diameter whose centre was the colony centre (usually the spore site). The length of mycelium in each ring was stepped off with dividers and a density figure calculated. When the whole surface area could not be measured satisfactorily, as was usual with large colonies, one or more sectors were measured. Usually measurements were taken of a single colony from time to time so that change in density could be determined; measurement on different colonies gave a crude picture of density change because different colonies of the same age, particularly young colonies, showed differences in development due to differences in the pre-germination period, in environmental conditions and so on.

Individual Hyphae - Hyphae were either measured with microscope and eyepiece micrometer, or were photographed and sketches made. Small colonies were measured similarly.

Measurements were usually made on the living colony but some preparations were fixed and stained. Colonies for the latter were obtained by plating single spores on microscope slides covered with a thin layer of agar.. When the colonies had grown to the required stage, they were fixed in Flemming's weak solution and stained with iron alum haematoxylin or Delafield's Haemotoxylin; erthrosin in clove oil or safranin were sometimes used as counter-stains.

MORPHOLOGY

1. The Colony. The thick walled spore, an ovoid with average dimensions 9.2μ x 7.0μ germinates four to six hours after plating, the germ-tube being protruded from a pore at one end. The very young colony is made up of one or two hyphae. These remain unbranched for a time so that the young colony is usually much longer than broad. After six to eight hours side branches develop from the original hyphae, growing out more or less at right angles to them. (Plate 1A). As the colony grows the hyphae increase in number and the colony rounds off, this form being reached about 30 hours after plating (Plate 1B). At this stage aerial hypnae begin to appear first at the centre of the colony. As growth proceeds and the margin expands they extend over more and more of the surface. In the two-day old colony the aerial mycelium is quite dense, especially at the centre. The colony is now increasing in diameter at a constant rate and the mycelium around the edge has taken on a combed appearance; a portion of the growing edge of a 46 hour colonu is shown in Plate 1D.

In the very young colony the diameter of the hyphae ranged from 2.8 - 4.2μ (Table 1). As the colony aged, the hyphae formed in the peripheral growing fringe were of this size, but those formed within the colony were more and more fine, having a diameter of only 0.8 - 2.2 μ.

When the colony was starved by growing it on plain agar the mycelium was not only much more sparse than that in a colony having adequate food, but the hyphae were much finer; tne original hyphae and those of the growing fringe had a diameter of 1.9 - 3.5u and branch hyphae only 0.32- 1.1 μ. Although starvation had a very marked effect on the size of tne hyphae and density of the mycelium, that is, on the amount of growing substance, the diameters of normal and starved colonies of the same age were not very different (Plate 1C). The older starved colonies had irregular and indistinct outlines.

The smaller size of branch hyphae in all colonies, and of the hyphae generally in the starved colony than those in the normal colony, is due to starvation. The hyphae are fixed in the agar medium; within the colony food becomes depleted (and waste products accumulate). Growth is therefore restricted and this shows itself not only as a slowing of growth in length but also a diminution of hyphal diameter, which permits greater efficiency in growth.

2. Structure of the Mycelium - The mycelium is septate and multinucleate. The first septum develops 10-12 hours after plating (4-6 hours after germination), and is laid down 46 μ more than 100 μ behind the growing tip.

Table 1 records data concerning hyphal structure. It is evident that there is no constancy in length of cell and number of nuclei to each cell; however, the number of nuclei and septa in colonies of the same age is approximately the same. The apical cells are longer than the others and possess more nuclei. Aerial hyphae are often finer than branch hyphae; they are difficult to examine for cell structure.

Cell dimensions and number of nuclei in the starved colony are given in Table 1. The hyphae are finer than those of normal colonies.

GROWTH OF THE COLONY

The fungal hypha usually grows by elongation of its tip (Smith, 1924); it grows in this way in C. globosum. In a series of measurements of portions of hyphae between successive side branches, no evidence was obtained of any elongation. Crinkling of the hyphae was not noticed in the colonies and this would be expected if elongation took place behind the hyphal tip, because side branches would act as anchors preventing a tip being pushed forward.

(a) GROWTH OF A SINGLE HYPHA

The growth of the individual hypha was determined from

measurements made from time to time of the length of a selected hypha and its side branches (Table 2). Length was measured from the tip of the hypha back to some arbitrary fixed point, usually its junction with another hypha; side branches were, of course, measured from tip to junction with parent hypha.

The data are plotted in Graph 1, logarithm of length against period of growth, for the selected hypha and side branches; growth of one of the branch hyphae is shown separately also. Each of these graphs may be considered in three parts, firstly, the part AB when growth is proceeding very rapidly in an unbranched hypha; secondly, the part AC showing the growth of the hypha plus its branches; and thirdly, the part AD showing the growth of the original hypha alone, without its branches. In the graph for the branch hypha the corresponding regions are A'B', A'C', and A'D'; growth in this system has the same characteristics as that in the system A B C D.

For convenience the part AC of the graph will be considered first; it includes all measurements on hypha plus branches except the first two (AB). The regression equation of period of growth X against logarithm of hyphal length Y for these five points is:-

$$Y = 0.1023\,X + 1.7792.$$

Correlation is highly significant at the 1% level, that is, growth is logarithmic. Exponential growth is fundamental to the hyphae, either as a whole (AC) or alone (A'C'). The departure from the exponential shown in AB and AD is not real; in AB a contribution to growth is coming from mycelium outside the hypha, while in AD the apparent falling off in logarithmic growth shown by the original hypha when branch hyphae appear is the result of the increase in the number of growing points.

In AB a part of the growth being measured is a contribution of growing substance from beyond the point of junction of the branch with the main stem; and the same effect is evident when some arbitrary point on the hypha is chosen from which to take measurements. The curve AD, on the other hand, is a complex function related to the number of growing points, that is, to the

number of branch hyphae present in the system. The growing point is, as it were, supplied by a "catchment area" of mycelium; when the amount of growth exceeds the capacity of the growing point other growing points appear, and when measurements are taken from some arbitrary point on the hypha, growth rate appears to exceed the normal rate of logarithmic increase until the whole area concerned in growth of the hyphal tip is being measured. It follows, that the unbranched growing hypha has a functional cell length, this being the length at which division occurs by the putting out of a branch hypha; **it is the point of inflection A,A' in Graph 1.**

Growth of a hypha is therefore fundamentally growth of individual cell units. Growth of these units is logarithmic and this character is shown by the hyphae themselves so long as only a single growing point is being considered, or the total length of mycelium growing without restriction in the system; when the hypha has branches, growth of the hypha alone does not appear to be logarithmic because in the length of hypha measured are parts, having constant value, of other cell units. As will be shown later, when the system becomes stabilised so that the number of growing points in the area is constant, as in the growing fringe, the rate of growth of any hypha appears to be constant and the rate of expansion of the fringe is linear not exponential.

(b) GROWTH OF A HYPHAL TIP AFTER SECTION FROM THE PARENT COLONY

Difficulty was experienced in growing hyphal tips cut from an actively growing colony. Although tips as small as about 34 μ could be cut off, they could not be grown. The smallest piece of mycelium that we have been able to grow after section was a hyphal tip about 300 μ long. This is much longer than the apical cell (Table 1).

Growth data for a hypha after section from an actively growing colony are summarised in Table 3 and Graph 2 and the form of the colony is shown in Figure 1. The mycelium is considered arbitrarily in two pieces to right and left of a point 'a' (Figure 1), which came to be the approximate centre of the new colony. Such division was also necessary because

the mycelium in the left hand piece next to the point of section did not recover normal growth for some time.

Growth in the right hand piece shows the same characteristics as growth of the individual hyphae (Graph 1), that is, in the part AB of the growth curve a contribution to growth is coming from mycelium in the left hand piece, and in the part AC growth of the mycelium is logarithmic. Eventually growth rate starts to decline from logarithmic at D. While the corresponding part of Graph 1 shows the effect on the data of the inclusion of mycelium in the length measured which is not contributing to the growth of the tip, the section A'D here represents the effect of restriction of growth rate within the colony. Due to change in the environment within the colony the amount of new mycelium formed there is not the same as in areas in which the original environmental conditions continue to hold. There is not only this falling off in growth rate, which is shown particularly by the density measurements, but further diminution in actual growth follows the decrease in diameter of hyphae formed with the colony.

(c) INCREASE IN SIZE OF THE COLONY

1. Mean Dimension of [illegible]

Typical measurements of mean colony diameter for growth on 1% malt agar and plain agar are plotted in Graph 4, the linear and logarithmic forms being shown.

Mean diameter of the colony increases in the following way:-

(1) Rate of increase in mean diameter is logarithmic at first.
(2) After a colony diameter of about 0.8 mm (about a 30 hour colony) has been reached this logarithmic rate diminishes until the rate becomes uniform and the colony spreads over the surface of the medium at a constant rate, which, in C. globosum can continue indefinitely.

A few observations have been made on growth just after germination of the spore (Graph 5). Growth during this period

also is logaritnmic. If tne graph is drawn back to the equivalent length of the spore contents (about 5 μ), the time scale is cut at 2-3 hours. This point will represent the time after plating at wnich growth began in the spore.

2. Growth in Depth - when the colony was grown in a petri dish, the rate of growth downwards into tne medium was tne same as the rate of growth over the surface of tne medium (Graph 6). Growth was three dimensional from the time of germination of the spore, hyphae growing into the agar as well as over the surface; and the rates of growth into and over the surface of tne medium were the same so that the colony was hemispherical.

In the petri dish growth continued in this way until the bottom of tne dish was reached, after which the colony had the shape of a truncated hemisphere. Growth proceeded regularly on the surface and tnrough tne medium. Surface growth did not appear to be altered when the bottom was reached; no such effect should occur unless there were staling (vide Brown (1923, 1926) who investigated the growth of staling fungi on deep and shallow plates). However, when the agar was deeper or its surface limited, growth downwards finally stopped. When colonies were grown in tubes, downward growth slowed or ceased when tne surface mycelium reached the sides of tne rubes (Graph 7)(1). Th. The abrupt change in rate of downward growth showed tnat the effect followed occlusion of the surface, i.e. tnat no gaseous or otner excnange took place through tne mycelium itself.

Y

Footnote 1: Colonies were grown in tubes of agar and after a time depth of growth measured by cutting sections. Such measurements however, were subject not only to an error as to the deepest point of growth, which was difficult to determine, but also to that of contraction of the agar during tne experiment, which was as much as 6-7 mm. The appearance of these tube colonies suggests tnat most of the shrinkage was in the surface agar; there was a surface layer of very dense and matted hyphae, then a zone in wnich the hyphae ran parallel to tne surface and were very densely packed, while below tnis tne hyphae radiated normally. Such packing was not found in the normal colony and the parallel layers of hyphae could only have resulted from snrinkage in the surface of the colony. Arbitrarily 60 per cent of the contraction has been considered as being in the surface, and has been added to tne crude depth measurement.

3. Density: the distribution of the hyphae in the colony

Final evidence concerning the growth of hyphae within the colony and the method of colony formation was provided by data showing the way in which the hyphae were distributed in the colony. Because growth within and on the surface of the medium were the same, measurement of the distribution of hyphae over the surface of the colony would describe their distribution in the whole colony. Measurements were therefore made of the density of hyphae in a surface layer, figures being obtained for areas at equal increments of distance from the centre of the colony. Although measurements were actually made in respect to a volume, the hyphae included in the measurement extending below the surface to a depth of 0.16 mm, the depth of focus of the optical system, the distribution of hyphae radially across the space rather than haphazardly through it gave a measurement in respect to surface density rather than to volume density.

Density over the surface area of the colony was calculated from measurements of the length of mycelium in each concentric ring dividing the surface from the formula:-

$$d_1 = \frac{l_1}{\pi (r_1 - r_o)^2}$$

where d_1 = density over the ring; l_1 = total length of mycelium; r_1 = outer radius of the ring; r_o = inner radius of the ring. Densities measured over a number of colonies are show in Graph 8. The graphs show the colony to consist of an outer zone 100 - 200 μ wide, in which density increases very rapidly and an inner zone which is the main mass of the colony. The outer zone comprises a few hyphae which have grown out here and there in advance of the general colony margin.

Density within the colony increases with time until it reaches a maximum value. Density change is rapid at first but becomes more and more slow as the maximum value is approached. The form of the curve is not homogeneous. When density change was plotted for a particular site (Graphs 9 and 10) a "sigmoid" curve was

obtained. This "sigmoid" comprises three regions, growth at the site going through three general phases, at first logarithmic, then constant, and finally falling off to zero when the maximum value is reached. These curves have the same form as those for growth of the hypha itself (Graph 1), though they are undoubtedly complicated by the presence of the secondary hyphae when they appear and by the final phase as the saturation density is approached.

Density in the very young colony (vide 18 hours 30 minutes colony, Graph 8) departs somewhat from its form in the older colony and is logarithmic to the centre. In the young colony more free space is available for colonisation by the hyphae, whereas once the colony has rounded off all hyphae compete equally for the space available and in the environment generally.

When the colony is starved, the density curves have the same form as those of the normal colony. Rate of change of density is slower than in the normal colony, and the saturation density less.

4. Colony Size - Because growth within and over the surface of the colony is the same, the amount of mycelium in the whole colony can be determined from the measurements of surface density by calculating the lengths of mycelium in each hemispherical shell projected from the concentric rings dividing the colony surface, using the equation:-

Length of mycelium in the shell, L_s

$= - \quad (r_1 - r_o)^3 \, d_v$, where d_v is the density of mycelium in unit volume of the shell.

d_v may be determined directly from the figure for density per unit area at the surface of the colony; it is $(d_1)^3$.

$$L_s = \tfrac{2}{3}\pi (r_1 - r_o)^3 . \; (\sqrt{d_1})^3$$

$$= \tfrac{2}{3}\pi (r_1 - r_o)^3 \left[\sqrt{\frac{l_1}{(r_1 - r_o)^2 \pi}}\right]^3$$

$$= \tfrac{2}{3}\pi (r_1 - r_o)^3 . \frac{l_1}{\pi (r_1 - r_o)^3} . \sqrt{\frac{l_1}{\pi}}$$

$$= \frac{2}{3\sqrt{\pi}} l_1 \sqrt{l_1}$$
$$= 0.376\, l_1 \sqrt{l_1} \text{ approx.}$$

Summation gave the total quantity of mycelium in the colony. Volume change has the same characteristics as the other features of colony formation (Graphs 11-13), that is:-

(a) The hyphal mass grows logarithmically at first but eventually the rate declines, presumably to a constant value.

(b) Growth in the starved colony differs from that in the normal colony by occurring at a slower rate.

DISCUSSION

In biology much attention has been given to two functions of living things, the growth of individual organisms and the increase of populations. Both are affected by the environment and both are closed systems in that they have limits of existence. The individual will grow to a size which will allow it to carry on its functions most efficiently. Because cell size is determined by surface-volume relations, the organism can overcome these limitations only by obligate association (and specialisation) of cells. The growth of an individual organism is therefore a study of the changes occurring in the organism in time, from whatever cause. On the other hand, increase in numbers of a population is essentially a study of the effects of the environment, physical and biological, upon the reproduction of the organism,

Because of their tubular form, fungal hyphae can grow unrestricted by surface-volume relations (Bower, 1930). Growth of the hypha, therefore, can follow the logarithmic law indefinitely, rate of growth being proportional to the mass of growing tissue. Logarithmic growth of a tubular cell form has been found in certain rod-form bacteria (Schmalhausen and Bordzilowskaja, 1930) and has been reported for the hyphae of Botrytis (Smith, 1924). On the other hand, the sporangiophore of Phycomyces grows at a constant rate, being wholly dependent

for material for growth upon other hyphae (Castle, 1940).

Smith's detailed study of the growth of the hyphae in Botrytis showed (a) that growth rate of a hypha was for a time proportional to its total length, that is logarithmic, but that this rate declined later;

(b) that growth of a branch hypha resembled that of its parent;

(c) that the growth of the hypha plus its branches continued logarithmically for much longer than each of them alone. He explained the eventual falling off in growth rate as being due to incomplete transfer of nutrient from the older parts of the hyphae to the tip, that is he assumed absorption to be constnat. In C. globosum, however, the decline in growth rate is considered as being due to local starvation of the units of which the colony is composed because they are fixed in the environment.

In C. globosum we have seen that growth of the colony is for a time logarithmic, the amount of growing material being proportional to material already formed, but that eventually growth falls off from the logarithmic and marginal growth proceeds at constant rate (Graph 3). This change in growth of the colony occurs in spite of potential logarithmic growth of the individual hyphae, which is seen not only in the mycelium of the germinating spore (Graph 5) and in the hyphae (Graph 1) but may be deduced from the fact that growth rate remains unchanged when mycelium is subcultured, from whatever part of the colony it may be taken. The apparent anomaly is resolved by the density measurements which show that within the colony (a) growth proceeds until a maximum density is reached, and (b) growth at a particular site is at first logarithmic but later falls off (until the maximum value is reached). Growth within the colony, therefore, proceeds logarithmically until prevented from doing so. Since growth of the colony is essentially growth of the hyphal tips and not cell enlargement subsequent to cell division, and since all hyphal tips can grow logarithmically, the restraint must come

from the environment, in which the tip is growing. ~~The environment, in which the tip is growing.~~ That environment can influence the rate of growth is shown by the slower rate on an incomplete medium (Graphs 3, 12, 13). A parallel example is the effect of environment on the rate of population increase in Scenedesmus, the rate differing according to the environmental conditions (Roach, 1928). Morphological evidence is in agreement: as a part of the colony ages, the mycelium formed consists of finer and finer hyphae. Moreover, it is characteristic of starved growth that the diameters of the hyphae are less than those of the colony grown on the complete medium (Table 1). Formation of the finer hyphae is an economy by which maximum exploration of the environment is possible for the least expenditure of growth and is comparable with the finding of Gould, Pearl, Edwards and Miner (1934) that translocation of reserve food material from the cotyledons of canteloup seedlings to the growing plant was more efficient in seedlings in which cotyledonary tissue had been removed than in the normal unoperated seedling.

The change from logarithmic increase to a constant rate is most clearly shown by the margin (Graph 3), but it can be seen in the measurements of total hyphal length (Graphs 11,12). Marginal density increases steadily as the size of the colony increases (Graph 8) and in the 31½ hour colony has reached a steady state; by this time marginal growth rate is also constant (Graph 3). The hyphae in the margin are evenly distributed and of the same size and appearance (Plate 1D).

In examining the density figures it has been noted that some hyphae grow out in advance of the main mass of the colony. Because they are thereby freed from the restriction of neighbouring hyphae, they will be able to grow in all directions. In effect each such hypha will act as did the isolated hypha after section from the colony, that is, it will tend to form a rounded colony and there will be apparent slowing of the

forward growth rate. In this way the colony edge will keep a regular line of advance. Any hypha which, from some advantage, has grown beyond the colony edge, will dissipate its energy in the formation of side branches. Thus it will be overtaken by the hyphae of the main colony mass which, restricted in their growth to minimum lateral branching, now have the greater forward growth rate. A dynamic balance is therefore set up in the margin which functions to keep the hyphae in line.

The margin is that region of the colony contributing to constant forward growth. It is, therefore, a region in which growth is linear; its width may be determined from the density figures:

Age of Colony Hours	Mins.	Colony Radius	Total Width of Margin	Region of Linear Growth (less outer zone 100 μ)
18	30	0.22 mm	Whole colony logarithmic	
26	00	0.50	0.384 mm.	0.284 mm.
26	15	0.48	.360	.260
31	30	0.84	.360	.260
34	00	1.08	.330	.230
40	00	2.39	.290	.190
42	10	2.69	.190	.090

While these figures are only approximate and, moreover, for young colonies, they do show that the margin is a functional unit rather than a morphological unit, its width decreasing as the colony ages. This is in accordance with other data. In the very young colony all growth is logarithmic and no true margin has been established. With increasing colony size (e.g. 26 hour colony) marginal growth becomes linear; the margin is wide at first but is reduced as the amount of lateral space available for colonisation becomes smaller. This is the phase of rounding off the colony, which is complete when colony radius is about 0.4 mm. (0.8 mm, diameter : Graph 4). At the same time logarithmic increase in colony diameter is declining to constant rate. The margin is finally reduced to a width of about 100 μ (42 hour colony), by which time the steady state has been set up. This

stable width of margin corresponds to functional cell length, that is, the length of a hypha which will grow logarithmically. Such a hypha will grow in this way to a length of 100 - 120 μ (Graph 1; compare also length of apical cell, Table 1).

Two principal factors are involved therefore in the development of a margin growing at constant rate, firstly logarithmic growth of hyphae whose functional length is limited, and secondly, restriction of branching to a minimum. Absorption of nutrient controls the value of this constant rate by its effect on hyphal density and on the rate of logarithmic growth. Competition for the space available for growth is closely associated with nutrient absorption. Moreover, in the rounded colony the new space available for colonisation by each hypha is a sector of the expanding circumference. This area, however, will remain virtually unchanged with change in colony radius because growth is dependent only on marginal width.

The independent growth of the hyphae is further shown by the data on growth in depth. Such growth (a) takes place at the same rate as surface growth, (b) is affected by occlusion of the surface, and (c) does not seem to influence surface growth. The mycelium within the medium is not acting as a root system for the colony but is merely a part of the colonisation of all the medium which mycelium can inhabit.

It has been shown in the foregoing discussion that the colony in C. globosum has been built up as a result of growth of its constituent hyphae, each hypha growing independently of the others of the colony, yet forming a regular pattern because each element of the population is fixed in space in its environment. Growth of each hypha is logarithmic but such a growth rate is effective only while there is unlimited freedom of growth in the environment. A colony is therefore formed in which (a) growth within the colony declines from logarithmic to zero, so that maximum hyphal density is reached eventually and, (b) growth at the margin settles down to a constant rate. This

fungal colony is a cell population and in any consideration of its functions should be treated as such. On the one hand, measurements of environmental effects should be based on measurements of the total population of cells, or, at any rate, on the cell pattern of the colony. On the other hand, the physiology of the colony is to be considered in terms of a cell population fixed spatially in its environment.

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TABLE 1

Hyphal structure in colonies grown on (a) 1 per cent malt agar and (b) plain agar.

Hyphae	Length of cells μ	Length Apical Cell μ	Diameter hyphae μ	No. Nuclei to Cells.
(a) 1 per cent malt agar - normal colony				
Main Hyphae	12.8-48.0	46-120	2.8-4.2	3-13
Branch Hyphae	19.0-28.0	48-100	0.8-2.2	3-5
(b) Plain agar - Starved Colony				
Main Hyphae	11-32	66-130	1.9-3.5	3-7
Branch Hyphae	-	-	0.32-1.1	-

Spore dimensions were 9.2 μ x 7.0 μ (8.8-10.1 x 6.2 - 7.7) (slightly less than Chivers (1915) measurements of 10.5 μ x 8.5 μ (9.5 - 13.0 x 6.3 - 9.5).

All measurements are from approximate minimum to approximate maximum dimensions. Measurements on the starved colony are for one or two examples only.

TABLE 11

Growth of a single hypha: increase in length of a selected hypha, including the branches arising from it.

Time Hours	Time Mins.	Main Hypha	1st Branch Hypha	Other Branch Hyphae	Total Branch Hyphae	Total Hyphal Length
0	0	43	-	-	-	43
1	15	67	-	-	-	67
2	50	115	-	-	-	115
4	30	168	14	-	14	182
6	30	240	34	-	34	274
9	50	341	140	105	245	586
12	40	422	250) 28)	519	797	1219

All measurements in μ

The measurements are made on a selected hypha in a hyphal tip inoculation (see Table 111). This hypha is that marked 'b' in Figure 1.

TABLE III

Growth of a hypha after section from an actively growing colony increase in total length of the (surface) mycelium. The mycelium has been considered arbitrarily in two pieces, to left and right of 'a' (Figure 1).

Times Hours	Mins.	Left Hand Piece	Right Hand Piece	Total length Mycelium
2	30	240	163	403
3	45	240	336	576
5	20	240	518	758
7	-	259	787	1046
9	-	384	1190	1574
12	20	749	2813	3562
15	10	2563	4022	6585

All measurements in μ

PLATE 1

Explanation

A - 21 hour colony of C. globosum approximate dimensions 0.36 x 0.28 mm.

B - 28 hour colony of C. globosum approximate dimensions 0.8 x 0.8 mm.

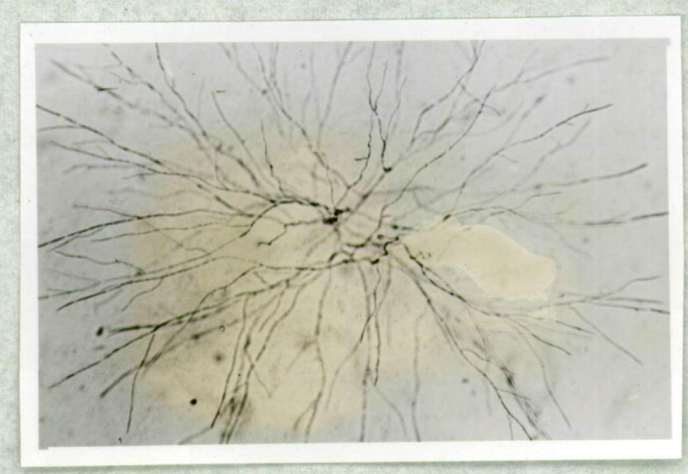
C - 28 hour starved colony of C. globosum approximate dimensions 0.72 x 0.81 mm.

D - 46 hour colony - portion of margin.

Colonies A, B and D grown on 1 percent malt agar, Colony C on plain agar (starved).

PLATE I.

A



B.

C.

D.

FIGURE I

Explanation

Sketches (1-7) showing growth of a hypha after section from an actively growing colony. The hypha was cut off from the parent colony at 'c' when it was growing in the general direction shown by the arrow. It continued to grow in this direction for a time, but later the point 'a' became the growth centre, the colony radiating about it. The proximal end 'c' - 'ci' of the left hand piece, close to the point of section, gave some appearance of injury and after 9 hours it was partly cut away. The hypha (and its branches) marked 'b', is that referred to in Table 11.

FIGURE 1.

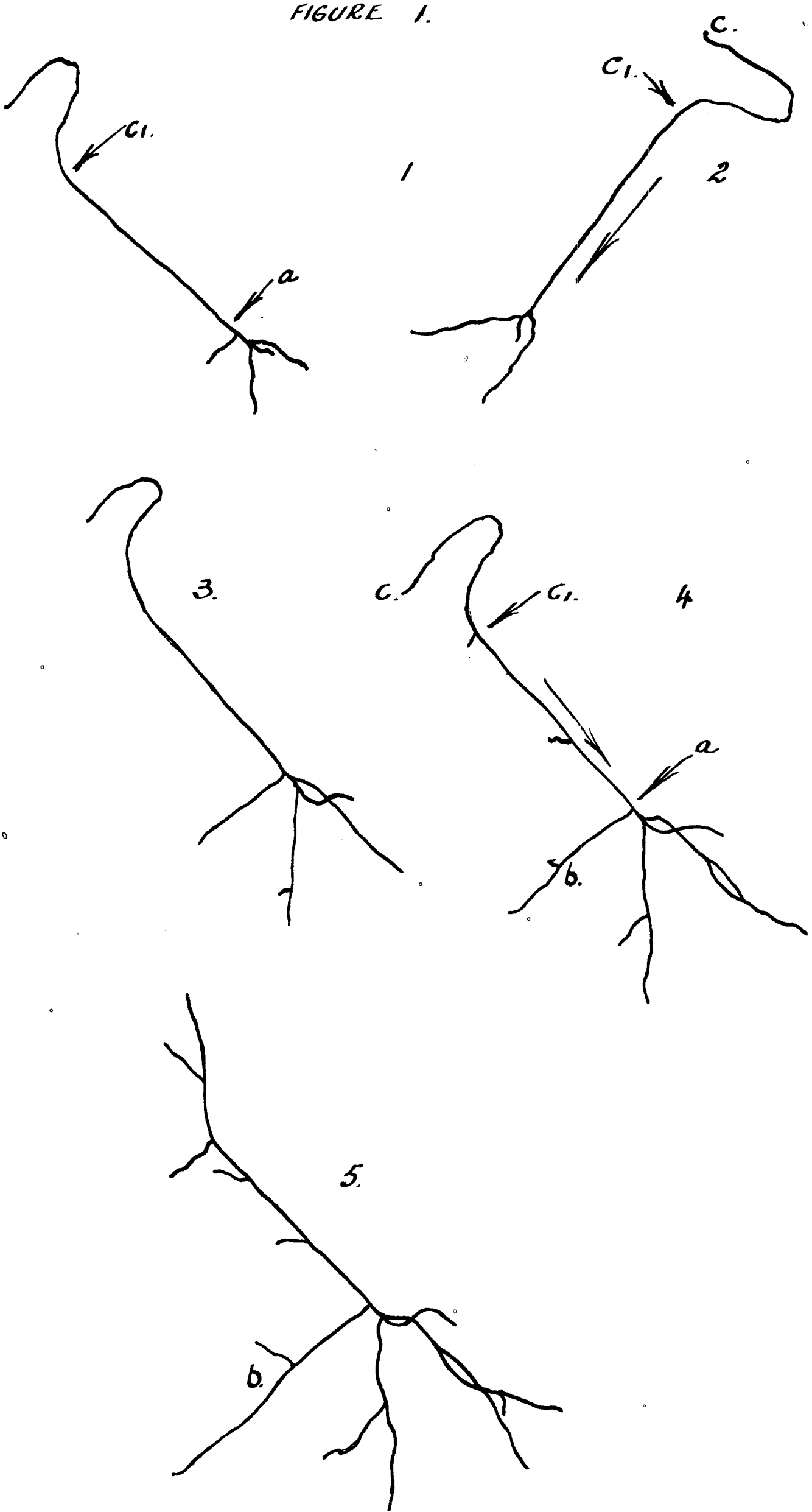
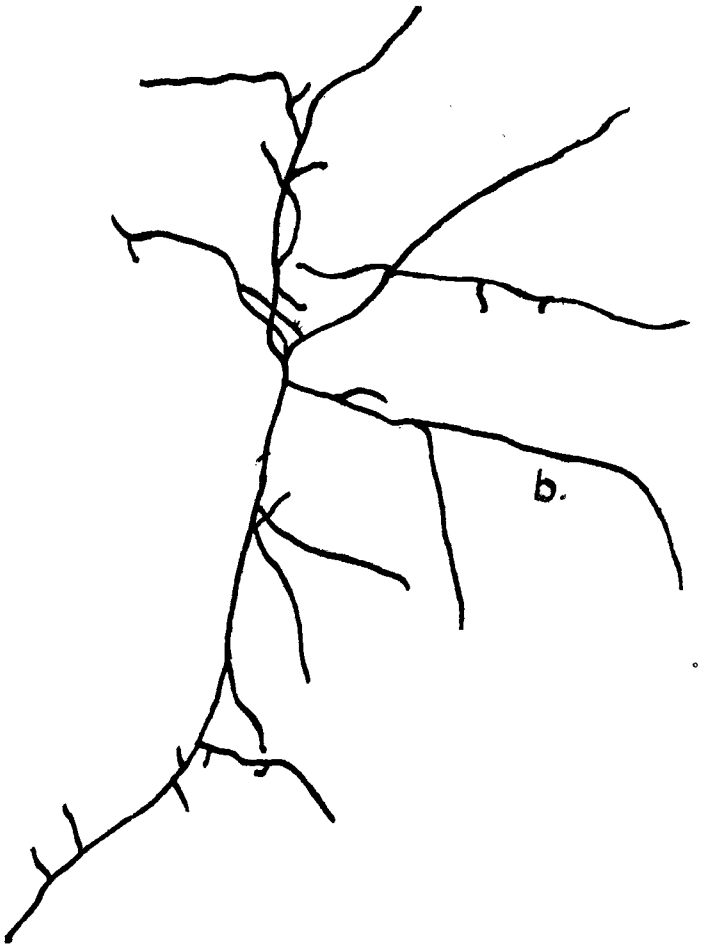
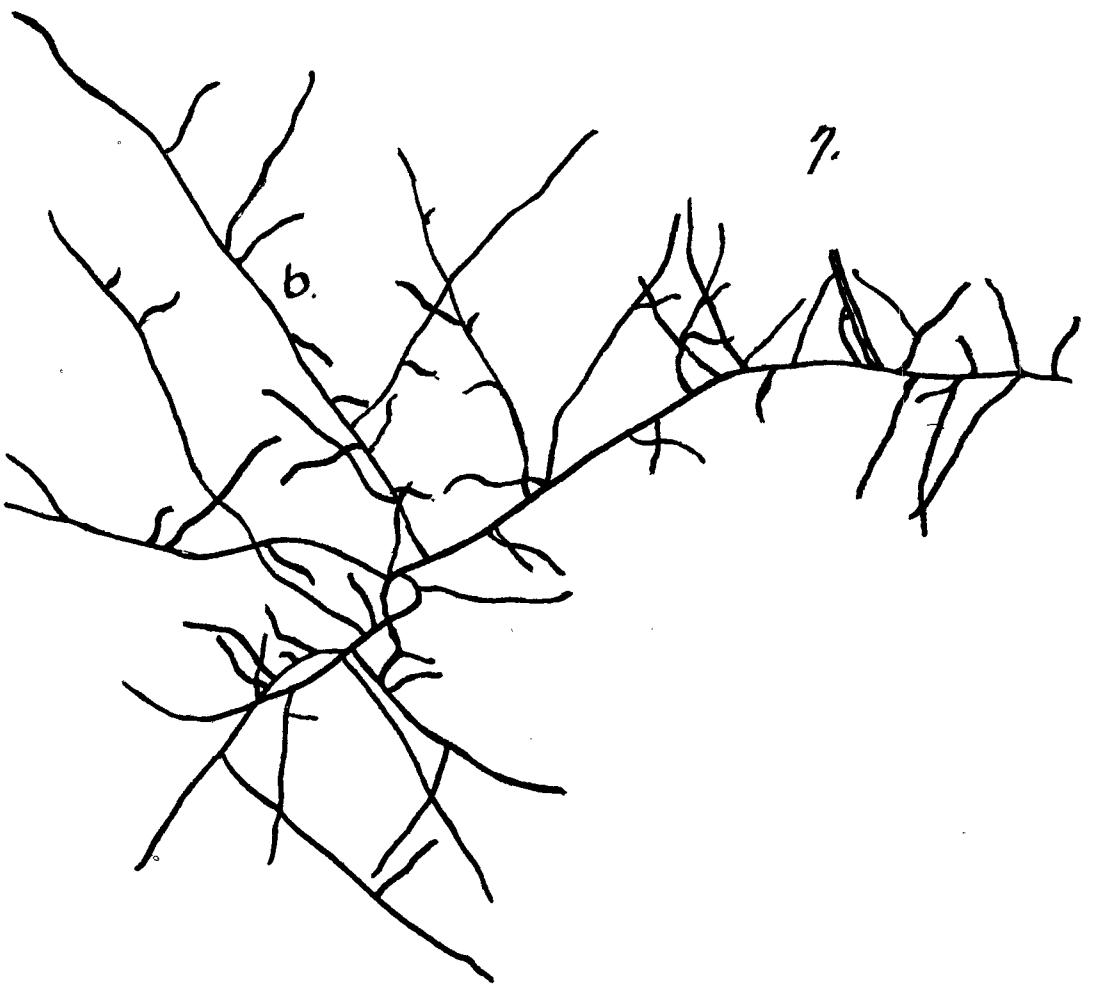


FIGURE 1.



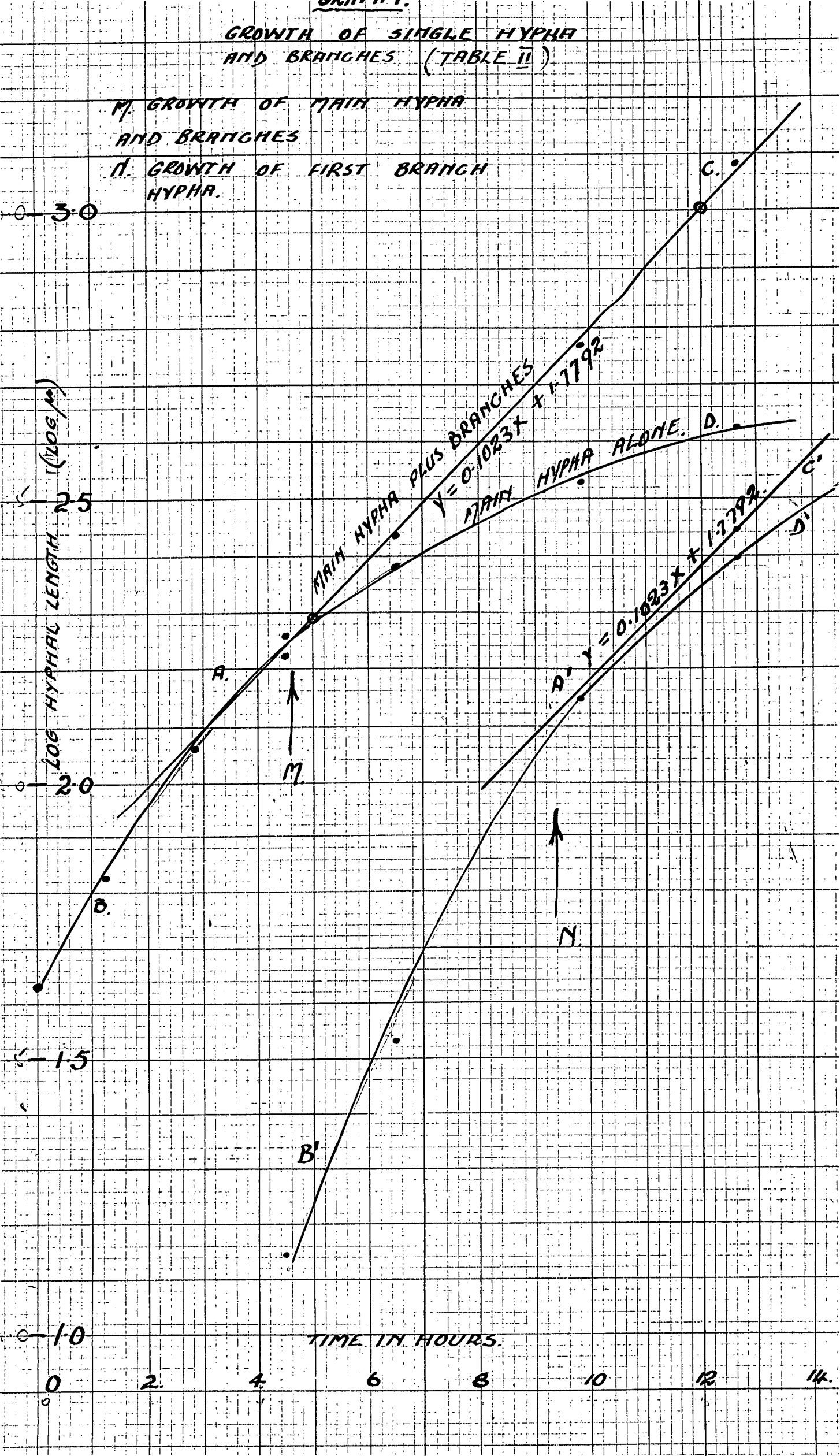


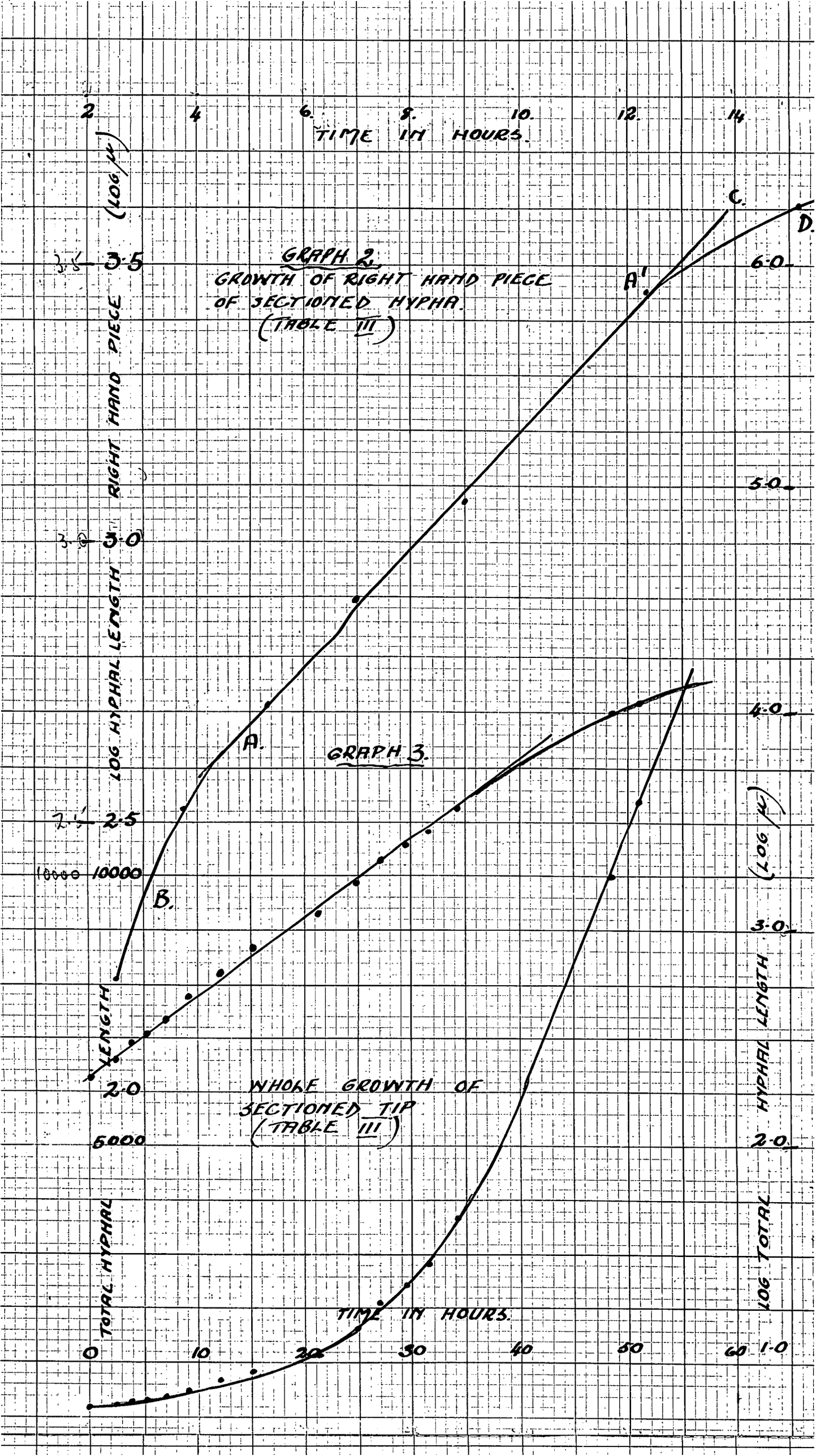
GRAPH I.

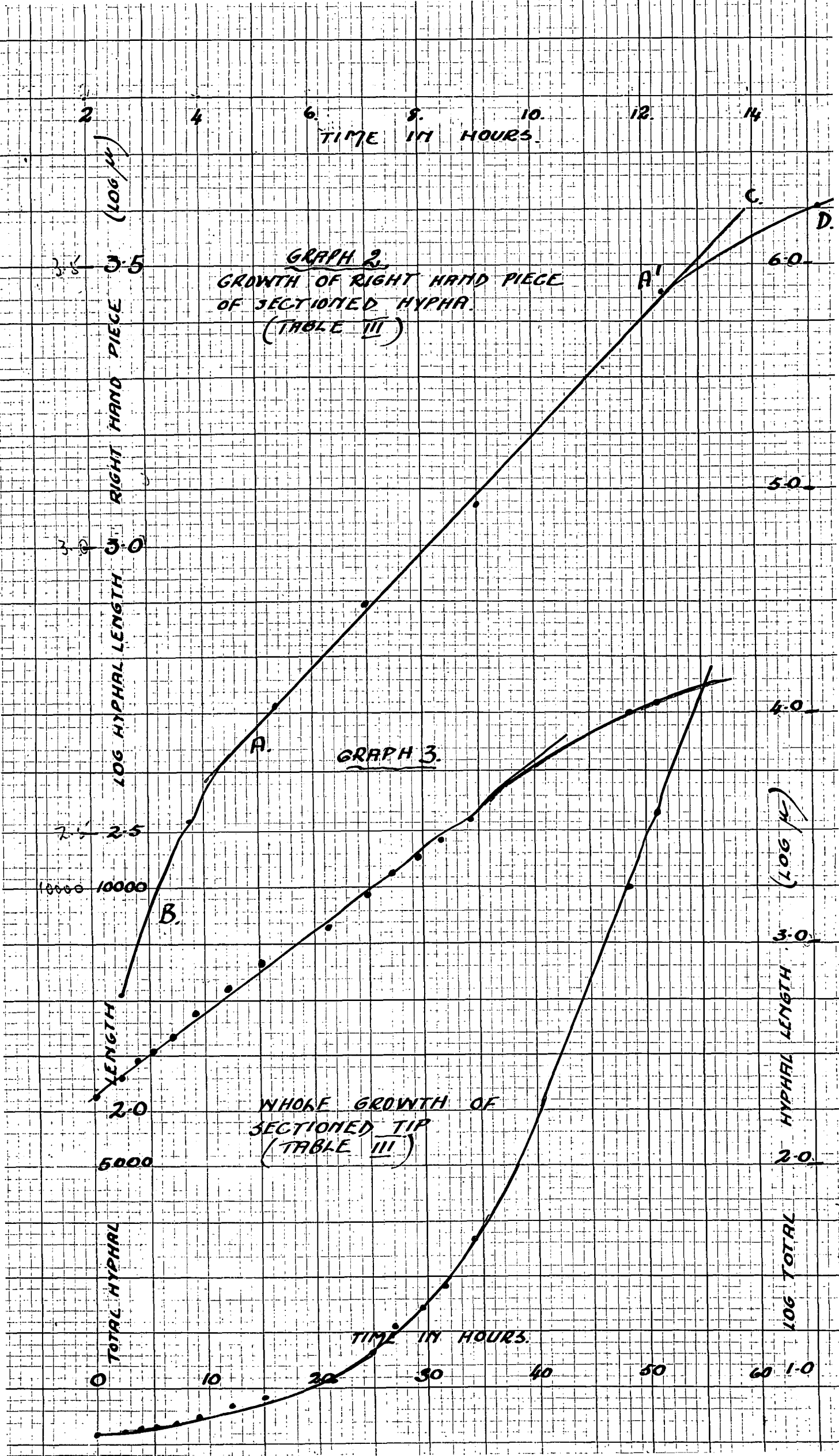
GROWTH OF SINGLE HYPHA AND BRANCHES (TABLE II)

M. GROWTH OF MAIN HYPHA AND BRANCHES

N. GROWTH OF FIRST BRANCH HYPHA.




2
4
6.
8.
10.
12.
14
TIME IN HOURS.
LOG HYPHAL LENGTH RIGHT HAND PIECE (LOG μ)
3.5
3.0
2.5
2.0
10000
5000
0
GRAPH 2.
GROWTH OF RIGHT HAND PIECE OF SECTIONED HYPHA.
(TABLE III)
A.
B.
C.
D.
A'
GRAPH 3.
WHOLE GROWTH OF SECTIONED TIP
(TABLE III)
TOTAL HYPHAL LENGTH
LOG TOTAL HYPHAL LENGTH (LOG μ)
6.0
5.0
4.0
3.0
2.0
1.0
10
20
30
40
50
60
TIME IN HOURS.


TIME IN HOURS.
2
4
6.
8.
10.
12.
14
GRAPH 2.
GROWTH OF RIGHT HAND PIECE
OF SECTIONED HYPHA.
(TABLE III)
LOG HYPHAL LENGTH RIGHT HAND PIECE (10⁶ μ)
3.5
3.0
2.5
2.0
A.
B.
C.
D.
A'
6.0
5.0
4.0
3.0
2.0
1.0
GRAPH 3.
LENGTH
10000
5000
0
WHOLE GROWTH OF
SECTIONED TIP
(TABLE III)
TOTAL HYPHAL LENGTH (10⁶ μ)
TIME IN HOURS.
10
20
30
40
50
60

GRAPH 4.

INCREASE IN MEAN SURFACE DIAMETER OF COLONY

MEAN DIAMETER OF COLONY (log mm)

LOGARITHM OF MEAN DIAMETER OF COLONY (log mm)

TIME IN HOURS

NORMAL

STRAVED

NORMAL

STRAVED

GRAPH 5

GROWTH IN DEPTH

SURFACE RADIUS OF COLONY (mm)

DEPTH OF MYCELIUM (mm)

GROWTH OF MYCELIUM (SURFACE) FROM TIME OF GERMINATION

LENGTH EQUIVALENT OF SPORE CONTENTS (5 μ)

LOGARITHM OF HYPHAL LENGTH (LOG μ)

GRAPH 1.

GROWTH IN DEPTH WHEN SURFACE RESTRICTED.

MYCELIAL DEPTHS ARE PLOTTED AGAINST SURFACE DIMENSION WHICH MAY BE REAL (BEFORE THE SIDES OF THE TUBE ARE REACHED) OR CALCULATED FROM THE NORMAL GROWTH CURVE AS ESTIMATED EXTENSION OF THE MARGIN IF UNRESTRICTED (GRAPH 4)

CALCULATED COLONY DIAMETER (mm)

10 20 30 40 50 60 70 80

SURFACE DIAMETER (14 mm.)

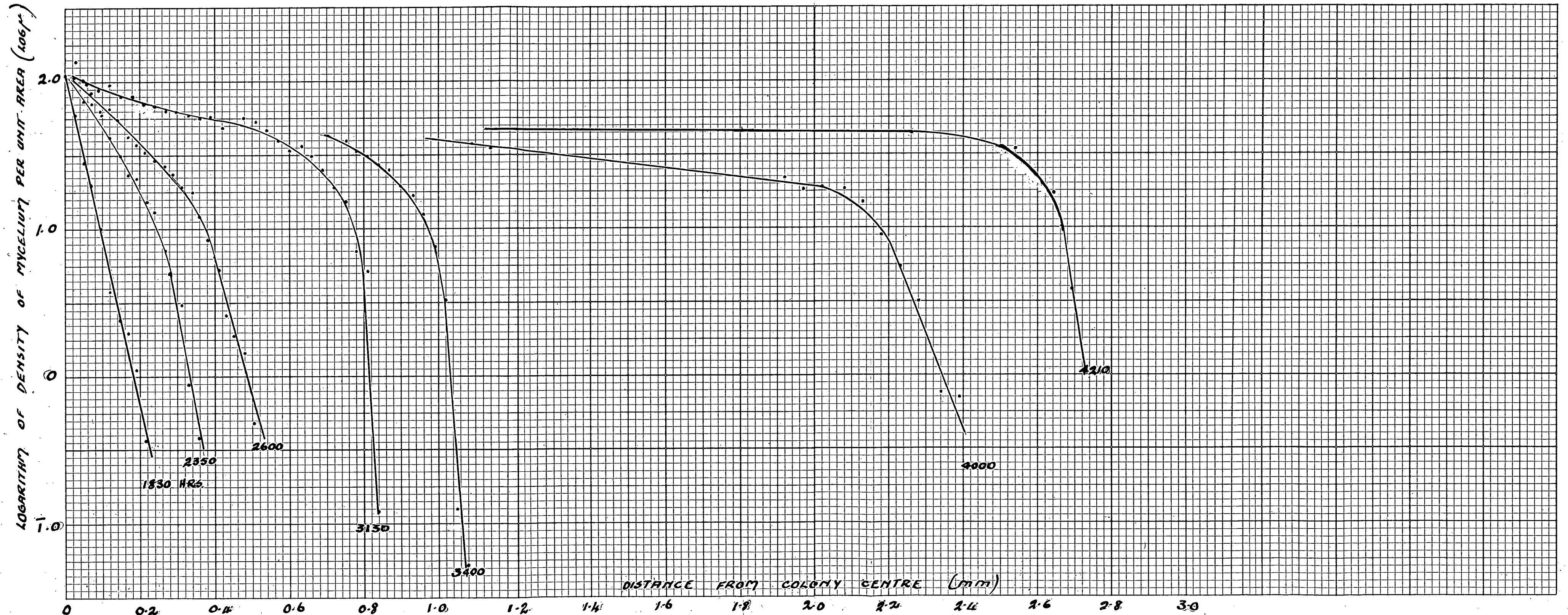
SURFACE DIAMETER (23·5 mm)

DEPTH OF MYCELIUM (mm)

2 4 6 8 10 12 14

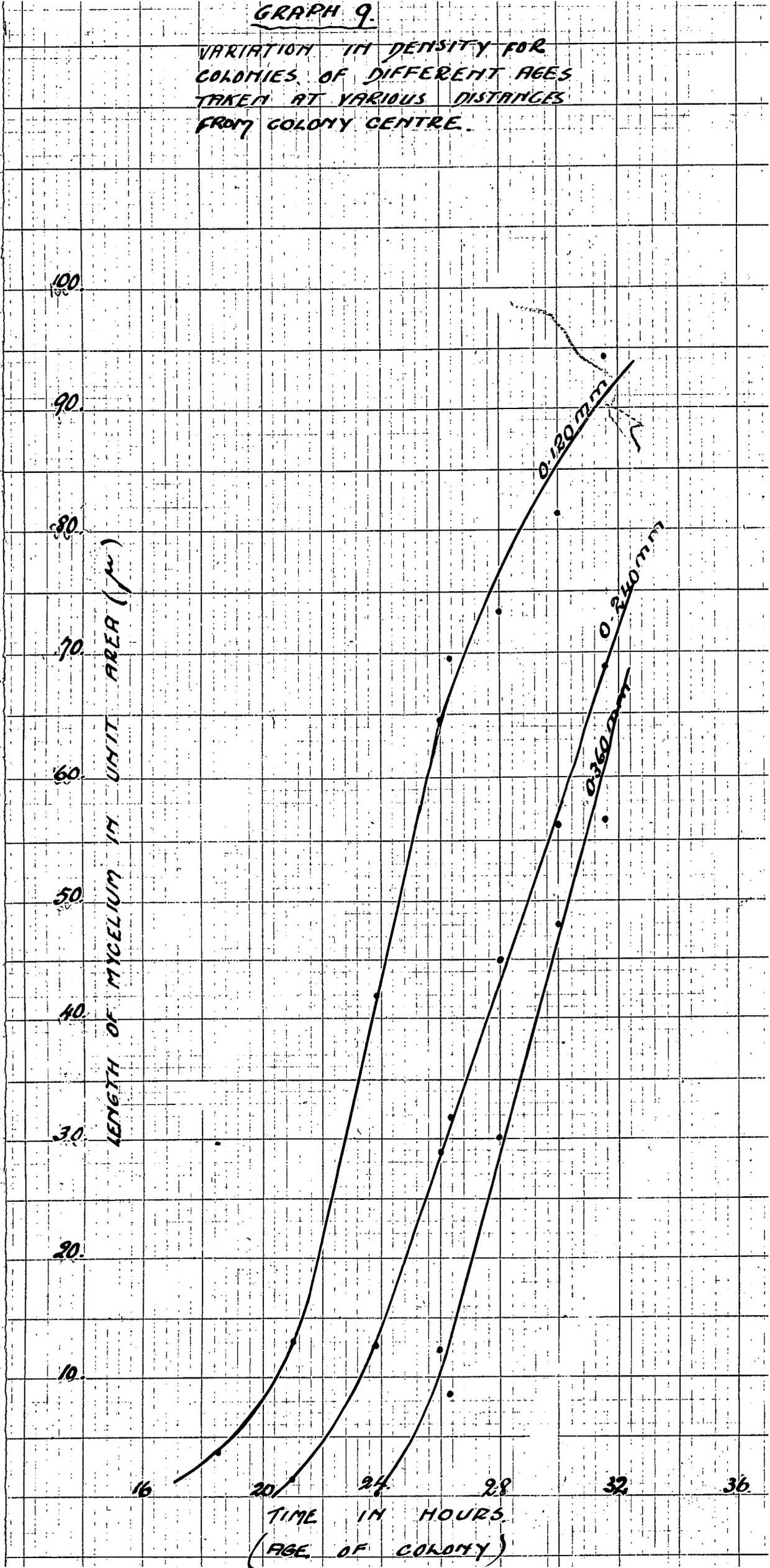
GRAPH 8.

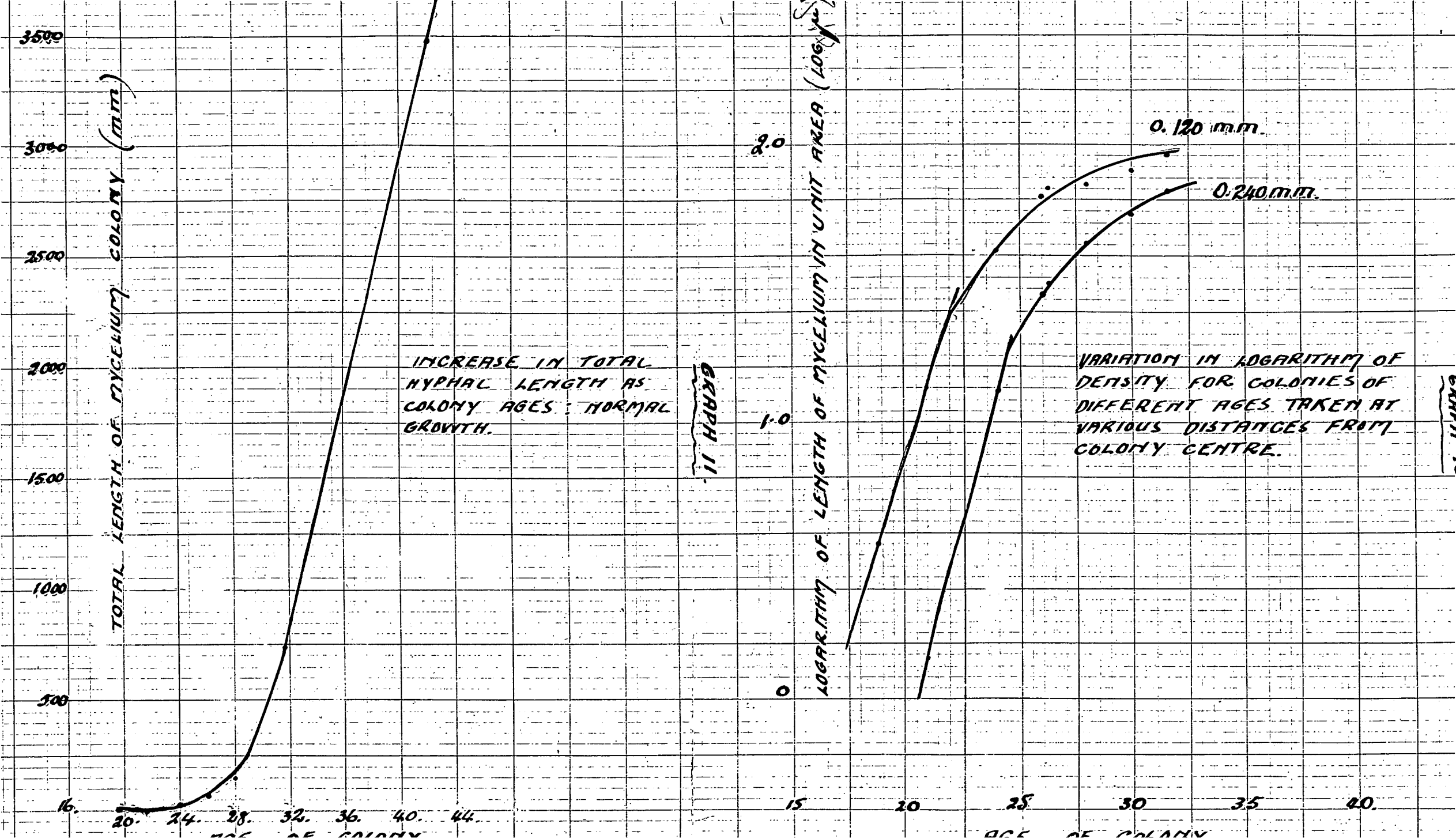
VARIATION IN DENSITY OVER SURFACE FOR COLONIES OF VARIOUS AGES.



GRAPH 9.

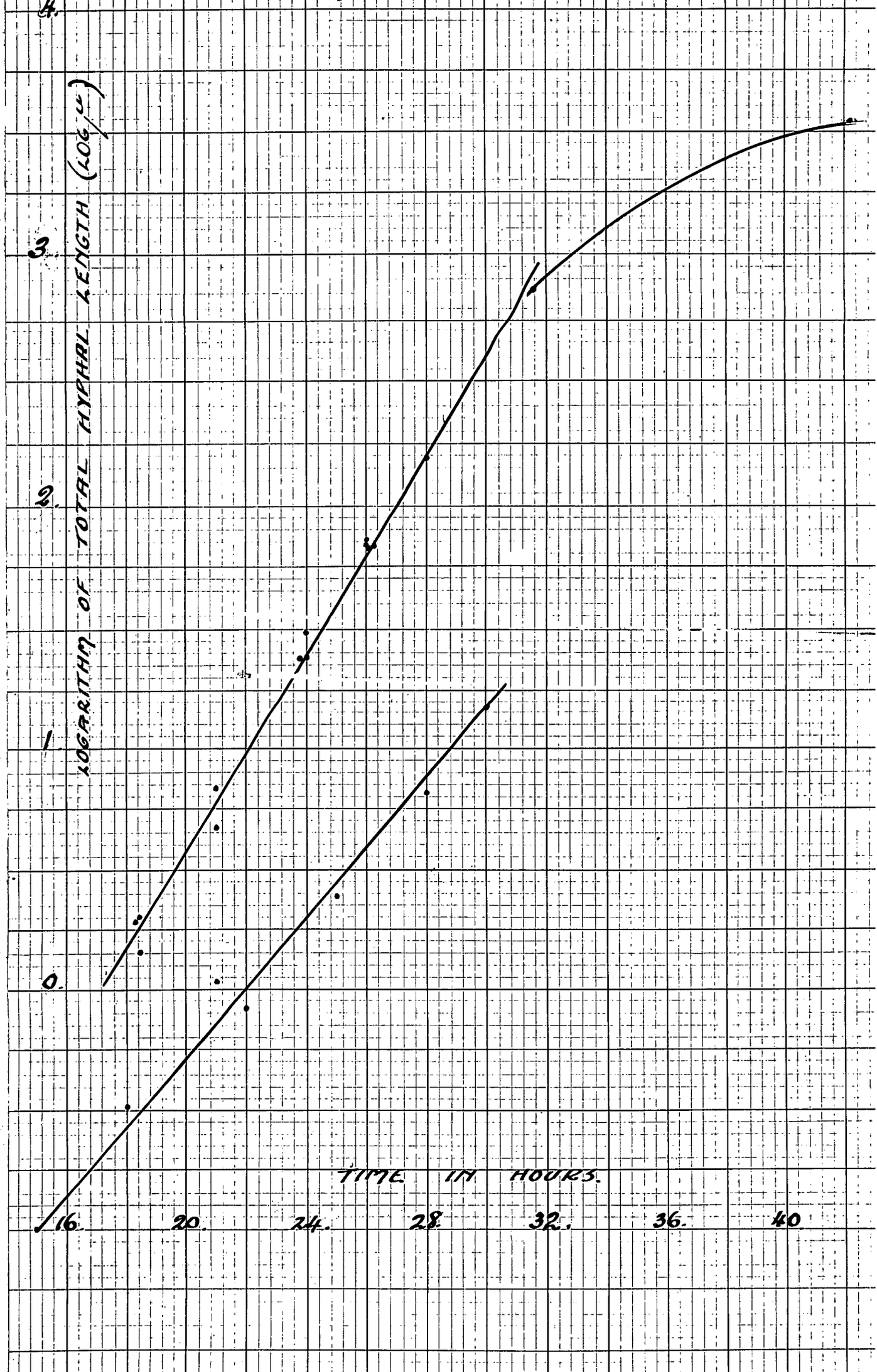
VARIATION IN DENSITY FOR COLONIES OF DIFFERENT AGES TAKEN AT VARIOUS DISTANCES FROM COLONY CENTRE.




TOTAL LENGTH OF MYCELIUM/COLONY (mm)
3500
3000
2500
2000
1500
1000
500
16.
20.
24.
28.
32.
36.
40.
44.
INCREASE IN TOTAL HYPHAL LENGTH AS COLONY AGES: NORMAL GROWTH.
FIG. 11.
LOGARITHM OF LENGTH OF MYCELIUM IN UNIT AREA (LOG
2.0
1.0
0
0.120 mm.
0.240 mm.
VARIATION IN LOGARITHM OF DENSITY FOR COLONIES OF DIFFERENT AGES TAKEN AT VARIOUS DISTANCES FROM COLONY CENTRE.
15
20
25
30
35
40.

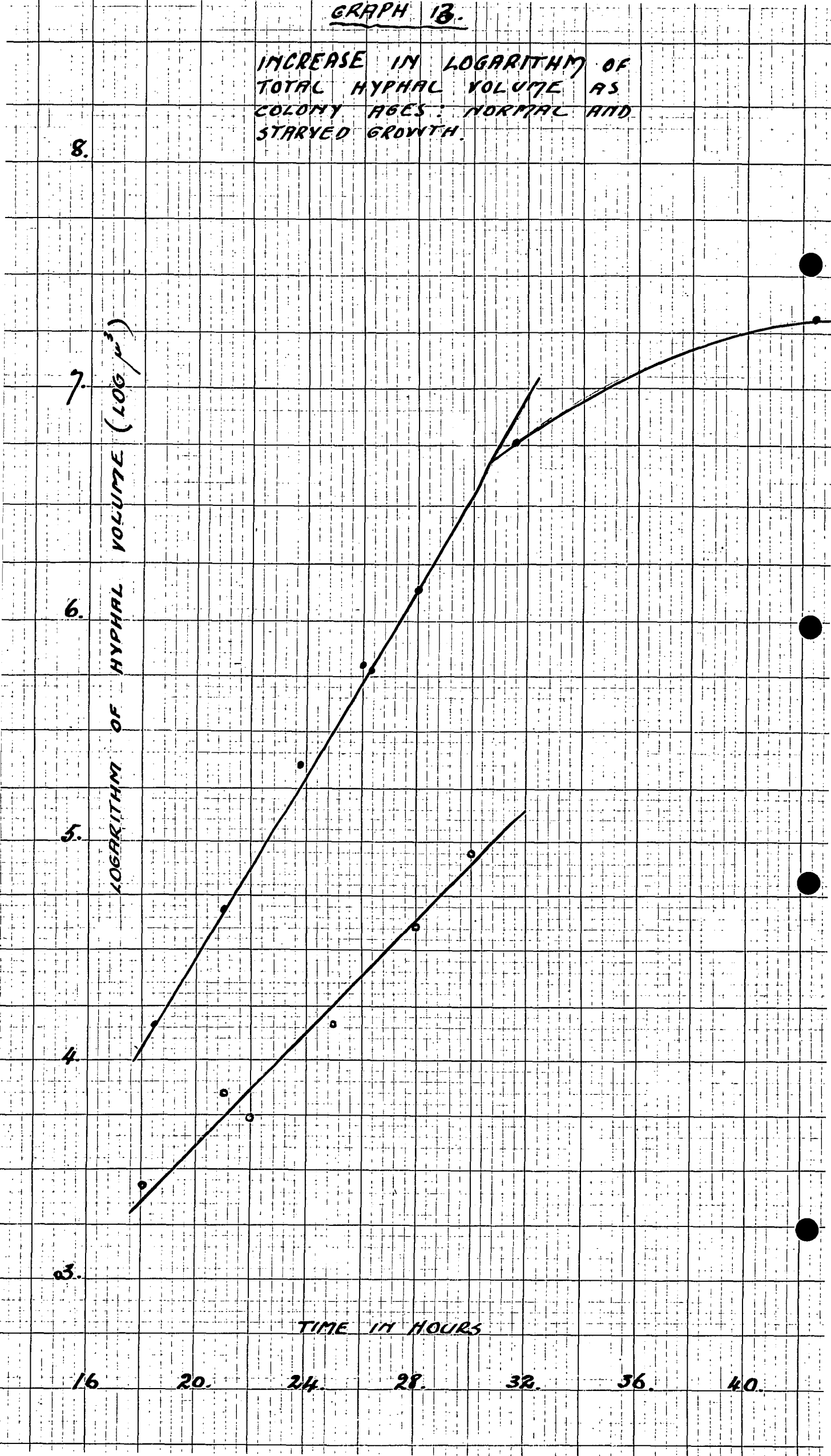
GRAPH 12.

INCREASE IN LOGARITHM OF TOTAL HYPHAL LENGTH AS COLONY AGES: NORMAL AND STARVED GROWTH.



GRAPH 13.

INCREASE IN LOGARITHM OF TOTAL HYPHAL VOLUME AS COLONY AGES: NORMAL AND STARVED GROWTH.



APPENDIX 2.

SECTORING IN THE ASCOMYCETOUS FUNGUS CHAETOMIUM GLOBOSUM KUNZE.

BY

N.J.B. PLOMLEY

APPENDIX 2.

Sectoring in the ascomycetous fungus *Chaetomium globosum* Kunze

by

M.J.E.PLOMLEY

The mechanism of sector formation in fungi has recently been described by Pontecorvo and Gemmell (1944). These authors describe the sectors induced (and occurring spontaneously) in colonies of *Penicillium notatum*. Growth in the colony was seen as growth of competing individual hyphae (see also Plomley and Fora, Appendix 1). The geometrical form of the sectors depended on whether growth rate in the sector was the same as, greater or less than that of the parent mycelium; complex forms could be built up from these basic forms (Text-fig. 1). If the variant growth rate was the higher, a sector of type 'a' was formed, "the boundaries of which are parts of equiangular spirals, with characteristics depending on the ratio of the two growth rates". When the growth rate in the sector was the same as that in the parent, the sector had straight sides (type 'b'), and when the variant rate was slower the parent came to enclose the variant (type 'c'). In this latter case the variant sector could only be formed under special conditions; normally the variant hyphae would soon be surrounded by neighbouring hyphae and evidence of its presence found only by inoculating from the site.(1). These special conditions must give the slow-growing variant some initial advantage in the colony. Pontecorvo and Gemmell showed that the sector types could be imitated when

---

Footnote 1: This may be compared with masked saltation in *Fusarium* D (Brown, 1926), and with the very common finding of saltants when subcultures, both spore and mycelial, are made from fungal colonies, the change occurring spontaneously, or induced by age or some other factor of the environment.

spore mixtures were plated. When the growth rates of the two spore types in the mixture were the same, type 'b' sectors were formed, the areas of sector and normal being proportional to the numbers of spores of each type in the mixture. When the spore types had different growth rates sectors of type 'a' or type 'c' resulted. Many of the types 'b' and 'c' sectors in irradiated material had blunt apices. The type 'b' sector with blunt apex could be imitated by plating two colonies close together: "the results are given by the intersection of two systems of concentric circles...; the intersections are hyperbolae and the angle between the asymptotes depends only on the ratio of the two diameters when the systems first meet". It was assumed that the variant hyphae in the irradiated colony had some positional advantage over neighbouring normal hyphae to produce this effect.

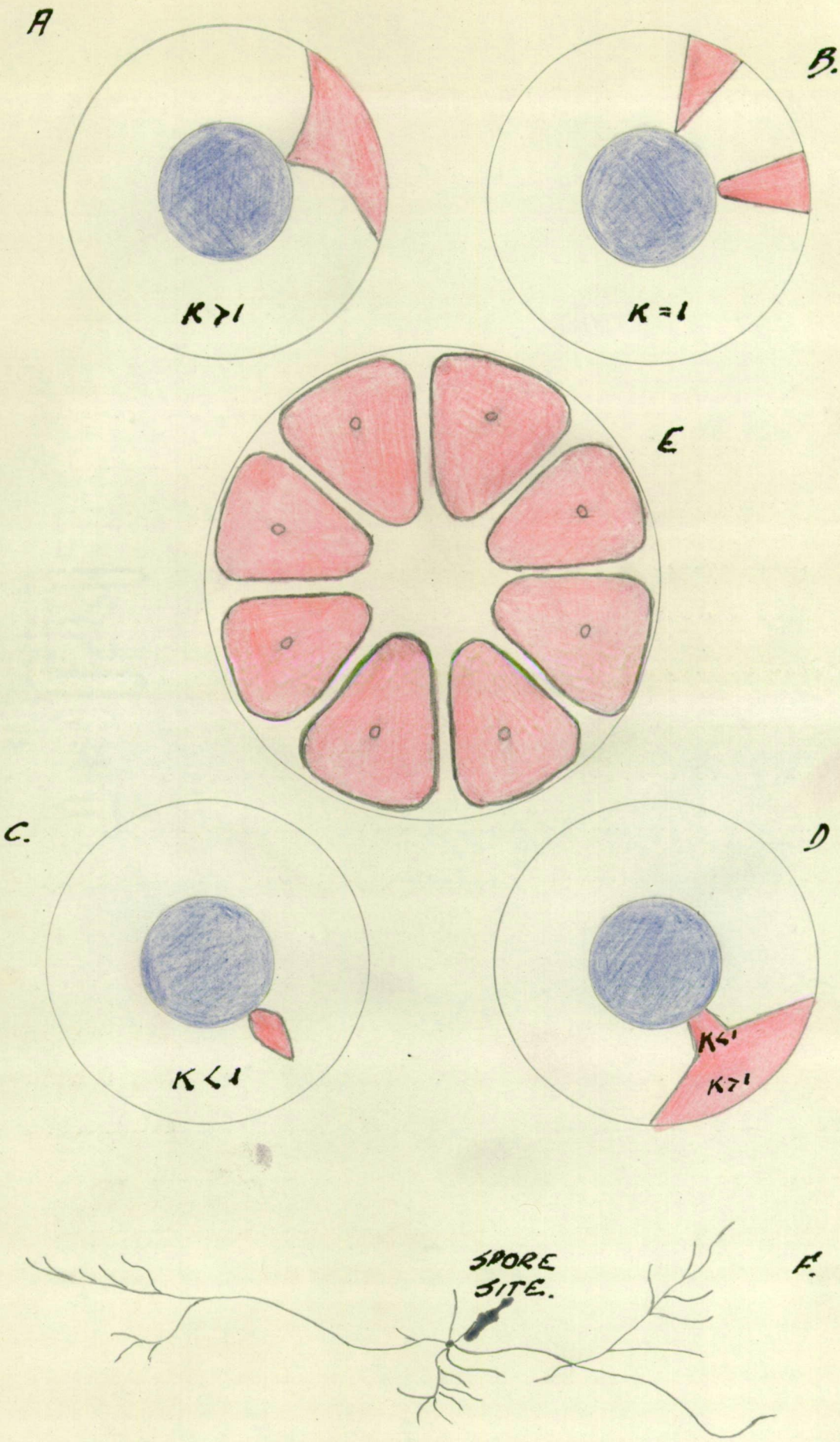
In Chaetomium globosum the same three basic types of sector have been found in both irradiated and control colonies. These sectoring colonies originated in single spores. The type 'a' sector is seen in Fig. 8, type 'b' sector in Fig. 9, and type 'c' sector in Fig. 36. This occurrence of the type 'c' sector in a colony of single spore origin, however, requires some modification of Pontecorvo and Gemmell's concept of "positional advantage". These authors pointed out that in the sectors developing after irradiation the slow growing mycelium (type 'c' sector) had to have some initial advantage over the faster growing normal mycelium for a sector to be formed. Because type 'b' sectors could have blunt apices, which were simulated by sectors formed when spore mixtures were plated in such a way that the spores were few in number and widely scattered, and also by plating colonies some distance apart, it was concluded that this initial advantage was positional. This could be the case in the irradiated colony, but it is difficult to conceive how it could have been so in spore mixtures. Irradiation of mycelium inhibits its growth, so that if the variant hypha starts growing before the normal mycelium a positional

advantage will be obtained. This advantage is not that the hyphae grows forward beyond the general colony edge, but that having freed itself from neighbouring hyphae it will grow sideways, thus forming a mass of mycelium from which the sector will arise. It may be noted here that the very early growth of irradiated spores may be quite unlike that in the normal colony (Text-fig. 1); the germinating hypha may grow slowly into a long, straggling 'colony' and then at some point or other hyphae start to grow normally, forming the true colony. By this means considerable positional advantage may be obtained.

In the colonies from the spore mixtures (other than the special case where spores were spread over a wide area) it is difficult to see how any positional effect could operate. Moreover, Pontecorvo and Gemmell explain the origin of type 'a' sectors, in which fast growing sector occurs in slow growing colony, as being due to a larger number of spores of the slow type in the mixture, yet they require an initial positional advantage in the complementary case, the type 'c' sector, Examination of the young colony, however, resolves the question; at this time hyphae are occupying growing space chiefly by lateral growth into it from one or two first formed hyphae (Plomley and Ford, Appendix 1). This early growth determines the spherical form of the colony, so that even if some of the hyphae are slow growing they can still block out sufficient area to form potentially an appreciable sector of the colony. It is only when the colony is expanding at constant rate that this slow growing mycelium will be at any real disadvantage and will come to be enclosed by the fast growing mycelium.

Brown, W. (1926). Studies in the genus Fusarium. IV. On the occurrence of saltations. Ann. Bot., 40. p. 223/

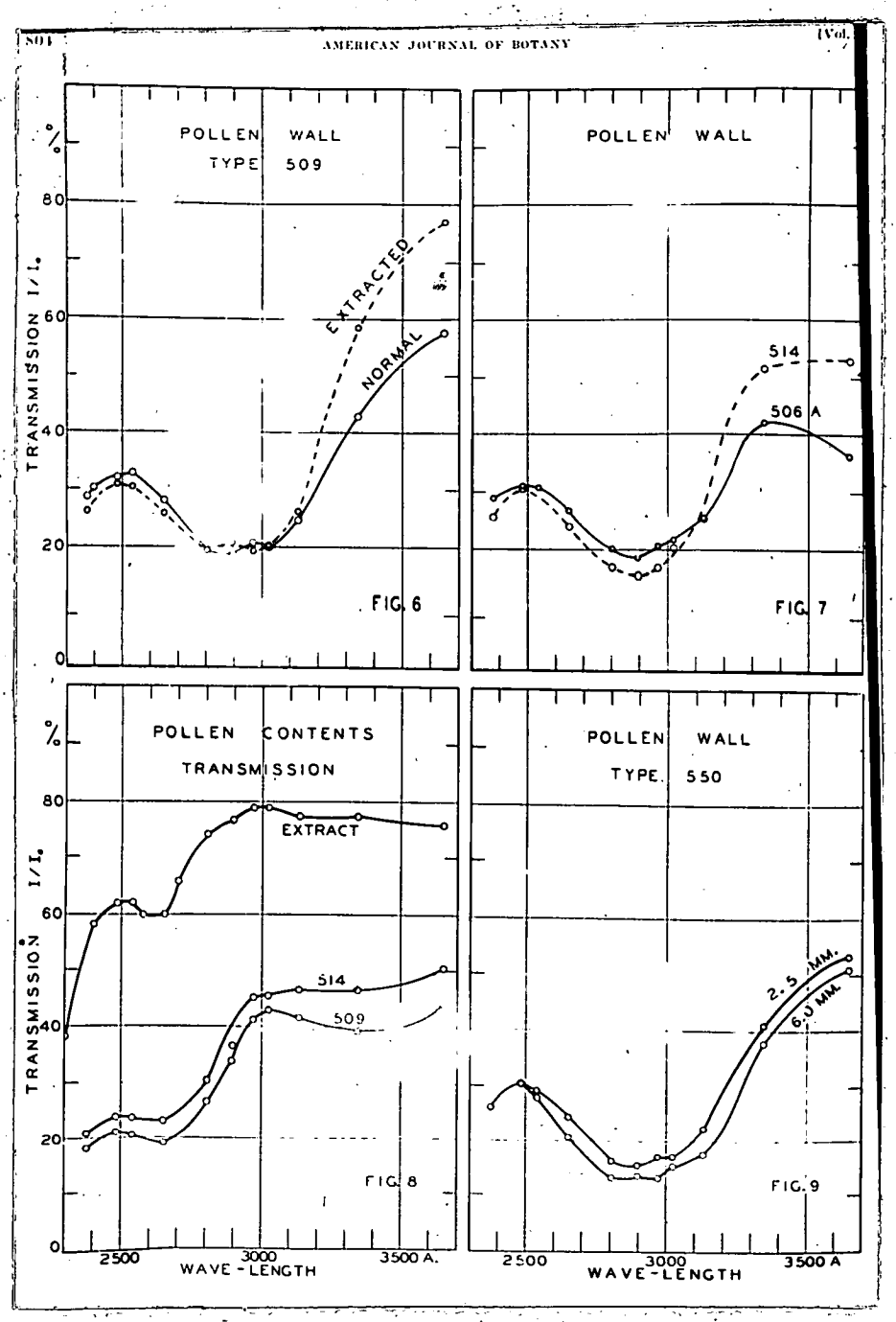
Pontecorvo, G. and Gemmell, A.R. (1944). Colonies of Penicillium notatum and other moulds as models for the study of population genetics. Nature. 154/3913. p. 532.



A-D: Basic sector types. Colonies were irradiated and variant sectors became apparent as growth continued in the colony. Blue area represents colony at time of irradiation; red sectors are variant sectors. A: type 'a' sector, variant hyphae having higher growth than normal. B: type 'b' sector, variant hyphae having same growth rate as normal; sectors may have acute (a) or blunted (b) origins. C: type 'c' sector, variant having slower growth rate than normal. D: complex sectorial form. After Pontecorvo and Gemmell (1944).

E: Imitation of sectorial form when colonies of a Chaetomium saltant developed from spores inoculated at equal spacing.

F: Abnormal growth in a young Chaetomium colony grown from an irradiated spore. Note the straggling form which could lead to positional advantage in later growth.



6. Transmission curves for individual pollen walls, normal and after extraction for pectin and water soluble constituents. 2.5 mm objective.

7. Transmission curves for individual walls of two distinct types of pollen; 514, corn pollen; 506A, "nojoya teosinte". 2.5 mm objective.

8. Transmission curves for 8 micron layers of pollen contents for types 509 and 514 corn. The top curve is for an aqueous plooen extract in phosphate buffer solution at pH 8. 6.0 mm objective.

9. Pollen wall transmission curves as measured with 2.5 mm and 6.0 mm focal length objectives.

Uber (1939).

APPENDIX 4.

SALTANTS PRODUCED IN THE FUNGUS CHAETOMIUM GLOBOSUM BY MONOCHROMATIC ULTRA-VIOLET IRRADIATION AND A GROWTH EFFECT CHARACTERISTIC OF WAVELENGTH

BY A.L.MCAULAY, E.J.B.PLOMLEY, AND J.M.FORD

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by A. L. McAULAY, N. J. B. PLOMLEY, and J. M. FORD

SALTANTS PRODUCED IN THE FUNGUS *CHAETOMIUM GLOBOSUM* BY MONOCHROMATIC ULTRA-VIOLET IRRADIATION AND A GROWTH EFFECT CHARACTERISTIC OF WAVELENGTH

by A. L. McAULAY, N. J. B. PLOMLEY, AND J. M. FORD[1]

(From the Physics Department, University of Tasmania).

(Accepted for publication 1st November, 1944.)

Spores of *Chaetomium globosum* have been subjected to monochromatic ultra-violet irradiation and colonies have been grown from single spores which were irradiated dry. Special attention has been given to experiments in which the mercury lines at 265 mμ, 313 mμ and 334 mμ were used. In all these cases many saltants were produced as the result of the treatment of the spores. The primary object of the investigation was to observe any selective appearance of saltants at different wavelengths. Evidence for such a selective effect has been given by one of us, McAulay (1938) and the present experiments were made with more refined methods to obtain further information on this question.

A very marked selective effect was found in which a certain easily recognized type of change was produced in large numbers of colonies grown from spores irradiated by short wavelengths, but in a very few irradiated by long wavelengths. This change is more in the nature of a growth modification than of a saltant. A part of the colony is normal but a sector appears whose vertex usually is not at the centre of the circular growing colony. The sector is nearly clear of aerial mycelium and is frequently edged with dense brown in the substratum. The aerial mycelium is often dense at the edge of the sector and may form white knots. The modification has been designated by the letter "K."

There is a particular feature of interest about the K type. The colony, grown from a single spore, comprises distinct parts each with quite different characteristics. It would appear that the mycelium growing from the single spores is unstable in the sense that a colony derived from it may have two quite distinct forms.

On several occasions the same single spore gave rise to three or more types, normal, K and a saltant (Fig. 2, No. 6).

A very large number of saltant types is produced in *Chaetomium globosum* by irradiation with wavelengths over the range 230 mμ to 334 mμ. The percentage of colonies saltating is roughly constant over this range provided the energy per sq. cm. applied to the spore is a constant proportion of the lethal dose, in striking contrast to the "K" growth modification which appears selectively at short wavelengths.

EXPERIMENTAL METHOD.

The monochromator used in these experiments was constructed in the laboratory, and has been described by McAulay and Taylor (1939). A 125 watt mercury discharge lamp, a commercial unit with outer envelope removed, was used as a source of ultra-volet light.

Spores were carefully spread on a coverslip in a narrow arc within the limits of the wavelengths and separated from one another so that all would have an equal opportunity of being irradiated. The spectrum was focussed on the spores by fluorescence of an underlying uranium

[1] The funds required for this work came from the Commonwealth Research Grant to the University.

glass mount; the control spores on another coverslip were placed near, but in the visible end of the spectrum. When the spores were treated at long wavelengths, short wavelengths were screened by means of glass microscope slides in order to eliminate the small amount of short wave radiation which was found to be present over the whole spectrum. One microscope slide was used for 313 mμ and three for 334 mμ. Even when these precautions were taken a certain amount of leakage occurred, and this is the possible explanation of a few K type appearing at the longer wavelengths.

An airstream was passed over the spores during irradiation to prevent the occurrence of secondary effects due to ozone accumulation, lack of aeration, high temperature and so on.

The spores were irradiated for varying lengths of time, for a few minutes at 265 mμ; a few hours at 313 mμ; and a few days at 334 mμ. Intensity measurements were made by means of a sensitive galvanometer and a vacuum thermopile. The average doses to give equivalent biological effects, exclusive of the K modification, were:

	265 mμ.	313 mμ.	334 mμ.
Average dose in joules/cm²	0·1	25	150

Irradiated and control spores were plated singly in separate Petrie dishes so that the numbers of saltant colonies could be determined and each saltant obtained unassociated with mycelium from other spores.

Two techniques were used, the first a strictly controlled single spore method, and the second a dilution plate method. In the first dabs of spores were taken from the irradiated and control spore lines and placed in the centre of Petrie dishes on 1 p.c. clear malt agar. Single spores were picked up with a pointed platinum needle and placed at marked intervals on the agar. This plating was carried out under the high power of a dissecting microscope so that the spores, in their plated position, were easily visible and there was no possibility of more than one spore giving rise to the adult colony. All the leading features of mixed growth, etc., which are later to be described, were observed when this technique was used.

Spores remaining after the above plating were made into dilution plates, the amount of dilution depending on the percentage germination and visible colonies obtained from the plated single spores. When the colonies were visible to the naked eye they were cut out and placed in fresh dishes of malt agar, a single colony to a Petrie dish.

Irradiation Dosage.

In the earlier experiments 100 irradiated and 100 control spores were plated singly on 1 p.c. malt agar in Petri dishes, while in later experiments only 50 control spores were used, the larger number being considered unnecessary. The number of spores germinating was counted in this way, and also the number germinating and continuing to grow to form adult colonies. Percentage germination did not give a good indication of the effect of irradiation because in some experiments the control germination was very low. The percentage adult colonies from germinating spores gave a much better measure of the biological effectiveness of the dose.

ANALYSIS OF SALTANTS.

Two strains of *Chaetomium globosum*, one, *Lj*, from Holland and the other, *KB*, from Sydney, and a globosum saltant, *Fld*, obtained in previous work, were used in these experiments. Fig. 1, No. 1, shows a normal colony of *C. globosum Fld*. A few additional experiments were performed with another saltant of *Chaetomium globosum* and with a strain of *Chaetomium elatum*, but owing to the difficulty of analysing the saltants of these two types they have not been included in the quantitative analysis (Table 1).

The changes which resulted from irradiating spores at 265 mμ, 313 mμ and 334 mμ were many and varied, but can be grouped into three rather broad classes, growth modifications, mycelial changes and perithecial changes. The first class contains those saltants or changes in which rate of growth or form is markedly different from that of the normal colony. Division of the growth types can be made into four sub-classes: photographs of typical examples of three of these are shown in Fig. 1, Nos. 2–4. The second class contains saltants in which the mycelium is very different from the normal. Here again division may be made into a number of sub-classes, and an example is shown in Fig 1, No. 5. Colonies in which the perithecia show a change in distribution, colour or size, make up the third class of saltants. An example is shown in Fig. 1, No. 6.

The mycelial and perithecial changes frequently appear together, and colonies are commonly found with marked growth modifications as well as perithecial and mycelial changes.

A point of interest is that saltants from irradiated spores often approach in appearance other species of *Chaetomium* (McAulay, 1938). Such resemblances may, however, be superficial only, no critical examination of material having been made.

Single irradiated spores would sometimes yield colonies in which a part was normal and a part saltant (Fig. 1, No. 6); less frequently, mixed saltants were obtained (Fig. 1, No. 7).

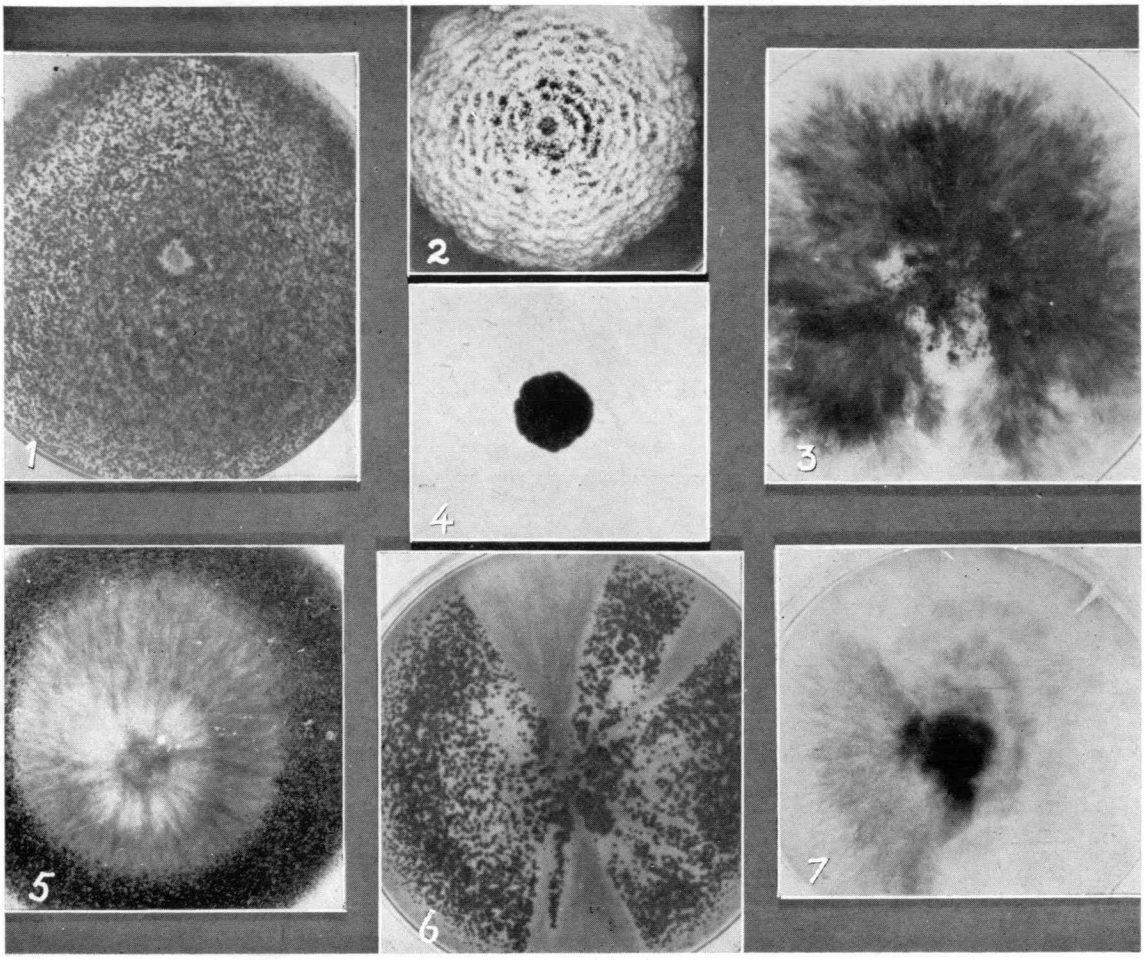

Fig. 1.

No. 1. *Chaetomium globosum* strain *Fld*: a normal colony grown from a single spore.

No. 2 Growth modification saltant resembling a succession of small scallops.

No. 3. Growth modification saltant with flares of mycelium growing from several central points, and with smaller flares further out giving the appearance of a red seaweed.

No. 4. Saltant colony which is small and button-like in form.

No. 5. Mycelial saltant with the central mycelium white and flat at first soon changing to fluffy; no perithecia. Saltant region surrounded by normal.

No. 6. Perithecial saltant sectors where mycelium is normal but perithecia are lacking: remainder normal. Colony grown from a single spore.

No. 7. A mixed colony grown from a single spore with two saltant types: scallop and perithecial saltant sectors.

All illustrations except No. 1 in Fig. 1 represent colonies of *Chaetomium globosum* grown from spores irradiated with monochromatic ultra-violet light. No. 1 has not been irradiated. Colonies are approximately three-quarters natural size.

K-TYPE MODIFICATION.

Colonies from single spores showing the K type modification have characteristic areas, frequently sectors, in which growth is abnormal. Perithecia and aerial mycelium are not formed over most of the area, although they may be present at the edges. When the K-area is large the mycelium at its edge is compact and knotted, and the substratum is dense brown. A series of K-type colonies is shown in Fig. 2. Results obtained so far have shown that spore cultures from these colonies produce normal colonies while mycelium cultures produce either normal or K-type colonies. The fact that pure line spore cultures of K type cannot be made has prevented us from classing this modification as a saltant.

In Fig. 2 it will be noted that both normal and abnormal parts are present to a varying degree, indicating the instability of colonies from irradiated single spores. In Nos. 1 to 4 the normal and abnormal regions appear as sectors, the abnormal sectors arising very close to the centre of the colony and the normal originating at the centre.

It is seen from Table 1 that the K-type modification is produced commonly at 265 mμ, a short wavelength, but rarely at long wavelengths. When production of the K-type is compared on the basis of equal lethal effect of the irradiation,

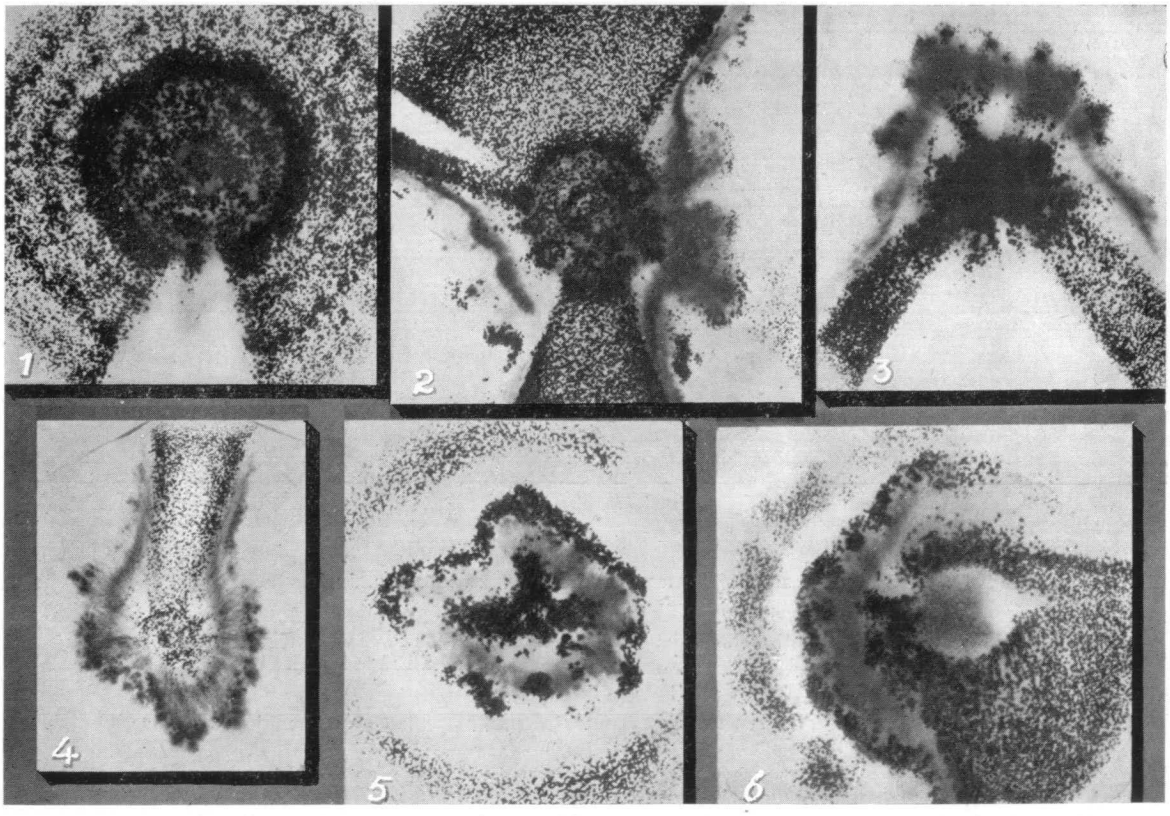

Fig. 2.

Nos. 1–6. Series of colonies of *Chaetomium globosum* showing the K-type modification characteristic of short wavelengths, ranging from a slight effect (No. 1) with little brown pigment, to a total effect (No. 5) with dense brown pigment round edge of affected area, an area devoid of aerial mycelium and perithecia. Growth normal only near limits of colony. No. 6 shows a mixture of 3 distinct types, normal, "K" and slow-growing saltant. Colonies represented in Nos. 4 and 6 are of single spore origin.

about 30 p.c. of the colonies growing from spores irradiated at 265 mμ show the modification, but less than 5 p.c. of the colonies when spores are irradiated at 313 mμ and 334 mμ (Table 1). The effect of irradiation is therefore selective in the production of K-type modification.

TABLE 1.

Table of occurrence of saltants and a special growth effect, the K type, in colonies grown from irradiated and control single spores of Chaetomium globosum Lj, Kb *and* Fld *(total of 41 experiments).*

Wavelength		265 mμ.				313 mμ.				334 mμ.			
		Lj.	KB.	Fld.	Total.	Lj.	KB.	Fld.	Total.	Lj.	KB.	Fld.	Total.
Total experimental colonies		527	121	617	1,265	409 (293)*	288	669	1,366 (1,250)*	—	197	1,069	1,266
Total control colonies					700				700				650
K type	exptl.	126	58	212	396	61 (14)*	3	20	84 (37)*	—	0	17	17
	control	0	0	0	0	0	0	0	0	—	0	0	0
Growth modification saltants	exptl.	12	4	5	21	10 (5)*	6	12	28 (23)*	—	11	13	24
	control	0	0	0	0	0	0	0	0	—	0	0	0
Mycelial saltants	exptl.	6	0	12	18	8 (5)*	6	15	29 (26)*	—	5	19	24
	control	0	0	0	0	0	1	0	1	—	0	0	0
Perithecial saltants	exptl.	2	3	5	10	5 (1)*	0	1	6 (2)*	—	0	8	8
	control	1	0	1	2	0	0	3	3	—	0	0	0
Exptl.	percentage K type				31·30				6·15 (2·96)*				1·34
	percentage true saltants				3·87				4·61 (4·08)*				4·42
Average dose in joules/cm²					0·1				25				150

* The figures in brackets omit an early 313 mμ experiment in which a large number of "K" type modifications appeared in contrast to all other 313 mμ and 334 mμ experiments. It is possible that contamination by 265 mμ occurred in this case.

SUMMARY.

Further results are reported of effects produced by monochromatic ultra-violet irradiation of *Chaetomium* spores.

A refined technique is used enabling the effect of the irradiation on individual spores to be studied.

Saltations involving modifications of growth rate and form, mycelium and perithecia are produced, as well as a growth modification, the K-type.

Mycelium from the irradiated spore frequently shows instability, having the capacity for development into more than one type of colony. This property is particularly marked in the K type.

For equal lethal effects of the irradiation, production of K-type amounts to 31·3 p.c. at 265 mμ, but less than 5 p.c. at 313 mμ and 334 mμ, while production of saltants is independent of wavelength.

A selective effect with wavelength has been established for ultra-violet irradiation of biological material.

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