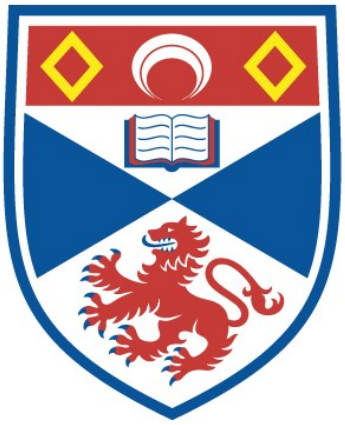
# A STUDY ON MARASMIUS ANDROSACEUS FR. AND MARASMIUS ROTULA (SCOP) FR

Edgar Julian Duncan

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1963

# A STUDY ON

# MARASMIUS ANDROSACEUS FR.

and

# MARASMIUS ROTULA (Scop.) FR.

by

Edgar Julian Duncan, B.Sc.

A thesis submitted to the University of St. Andrews for the degree of Doctor of Philosophy.

Department of Botany,
University of St. Andrews.

July, 1963.



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DECLARATION.

I hereby declare that the following Thesis is based on a record of work done by me, that the Thesis is my own composition, and that it has not previously been presented for a Higher Degree.

The research was carried out in the Department of Botany at St. Salvator's College of the University of St. Andrews under the direction of Professor J.A. Macdonald.

## CAREER.

I graduated from the University College of the West Indies, Kingston, Jamaica, in June 1960, with second class honours in the Upper Division in Botany and Zoology. I was awarded the Sir James Irvine Memorial Scholarship, tenable at the University of St. Andrews.

I matriculated at the University of St. Andrews and was admitted as a Research Student under Ordinances 16 and 61.

During the tenure of the Scholarship I undertook the research work presented here for the degree of Ph.D.

CERTIFICATE.

I certify that Edgar Julian Duncan has spent nine terms of research work under my direction and that he has fulfilled the conditions of Ordinance No. 16 (St. Andrews), and that he is qualified to submit the accompanying Thesis in application for the degree of Doctor of Philosophy.

ACKNOWLEDGEMENTS.

I wish to record my indebtedness to Professor J.A. Macdonald of the Department of Botany, St. Salvator's College, for supervising the work presented in this Thesis, and for the continued interest he has shown in the investigation.

I am also indebted to the University of the West Indies, for awarding me the Sir James Irvine Memorial Scholarship, thus making it possible for me to carry out the work presented here.

CONTENTS.

# INTRODUCTION.

Wager in 1893 wrote "The question of the structure and division of the nuclei in the lower plants is one of considerable interest to histologists, and has attracted the attention of numerous observers in recent years. The results obtained, however, leave much to be desired, especially as regards the nuclei of the Fungi."

The great impetus to the study of the cytology of the basidiomycetes came, however, only after the fundamental investigations of Kniep (1928). In the following two decades, much was written on the cytology of the basidium, but the basidiomycetes "attracted less and less interest", as "other fungi have come to the foreground, a process which has been brought about by the demands of industry, genetics and physiology of metabolism (Yeasts, Aspergillus, Penicillium, Neurospora)." (Girbardt, 1955).

With the development of new techniques and more efficient optical instruments, a new phase in the cytology of the basidiomycetes as indeed in all fungal cytology was ushered in. The earliest work published in the new era appears to be by Macdonald (1949) who described the appearance of the living nucleus in the

hyphae of Marasmius androsaceus as seen under phase contrast; this was complemented with a study of the nucleus in fixed and stained preparations of the hyphae.

The study of nuclear phenomena in the fungi can thus be divided roughly into two phases; (a) the early study of the basidium, (b) the later study of the nucleus of the vegetative hyphae as seen in live observations under phase contrast, and by a study of fixed and stained preparations of the hyphae.

(a) The study of the cytology of the basidium.

Wager (1893) working on Agaricus (Stropharia) stercorarius and Agaricus (Amanita) muscarius, found that the single nucleus of the basidium was formed by the fusion of two or more pre-existing nuclei; the structure of the nucleus was similar to that of higher plants; the division of the nucleus was karyokinetic, resembling generally that which takes place in higher plants but with slight differences of detail; the nucleolus did not disappear entirely until the division was nearly complete; a spindle was formed in connection with the chromatic elements, the latter dividing into two groups and passing along the threads of the former to the poles, where they fused together; the daughter

nuclei divided in the same manner as the parent nucleus.

The same author (1894) working on Agaricus (Mycena) galericulatus, reported the presence of centrospheres in the basidium. Marie (1902)[1], Lewis (1902)[1], Sass (1928)[1], and Buhr (1932)[1], all working on the cytology of Psalliota campestris mainly in relation to spore formation, and Colson (1935) working on the same species, all found meiosis to take place in the basidium as it did in higher plants, but Colson stated that the presence of centrosomes as described by Wager was not found.

Olive (1953) in his review of work done between the years 1928 - 1953 concluded that (a) "the nucleus of all the major groups of the fungi is essentially the same in structure and is similar in structure to the nucleus of higher plants. (b) Meiotic nuclear divisions have been the best source of information concerning the structure and behaviour of fungus nuclei. Meiosis is generally directly preceded by karyogamy. Karyogamy, stages in meiotic prophase and the two meiotic divisions proceed very much as in higher organisms. The spindles are intranuclear in origin and are frequently described as being provided with polar centrosomes."

Olive made mention of the fact that Kharbush, studying three species of Exobasidium, described the

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[1]Colson (1935)

chromatin during late prophase as taking the form of numerous granules, interpreted as 'protochromosomes', which eventually come together and form two large chromosomes. Olive felt that the protochromosomes were probably the true chromosomes or heterochromatic bodies on the chromosomes, and believed that the haploid chromosome number was probably greater than two.

Heim (1954) makes mention of the fact that Marie, Kühner in Psathyrella and Sebacina gloeocystidiata, and Chou-Chung Hwang in several members of the genus Coprinus, all describe 'caryosomes' or chromatic corpuscles which finally unite into two metaphase chromosomes. Heim herself, while not subscribing to the 'chromatic corpuscles, two metaphase chromosomes' theory, states that division is as it is in higher plants, but prophase takes place so rapidly that one is unable to follow the events which lead to the 'grouping of the small chromosomes in the centre of the nucleus at metaphase.'

Evans (1959) working on the nuclear behaviour in the cultivated mushroom confirmed the reports of earlier workers on that organism, stating that centrosomes were not observed. He writes that "observations on other basidiomycetes and reports by other investigators suggest

that the absence of centrosomes may be characteristic of the group."

(b) The study of the nucleus of the vegetative hyphae by live observations and from fixed and stained preparations of the hyphae.

Macdonald (1949) described the structure of the living nucleus in Marasmius androsaceus; Girbardt (1955) the structure of the living nucleus in Polystictus versicolor; Robinow (1957) that of the Mucorales, while Bakerspigel (1959) that of Schizophyllum commune. All these descriptions agree in general, stating in the main that the nucleus consists of a central grey spherical body surrounded by a clear halo of variable shape. The descriptions of the division of the nucleus do not agree as well as the description of its structure, and these can be divided into (a) those describing an elongation and constriction of the whole nucleus (Macdonald, 1949 and Robinow, 1957); and (b) those describing the disappearance of the nucleolus before division (Bakerspigel, 1960). Girbardt states that the nucleolus disappears as does the whole of the nucleus, and when it reappears two nuclei are seen in the place of the original one.

Data of a more definite nature have been obtained from fixed and stained preparations, and here again the literature can be divided into (a) that which describes a nuclear division in which the nucleolus persists throughout, (b) nuclear division in which the nucleus elongates and constricts and in which the nucleolus disappears, and (c) nuclear division which is a typical mitosis.

Robinow (1957 a & b) working on Phycomyces, Mucor and Saprolegnia found that the nuclei divided by elongation followed by constriction, the nucleolus dividing at the same time and in the same way, half passing to each daughter. He postulated that the "chromosomes of the resting nucleus are already divided and segregated to opposite sides and constriction is the consummation of a kind of endomitosis initiated during the terminal stages of the previous division." Bakerspigel (1958 & 1959 a & b) agreed in the main with Robinow's account, stating that the nucleus, a crescent ring or cap of chromatin and nucleolus, becomes angular, elongates, the chromatin separates into two portions situated at opposite ends of the elongating nucleolus, the nucleus constricts in the mid-region and sister nuclei move apart.

Turian and Cantino (1960) found division to be similar in Blastocladiella. They suggested that "endomitotic reproduction of the hereditary material in the fungus is normally, but not necessarily, followed by pseudo-amitotic intranuclear division."

Bakerspigel (1959), working on Schizophyllum commune, found that the nucleolus disappeared during division which took place by elongation and constriction of the chromatin. Dowding and Weijer (1961 a & b) found that the nucleolus disappeared, but the chromatin strand of which the nucleus is composed, split longitudinally, each half moving apart to form a daughter nucleus. McGinnis (1953) in rust fungi, and Ward and Ciurysek (1961, 1962) in an unidentified basidiomycete and in Neurospora crassa found that normal somatic mitosis took place.

Savile (1939) working on members of the Uredinales stated that "there are two distinct types of nuclei in the rusts;.....what is termed the unexpanded form is adopted in every part of the life cycle where migration of the nucleus through a narrow pore is necessary. In the transformation of the unexpanded nucleus into the expanded, a new nuclear sphere, the ectosphere, is formed about the original nucleus. The chromatin passes through

the original nuclear membrane and becomes distributed through the ectosphere, leaving the original nuclear sphere or endosphere, completely devoid of it." "In the unexpanded nucleus the spindle forms equatorially in the single endosphere. In the expanded nucleus it forms beside the endosphere as a cord to the ectosphere membrane. The nuclei of the mycelium usually have some of their chromatin outside the endosphere, but their division is essentially similar to that of unexpanded nuclei."

Looking at the literature one wonders whether Wager's words of 1893 are not still applicable today. Three main questions present themselves: (a) How do nuclei divide as seen in live observations? (b) How do nuclei of vegetative hyphae divide as seen from fixed and stained preparations? (c) How closely do meiotic divisions I and II in the basidium resemble meiosis found in higher plants?

With these questions in mind the present work was undertaken; it is realised that an investigation to try and solve these problems should be undertaken on a wide variety of fungi; the difficulties involved in fungal cytology have limited the investigation to two species of the genus Marasmius, Marasmius androsaceus and M. rotula, and it is hoped that the findings presented here will go towards adding to the scant but no less

valuable information at present available on the fungi.

The work is presented in four sections. Section I gives a brief resume of work that has been done on the organisms used in this study, Section II deals with the live observations carried out by the author, Section III with a study of the fructification, dealing with the cytology of the basidium and fructification clamps in the main, and Section IV deals with the study of the vegetative hyphae from fixed and stained preparations.

The photographs, drawings and graphs which illustrate the text have been mounted separately in Volume II.

# SECTION I.

# THE FUNGUS.

## 1. Classification and Affinities.

Androsaceus and rotula are species of the genus Marasmius which belongs to the family Agaricaceae of the order Agaricales of the sub-class Homobasidiomycetidae of the Basidiomycetes.

The fungi were named Agaricus androsaceus and A. rotula by Linnaeus, but were placed in the genus Marasmius by Fries; "Ce genre n'est pas homogêne et ses limites ne sont pas nettement tranchées avec les genres voisins: Collybia par les espèces les plus charnues et Mycena pour celles de petite taille. En fait, les Marasmius sont reliés aux uns et aux autres par des intermediaries que les auteurs classent tantôt dans l'un, tantôt dans l'autre genre." (Konrad et Maublanc, 1948). Fries divided the genus into two great sections: I. Collybarii, those with the margin of the pileus incurved at first; II. Mycenarii, those with the margin straight in the beginning and attached to the stipe. Into a smaller section, III. Apus, the single stemless species was put.

The section Mycenarii was sub-divided into two groups: (A) Chordales and (B) Rotulae, into the latter of which both M. androsaceus and M. rotula were placed.

Cooke (1871) and Stevenson (1886), who used the classification of Fries, added nothing new to either classification or description. Massee (1893), who also followed the classification and to a large extent the description of Fries, described M. androsaceus as follows: "Pileus up to ½ in. across, membraneous, dry, umbilicate, glabrous, striate, whitish; gills directly adnate to the stem without the intervention of a collar, simple, distinct, distant, narrow, whitish: stem 1½ - 2½ in. long, very slender and tough, equal, absolutely glabrous and polished, black; twisted and striate, due to contraction, when dry; spores pip-shaped 7 x 3-4 ." It is recorded as being found on fallen leaves, and he further says "Fries distinguishes two principal forms:- (A) on deciduous leaves; pileus whitish, deeply umbilicate, plicate; mycelium usually traversing the surface of the leaf; (B) on pine and juniper leaves, also on bark; pileus scarcely umbilicate, surface more even; mycelium usually more superficial."

M. rotula is described as having a "pileus about ¼ in. across, membraneous; slightly convex, umbilicate, plicate, entirely whitish or darker on the disc; gills few, broad, distant, joined to a collar that is free from the stipe; stem 1 - 1½ in. long, very slender, equal, horny, shining,

quite glabrous, blackish; spores pip-shaped 6 x 3-4 ."
It is described as growing on fallen twigs. There is often a blackish, creeping rhizomorphoid mycelium from which individuals spring at intervals.

Rea (1922) adopted the above classification, but included the genus Androsaceus which is attributed to Patouillard, who, observing the presence of hairy elements on the upper parts of the cells of the epicutis of the pileus in certain species of the genus Marasmius, suggested a modification of the classification put forward by Fries, and assembled these species (which included both androsaceus and rotula) into a new genus Androsaceus. The facts that M. androsaceus is found from April - December, and M. rotula from May - January and that both are common, are added to Rea's account of the fungi.

Kühner (1933) published a classification of the genus Marasmius which was more natural than that of either Fries or Patouillard. Basing his classification on macroscopic characters, anatomical structure and on chemical reactions, notably the iodine and cresyl blue reactions, he divided the genus into nine (9) sections of which Androsacei - 'petites espèces a pied greffé sur le support corné, et revêtement pileique forme de cellules irrégulières,

hérissées en brosse. Sans valeur; membraneuses,' - was section 6, and into which he placed M. androsaceus and M. splachnoides. Rotulae, section 8, was described as 'petites espèces à pied capillaire, glabre, corné, greffé sur le support, chapeau côtelé-sillonné, avec revêtement formé de cellules dressées en brosse, lamelles unies en collarium, sans cystides; hyphae amyloid. Sans valeur; membraneuses,'; into this section M. rotula; M. Wetsteinii (Saccardo and Sydow); M. Bulliardi (Quél.); M. graminum (Linert); and M. limosus (Bond and Quél.) were placed.

Singer (1950) was the necessity for a slight revision of the 'infrageneric classification' of the genus, and although his classification "still represents the Kühner system in basic ideals", there are certain minor "although not unimportant modifications." (Appendix I.)

2. Distribution and Host Range.

The genus Marasmius is world wide in its distribution but very few records of M. androsaceus and M. rotula appear to exist. M. androsaceus is recorded as occurring in Europe and America (Saccardo 1887); a detailed list of the places in which it occurs in Scotland is given by Macdonald (1949). M. rotula is recorded by Saccardo as occurring in Europe and South Africa. A later mention is

made of the occurrence of this species in Sweden (Commonwealth Mycological Institute 1950-60). Marasmius androsaceus possesses a wide host range and is reported to occur on Quercus, Juniperus, Pinus sylvestris, Fagus, Rubus, Olea europa (Saccardo) and on ferns, oak and beech (Massee)[1], Scripus (Dennis)[1], mosses (Lightfoot)[1] and on Calluna vulgaris (Rostrup, Lange, Dennis;1 Macdonald, 1949). The host range of M. rotula is not as well defined and is stated as being needles under spruce (C.M.I. 1950-60), and twigs and fallen branches.

3. General.

(a) Rhizomorphs. Macdonald (1949) writes, "the formation of rhizomorphs in Marasmius androsaceus is noted by Bulliard (1791), (Agaricus epiphylla) Fries (1821), Greville (1824), Berkeley (1860), Saccardo (1887), Ramsbottom (1923) and Lange (1935-40). Rhizomorphs have been recorded for M. rotula by Sowerby (1779), Fries (1821), Berkeley (1860), Stevenson (1886), Saccardo (1887), and Massee (1893)." Macdonald and Cartter (1961) found that in M. androsaceus rhizomorphs were formed on the dikariotic mycelium only; the presence of rhizomorphs restricted the development of new rhizomorphs only to a limited degree;

1 Macdonald (1949)

rhizomorph development was restricted when the pH value of the medium was as high as 7 or as low as 4, and was best at 20°C. over a range of temperature from 35 - 15°C.; rhizomorphs responded negatively to gravity and positively to light of sufficient intensity, and tips gave a positive reaction to Nobles' test for extracellular oxidase, indicating an ability to attack lignin.

(b) Lignin attacking capacity and ability to assimilate synthetic nutrient solutions. Besides the above mentioned report of the ability of the tips of the rhizomorphs and the mycelium to attack lignin, Lindeberg (1946) states that M. androsaceus decomposes proportionally two to three times more lignin than cellulose. He found (1948) that M. androsaceus and M. rotula grown on gallic acid and on tannic acid medium gave positive oxidase reactions on both media, and that the species both attacked lignin and cellulose experimentally. From his results he concluded that polyphenol oxidases occurred regularly and abundantly in these and other basidiomycetes worked on. He states, however, that the enzymes which catalyse the oxidation of gallic and tannic acids have been called polyphenol oxidases, "these, however, comprise two different enzymes:o-diphenol oxidase which catalyses the

oxidation of cathecol and other o-diphenols, and p-diphenol oxidase which catalyses the oxidation of diphenols of the hydroquinone type." He thus studied the behaviour of the fungi on cathecol- and hydroquinone-agar; both M. androsaceus and M. rotula gave positive reactions on the cathecol-agar, but M. rotula only of the two species gave a positive reaction on hydroquinone-agar; he thus concluded that M. androsaceus and M. rotula produced o-diphenol oxidase, in addition to which small amounts of p-diphenol oxidase were produced by M. rotula. In addition to this Lindeberg (1946) states that M. androsaceus is able to assimilate nutrient solution exclusively on the condition that both biotin and thiamin are added to the solution. Similar work does not appear to have been done with M. rotula.

(c) Reaction to Iodine and Cresyl Blue. Kühner (1933) states that in M. androsaceus the hyphae of the stipe are not amyloid, nor are the hyphae of the trama of the gills and the hyphae of the pileus flesh, whereas in M. rotula the internal hyphae of the stipe are definitely amyloid as are those of the pileus flesh in the disc region, and those of the trama of the gills. The internal hyphae of the stipe in M. androsaceus are not positive to cresyl

blue but the hyphae in the sub-cortical region stain pink - red, which colour is resistant to ammonia. In M. rotula, however, a positive reaction to cresyl blue was obtained in the hyphae of the cap and trama of the gills.

(d) Pigments. Kühner (1933) states that the pigment which imparts the fawn or brown colour to the cap in M. androsaceus, and which also occurs in the hyphae of the trama of the gills, is a membraneous pigment, fixed on to the surface of the thin walls of the hyphae, especially at the level of transverse septa. This superficial layer of pigment is found in patches and rings; a similar pigment of a finer texture is found on the superficial cells at the top of the stipe. The pigments found in M. rotula are uniformly distributed in the cell walls.

The samples of M. androsaceus used in this study conform to the description (B) given by Fries (Massee, 1893) for fructifications on pine and juniper. These fructifications were found growing on fallen needles of Pinus nigra. A sample found on Calluna vulgaris was in no way found to be different from those found on pine (Plate I, figs. 1, 2, 3 & 4). The samples of M. rotula

used were found on the bark of an elm, and agree with the description given for the species by Fries (Massee, 1893).

# SECTION II.

## LIVE OBSERVATIONS ON THE MYCELIUM

### 1. Materials and Methods.

#### (a) Organisms.

Cultures of *Marasmius androsaceus* were obtained from departmental stock, which had been isolated from the spores of fructifications found growing on the fallen needles of *Pinus nigra* on a plantation of mixed pine on the Forestry Commission land at Tentsmuir, Fife. Cultures of *M. rotula* were obtained from departmental stock received from two sources, (a) from the late E.W. Swanton, Keeper of Haslemere Museum in Surrey, and (b) from the Centraalbureau voor Schimmelcultures at Baarn, Holland, the culture No. being K 2661.

#### (b) Methods of Cultivation.

Stock cultures were maintained on malt-agar (2% Difco bacto agar, 2.5% malt extract, pH 5.8) and kept in an incubator at a temperature of 22°C. For purposes of studying the growth of the mycelium in culture, and checking on the purity of stocks, sub-cultures were made on to sterilised Petri-dishes of the above mentioned medium. For live observations the fungus was grown as follows:

No. 2 cover-slips (2 x 7/8") were placed on glass triangles lying on saturated filter papers on the bottom of deep Petri-dishes. The upper surfaces of the coverslips were coated with a thin layer of the medium, the thinness of the layer (necessary for clarity in viewing microscopically) being controlled by dropping on the the coverslips 18 drops of the medium from a pipette. It was found that this was sufficient to cover the coverslip evenly.

The petri-dishes were autoclaved at 14 lbs. pressure for 20 minutes. After they had been removed from the autoclave and had been allowed to cool, the petri dishes were transferred to a sterilised fume-cupboard to be inoculated; the covers of the dishes were tipped just wide enough to allow the inoculum to be placed on the coverslip. All precautions to maintain sterile conditions such as flaming the inoculating needle, which was kept standing in 70% alcohol when not in use, were observed.

To maintain as standard a size of inoculum as possible on all the coverslips, the following procedure was adopted: Two parallel lines 3 mm. apart were cut with the needle across the mycelium in the petri-dish, to a depth of about 3 mm. down into the medium; smaller cuts, 3 mm. apart, perpendicular to and between the main lines, were cut so the effect of a ladder was obtained on the

surface of the mycelium, the blocks between each rung being 3 mm. square. The blocks were lifted separately from the mycelium and used as inoculum, the depth being about 1.5 mm. Each bit of inoculum was thus approximately 3 x 3 x 1.5 mm. in size.

The pètri-dishes were transferred to an oven and incubated at 22°C. for three days before being examined. For examination the cover-slips were inverted, inoculated side downwards, over a rectangular cavity cut in a glass slide, at the other side of which was sealed a No. 1 cover slip, this being Macdonald's improvement (1949) of his modification (1947) of Nobles' (1937) improvement of the Sass technique. (Macdonald, 1949). The cell so formed was placed on the microscope stage and growth of individual hyphae, mitochondrial activity, and nuclear phenomena were observed under Phase contrast.

(c) Microscopy and Photomicrography.

Observations were made with a Cooke, Troughton and Simms CM 1741 Phase Contrast Microscope, using an Achromatic oil immersion objective (1.8 mm., N.A. 1.30; 95 x working distance 0.12 mm.) and compensating eyepieces x 6 and x 12.5; a Kodak Wratten No.74B filter 540 mμ was used. All drawings were made using a Watson Camera lucida,

with ocular x 12.5. Photographs were taken with a Watson eye-piece camera for 35 mm. roll film using compensating eyepiece (x 10) and an achromatic phase contrast objective. Ilford Micro Neg. Pan was used and was developed with Ilford PFP developer. Where time-lapse-photography was necessary, a Bolex Paillard 16 mm. cine-camera was used. Prints were made on Kodak photographic paper, Bromide single weight No. 3. The scale is given with the drawings and photographs.

## 2. The Mycelium in Culture.

The growth of the mycelium of Marasmius androsaceus in culture has been described in some detail by Macdonald (1949) and is included here only to add to what has already been said. The dikaryophase mycelium is slow growing. Incubated at 22°C., the optimum temperature for growth, it grows at a rate of about 5 mm. per day (Plate II) The mycelium spreads radially in all directions from the inoculum, keeping close to the surface which it covers with a dry, uniform, white growth. Five days after inoculation, the mycelium is seen to become banded with less dense white zones from the inoculum to the periphery. These zones, due to the production of branch hyphae which give a felt-like appearance to the fungus, are more

powdery the whiter and denser they are. After about 8 - 9 days, brown zones, independent of the white zones mentioned above, appear. These are banded on the side away from the inoculum by black lines which in time become raised above the surface level of the mycelium. Macdonald (1949) has found that in structure they agree with those described for Polyporus squamosus by Campbell and Munson (1936).

The above observations agree with the findings of Macdonald, except that he observed the brown lines in cultures upwards of two months old. Despite the presence of the brown zone, the hyphae behind the black lines often resume growth and produce a dense white line; the entire zone may later be covered with a fine white growth. Isolated brown patches also make their appearance on the mycelial surface; these too become bounded by black lines which also become raised above the surface of the mycelium, often to a greater height than the normal concentric lines. In plates two months and older, small white dome-shaped structures about 1 mm. high and 1 mm. in basal diameter are seen dotted all over the surface of the mycelium, which by this time has covered the entire plate. A microscopic examination of a squash of one of these shows them to be

composed of small tightly interwoven hyphae. Reference is made to these structures later in this section.

The dikaryophase mycelium of Marasmius rotula, although slow growing when compared with fungi such as Neurospora crassa (Zalokar, 1959), grows at a faster rate than that of M. androsaceus when incubated at the same temperature (Plate II). The mycelium grows radially from the inoculum and due to the production of branch hyphae, it exhibits the zoning seen in M. androsaceus of progressively less dense white bands from the inoculum to the periphery. Very little new growth appears on the inoculum itself in M. rotula, where as in M. androsaceus growth of the hyphae is quite profuse, giving it a white, fluffy appearance. The mycelium is not at first as closely appressed to the surface of the medium as is the case in M. androsaceus, due to the aerial hyphae produced, which are thickest nearest to the inoculum. Brown areas appear on the surface of the mycelium beyond the white tuft of aerial hyphae, and with their appearance comes a flattening of the mycelial growth. These areas are not bounded by black lines as is the case in M. androsaceus. The whole brown area in time becomes raised above the mycelial surface, and sooner or later becomes wrinkled, presenting

a skin-like appearance. ( Plate III, figs. 1 & 2 ). At this stage it is easy to peel the growth away from the medium where-ever the brown zone exists. An examination of a bit of mycelium from one of the brown zones shows it to consist of flattened scale plate-like pigmented cells, which interlock with each other forming a continuous plate.

When the mycelium reaches to the edge of the medium, it grows up against the side of the petri-dish or flask, if the culture is grown in a flask, in branching rhizomorphoid strands of associated hyphae, which turn dark brown then black with time. These rhizomorphoid strands which grow from the edge of the mycelium, and which appear to be a specialised form of the forward growing hyphae, are not very similar to the rhizomorphs of M. androsaceus, which are not shoe-lace like, but hair-like, nor are they formed specifically at the edge of the medium and become appressed to the glass, but appear anywhere on the surface of the mycelium, particularly on the inoculum, in any damaged area such as around the cavity left when a bit of the mycelium has been removed to be used as inoculum, and particularly along the black lines which border the brown zones. Structures such as the rhizomorphs that have just been described for M. androsaceus, have been obtained by the present author, in cultures of M. rotula. Monospore

cultures were isolated from fructifications of M. rotula for purposes of studying the mating behaviour dealt with on page 91 and for a comparison of the monokaryotic and dikaryotic mycelia. On some of the cultures obtained from the matings, structures resembling the rhizomorphs of M. androsaceus were seen (Plate IV, fig. 1). They responded negatively to gravity, and positively to light of sufficient intensity; when a change in the humidity of their environment was brought about by lifting the lid of the petri-dish, they exhibited twisting movements. An examination showed each to consist of long unbranched hyphae, twisted as the strands of a thread. (Plate IV, fig. 2). The tips of the hyphae terminated in claviform cells not unlike those to be seen on the epicutis of the cap of the fructification (Plate IV, fig. 3). The base was less loosely twisted than the rest of its length, and was covered in places by some of the flattened plate-like cells described in relation to the brown zone (Plate IV, fig. 4). These structures are dealt with in the discussion at the end of this section.

The monokaryophase mycelia of both M. androsaceus and M. rotula differ from the dikaryophase in having aerial, much branched hyphae, which gives the mycelial growth not

the flat look seen in that of the dikaryophase, but a rather woolly appearance.

3. Agar Film Mycelia.

The growth of the fungus on agar film is, as on plates, radial from the point of inoculation. Growth of individual hyphae can be watched under the oil-immersion lens; growth in the hyphae of both Marasmius androsaceus and M. rotula takes place only at the extreme tip, that is the hemispherical area at the end of the hyphae, occupying a length of about 5μ . Zalokar (1959) writes that this was noted as early as 1892, by Reinhardt. The growing tip exhibits a twisting motion, which gives the hyphae the appearance of a gently twisted ribbon. The twist appears to be always to the left in both M. androsaceus and M. rotula. The tip advances at a rate of about 90μ per hour in M. androsaceus and 105μ in M. rotula, proceeding steadily without stopping, even during the production of a clamp. This agrees with the finding of Girbardt (1955) in Polystictus versicolor who states that "Die von Macdonald (1949) bei Marasmius androsaceus beschriebene Sistierung des Spitzenwachstums während der Schnallenbildung konnte für Polystictus versicolor nicht bestätigt werden." On an examination of the literature

the present author has come to the conclusion that the above statement has arisen out of a misunderstanding on the part of Girbardt, of what Macdonald had written. To quote, he says, "at the point of origin of the clamp connection there is no longer any movement or growth of the hypha." This the author interprets as meaning that movement and growth have ceased at the point of the hypha where the clamp excresence makes its appearance; this thus ties in with the writer's findings that growth takes place only at the tip, and is uninterupted during the production of clamps.

The author has, however, observed that in M. androsaceus, hyphae within an area, cease growing, become bulbous at the tip as though some pressure were building up, then after 10 minutes or less growth would be resumed, not from the tip, but by a sub-terminal branch some 5 μ from the tip. (Plate V, fig. 1). During the arrestation of growth, the hyphae have been traced backwards in search of forming clamps, but on no occasion was this found to be taking place. Robertson (1958) in his observations on the effect of water on the hyphal apices of Fusarium oxysporum found that "if flooded with solutions of decreasing molarity from 0.76 M. an increasing number of the apices at the agar surface stop growing and branch

sub-terminally; in distilled water about 50% branch, this branching being preceeded by swelling, whereas in 0.5 M. sucrose more than 90% of the apices branch, and branching is not accompanied by swelling. In distilled water those hyphae which do not branch swell a little then grow on from the apex within 40 seconds." He concluded from further experiments that branching and swelling are not causally related, but branching always occurs following the arrestment of the hyphal apex for more than 60 seconds. The author has made attempts to repeat Robertson's experiments using his medium (mineral sucrose); these were unsuccessful as the fungus did not grow on the medium. Flooding the hyphal apices on malt agar results only in an arrestation of growth, swelling at, and bursting of the tips.

4. Mitochondria.

The hyphae in both Marasmius androsaceus and M. rotula, range in diameter from 5.5 μ in the large main hyphae to 2.5 μ in the newly formed secondary hyphae. The hyphae are regularly septate, a point to be discussed later in this section, the resulting cells ranging from 100 - 130 μ in length. Within each cell there is a gradation in the density of the cytoplasm from the distal end, where it is dark grey, to the proximal end, where it appears optically

empty. This property, the empty appearance of the proximal ends of the cells, allows careful observation of the mitochondria and their movement within the cells. The cytoplasm on the whole is sufficiently hyaline in appearance except in the extreme tip of, or, in some cases, the whole length of, the terminal cell, to permit accurate observations of mitochondria in good phase-contrast.

The mitochondria are seen as long filiform structures some of which are over 30 μ in length. They are seen occupying all the living cells throughout their length. In the terminal cell they extend to the extreme tip where they appear to end abruptly. The tip body seen by Girbardt (1955) in Polystictus versicolor, is not a regular feature of either Marasmius androsaceus or M. rotula, and has been seen in three tips only (in all cases in M. androsaceus) out of hundreds of tips examined. On these occasions the mitochondria appeared to coalesce and form a solid body at the tip, from which trailed a tail of the filiform structure. In the terminal cell, the distally placed mitochondria are relatively at rest, whereas those proximally placed are seen in lively sinuous motion round a position of rest. This is the condition in all the cells, although in the sub-terminal cells, the distally

positioned mitochondria exhibit more movement than those of the terminal cell. Singly, the mitochondria may travel for short distances, and have been seen within vacuoles. They often anastomose, then break up into single filaments after a while; in the filiform phase they take on various shapes (Plate V, fig.2). After the formation of the cross walls of the main hypha, and before the fusion of the clamp excresence with the main hypha, the vacuoles in the vicinity of the cross-wall retreat, leaving a length of unvacuolated cytoplasm about 30 $\mu$ long, into which the mitochondria in the neighbourhood proceed. Here they all coalesce, and go through a series of changes in shape, eventually becoming a spherical body, which may remain as such or become dumb-bell shaped, until fusion of the clamp with the hypha has taken place. Shortly before fusion of clamp and hypha, or after fusion in some cases, the mitochondria assume the threadlike form. (Plate V, figs. 3 & 4; Plate VI, figs. 1, 2, 3 and 4; Plate VII.). On becoming spherical the body moves towards the septum where it appears as though it would go through, but after a short interval moves to a position immediately beneath the point at which the clamp touches the main hypha. Girbardt (1955) has observed a similar phenomenon in Polystictus versicolor, the difference being that in

that fungus the time taken for the changes from the spherical body which he calls the 'wall body' back to the threadlike form is 36 minutes, whereas in Marasmius androsaceus the time taken was only 18 minutes, and M. rotula 12 - 15 minutes. Apart from this phenomenon, the mitochondria have been seen to curl round end to end, and become circular; on occasion those in a vacuole have become spherical. Where ever the mitochondria have taken on a string-of-pearls appearance, death of the cell in which they occurred has followed.

Heim (1947) found that there was a relationship between cell physiology and chondriosome morphology and wrote: "Ce rapport entre la physiologie cellulaire et l'aspect morphologique du chondriome est intimement lié à l'apparition du pigment. A l'état de repos fonctionnel, les éléments du chondriome restent sous forme de mitochondries. Ces dernières représent la stade initial, ce n'est qu'au moment de leur activité, pendant l'élaboration du pigment, que leur forme se modifie, devenant de granuleuse, filamenteuse. A ce state, le chondriosome devient un chromoplaste. Il représente l'étape finale."

Within the cells are also seen black highly refractive bodies, the order of size of these being about 0.3 $\mu$ , which exhibit Brownian movement or are seen at rest against the

against the inside of the walls of the hyphae. They have been seen to move rapidly both with and against the direction of flow of the cytoplasm. Similar bodies have been recorded by Macdonald (1949), Girbardt (1955) and Buller (1933) who states that they were recorded by Woronin in the cells of Ascobolus pulcherrimus in 1896, and by Ternetz in 1900. Buller has named these structures 'Woronin bodies', and states that "it is not to be supposed that they have any power of locomotion of their own, but are moved passively"; This is difficult to conceive, as they move at a speed greater than that of the flow of cytoplasm, judging the latter by the rate of movement of small vacuoles carried along by the cytoplasm. It is worthy of note that they move equally as fast against the cytoplasmic flow. In the vicinity of the forming clamp, they are seen in lively motion, and one or two have been seen to pass up into the clamp excrescence. Ever so often a group of these bodies come together and form a raft as it were and settle down against the wall of the cell. Mudd, Winterscheid, De Lameter and Henderson (1951) showed that the granules of Mycobacteria were loci of oxidative - reductive activities; that they contained phospholipid and gave in high dilutions of Janus green B,

the successive colour change characteristic for the staining of mitochondria. The author performed some of their experiments on the hyphae of Marasmius androsaceus and M. rotula, the methods and results of which are briefly summarised below.

Two reagents were chosen: Tetrazolium and the Nadi reagent, both of which demonstrate oxidative - reductive activities, the latter being specific for the presence of cytochrome oxidase.

A 1% solution of tetrazolium chloride was incorporated into liquid malt medium (2.5% malt extract in distilled water) to final concentrations of 0.005% and 0.025% of tetrazolium. Cultures of M. androsaceus and M. rotula which had previously been growing in liquid culture (medium as above less the tetrazolium) were transferred to the tetrazolium containing medium and incubated at 22°C. and examined at half-hourly intervals.

Equal parts of 1% aqueous solution of dimethyl-p-phenylene-diamine and 1% solution of -naphthol in 95% alcohol were added to the liquid malt medium to final concentrations of 0.005%, 0.025% and 0.05%. To these, cultures of M. androsaceus and M. rotula which had been grown in liquid culture were added, and incubated at 22°C.,

and examined at half-hourly intervals.

After 8 (eight) hours in the tetrazolium containing medium, the mycelium of both M. androsaceus and M. rotula appeared brick red, the colour being most intense around the periphery of the culture. Microscopic examination showed that a brick red halo had enveloped the granules within the cells of the hyphae. So large was the number of granules in the terminal cell, that the colour had spread throughout the cytoplasm of the cell, hence the density of colour at the periphery of the mycelium.

After half an hour in the medium with which the Nadi reagent had been incorporated, the hyphae of both species showed on examination, that the granules were surrounded by a bluish-purple halo of indophenol blue (Plate VIII, fig. 1). The above experiments have shown that the granules are sites of oxidative & reductive activities, and are thus likely to be mitochondria. If they are, it would seem that there are two types of mitochondria within the cells, the filiform type described earlier in the section, and the granular type that has just been dealt with.

5. The Nucleus.

Girbardt (1955) writes that "it now seems to be definitely established that the resting nucleus of different fungi is essentially like that of higher plants." The first description of the living nucleus seems to have been made by Macdonald (1949 a) in Marasmius androsaceus, who describes paired grey structures, round or oval in outline, corresponding in size and outline with the heterokaryotic pair seen in stained preparations. He goes on to describe dark moving granules, seen on either side of the pair of 'grey structures', and a region of concentration of these granules between the two structures. The grey structures he found were Feulgen negative, and counterstained with Fast Green. Girbardt (1955) describes in Polystictus versicolor and other basidiomycetes, 'grey structures' which he states appear almost black in phase-contrast, surrounded by a light area of extremely variable form. This light area he found when fixed and stained corresponded with the outer nucleus, the grey structures being the nucleolus. Robinow (1957 a & b) describes the nuclei of the vegetative hyphae of the Mucorales as having a well marked nucleolus surrounded by a variously shaped shell, the latter of which in stained preparations

consists of closely packed chromatin granules. Bakerspigel (1959) describes those of Schizophyllum commune as being composed of an optically dense spherical or oval central body, surrounded by an optically clear area which changes its shape continuously. A similar description is given for the nucleus of Saprolegnia parasitica and S. ferax. (Bakerspigel 1960).

In Marasmius androsaceus and M. rotula, the author has observed in the dikaryophase mycelium, two dark grey bodies which appear black in good phase-contrast, surrounded by an optically clear area quite distinct from the rest of the grey granular cytoplasm, though no limiting layer between these two areas has been perceived (Plate VIII, fig. 2). The same structure is seen in the monokaryophase mycelium, the difference being that there is one nucleus instead of two found in the dikaryophase mycelium (Plate VIII, fig. 3). Both the grey structures and the clear outer area continually change shape. In the sub-terminal cell and all cells behind it, the nucleus when it has ceased to move actively ( a condition made more explicit later in the section) is seen to have a rounded central grey structure eccentrically placed in a rounded clear outer shell. These nuclei are more or less

stationary in clear cytoplasm. In the terminal cell the nuclei differ in appearance from those of the sub-terminal cells. The central grey structure is more often than not tear-shaped, and has been seen to have a 'satellite' attached to it by a narrow isthmus. The clear outer shell which is often spindle-shaped also assumes various shapes and is often seen to elongate to considerable lengths. (Plate VIII, fig. 4 & Plate IX, figs. 1, 2 and 3). The position of the nuclei relative to each other and to the tip of the hypha, depends on their activity relative to clamp connection formation.

6. Nuclear movement.

If one measures 110 - 130 μ backwards from the tip of the hypha in either Marasmius androsaceus or M. rotula 30 - 40 minutes after the tip of the backward bending clamp excrescence has touched the main hypha, the forward nucleus of the conjugate pair is seen; the rear nucleus is found at varying distances from it, tracing backwards along the hypha. As the tip grows forwards, the fore nucleus moves at the same rate, keeping the distance between itself and the tip approximately constant. The movement of the rear nucleus is less constant and it may

even cease to move for short periods (Plate X). On occasion both nuclei have been observed to cease moving for a short period; this, however, had no effect on the progress of growth at the tip (Plate XI). When clamp connection formation is about to take place, the nuclei may be ahead of the position at which the clamp excrescence makes its appearance; the nuclei cease their forward progress and retreat until they are in the vicinity of the forming clamp. (Plates X & XI). Where the fore nucleus has been seen immediately after division, it has been observed to move more quickly than it normally does, until it attains its normal position relative to the tip of the hypha, then it travels at the rate at which the tip grows.

7. Nuclear division.

When division is about to take place the central grey structures enlarge many times their normal diameters and appear almost to fill the hyphal diameter. The nuclei take on various shapes, and a most constant feature is the great elongation which takes place followed by a contraction. The nuclei move towards the clamp where the fore nucleus positions itself in the neck of the clamp or

at the base of it, the hind nucleus remaining in the main hypha, usually at the base of the clamp. The nuclei again begin to expand, and the central grey structure becomes quite indistinct and finally disappears from view. At this stage it is difficult to observe precisely what happens, or exactly where the nucleus is positioned, as the grey structures were aids in locating the position of the nucleus. Observation is made more difficult by the fact that there is an increase in the concentration of mitochondria in the area, and that the cytoplasm becomes more optically dense. Where the clear area has been observed after the disappearance of the grey structures, it has been seen to be first elongate, then to contract; beyond this nothing definite has been seen. A short while after, daughter nuclei are seen. They are very small to begin with, but as time progresses they enlarge in size, each one consists of a grey central body surrounded by a small clear halo. One remains in the clamp, two are seen moving along towards the tip of the hypha and become the conjugate pair of the new terminal cell, and the fourth is seen in the sub-terminal cell.

8. Clamp Connection Formation.

The clamp excrescence makes its appearance as described before some distance behind the position of the nuclei, or at times at a point between the fore- and hind-nuclei. It often arises about 130-135 $\mu$ from the tip in Marasmius androsaceus and 130-140 $\mu$ in M. rotula. A small conical projection is seen to arise from the hypha; three minutes after its appearance it has attained the height of about 4$\mu$ and begins to bend backwards, that is, away from the direction in which the tip is growing. It touches the hypha 5 - 6 minutes after its first appearance, and begins to enlarge. By the time the clamp has touched the main hypha, the nuclei have retreated and are in the vicinity of the clamp. They undergo the changes described above, and about 15 minutes after the first appearance of the clamp excrescence, the fore nucleus moves into the neck of, or remains at the base of the clamp, and division takes place. The hind nucleus also divides, though it may do so immediately before or after the fore nucleus, as division is not always simultaneous, judging by the fact that the grey bodies do not disappear simultaneously. One of the daughter nuclei from the fore nucleus positions itself in the clamp, the other moves rapidly along the main hypha towards the tip. Of the daughter nuclei of the hind nucleus, one moves backwards and one forwards from the point of division.

9. Wall Formation.

The wall appears to be formed across the sites of nuclear division. This means that the cross wall dividing the clamp from the main hypha may either be in the neck of the clamp or at the junction of clamp and main hypha, depending on whether the division took place in the neck of the clamp or at the base. The first indication of the formation of a wall is the presence of what appears in optical focus to be two granules, one on either wall of the hypha. Careful focusing shows these to be parts of a complete ring around the hypha. This slowly closes inwards, like an iris diaphragm, making the pore in the centre smaller and smaller (Plate XII). Some of the vacuoles in the subterminal cell move with the cytoplasm, which exhibits a churning activity, into the terminal cell; as the pore in the septum becomes smaller, the vacuoles constrict as they pass through. The pore gets so small, about 0.5 μ that the vacuoles get through by forming a long finger-like projection to the pore, from which small vacuoles are budded off on the other side of the septum, where they eventually coalesce (Plate XII).

After about 40-45 minutes from the first appearance of the clamp excrescence, the phenomena referred to under the subsection 'Mitochondria', and depicted in Plate VII,

are seen to take place. The septum which was convex towards the tip of the hypha now becomes straight, and a thickening appears in the centre which sometimes resembles a plug. (Plate XIII, fig. 1). This suggests that a fine pore was present through which the cytoplasm passed to the terminal cell, the pressure causing the curvature of the septum; the fusion of the clamp and hypha having taken place, the cytoplasm is diverted into the clamp from which the secondary hypha grows, thus releasing the pressure on the septum, hence the straightening.

Moore and McAlear (1961) with the aid of electron microscopy have shown that in Merulius tremellosus "the septum instead of being interrupted by a simple hole, becomes flanged to produce an elongate channel." They have called the formation the dolipore septum. They further state that the dolipore septum "is enclosed on both sides by, in section, a variously crescent-shaped structure." From this characteristic shape they have called the structure the parenthesome. These dolipore-parenthesome septa were found to be characteristic of basidiocarpic, dikaryotic hyphae. This new information explains the structure of the thickened area of the septum.

The septum in the main hypha in Marasmius rotula is more often than not formed behind the point at which the tip of the clamp excrescence touches the main hypha. This means that if the clamp fuses with the main hypha it does so not with the sub-terminal cell, but with the terminal cell; which would then receive three nuclei. To obviate such a situation, a secondary projection grows out from the sub-terminal cell, and fuses with the clamp. (Plate XIII, fig. 2).

Clamps arise, spaced fairly regularly on the hypha, the distance between two of these varying between 100 - 130 μ. Cases have been seen where the distance between two clamps was only 45 - 50 μ; these are exceptional, and it has been observed that these occur when renewed growth takes place in the formation of a clamp on a cell other than the terminal cell (i.e. which has ceased growth) even after the branch has begun to grow out from the first-formed clamp of the cell. In cases such as these the nuclei revert to the condition found in terminal cell nuclei, from the rounded appearance of both central grey body and clear halo of the sub-terminal cell nuclei.

Another exceptional phenomenon encountered, which is even more rare than the above mentioned, is the simultaneous appearance of two clamps within 50 μ of each other on the

same hypha (Plate XIII, fig. 3). On the odd occasion on which this has been seen, formation of the fore clamp went on to completion, the other aborted.

10. Discussion.

The mycelium of Marasmius androsaceus produces in culture small dome-shaped bodies which, as has been described, proved on examination to consist of tightly interwoven hyphae. These structures closely resemble fruit-body initials; the question which then presents itself is why have they never come to maturity? Buller (1922) points out that on artificial mushroom beds a great many rudimentary fruit-bodies are produced for every one that comes to maturity; this no doubt stems from competition for available nutrient in the medium. In the cultures kept in the laboratory the mycelium had established itself quite successfully before producing the dome-shaped structures, and had no doubt greatly depleted the nutrient supply of the medium: unless production of new branches ceased, and all energy was directed to the fruit-body initials, it would be virtually impossible for very many to reach maturity. Coupled with the above mentioned fact is the lack of moisture which obtained in the culture. Buller (1922) points out that in dry seasons

no mushrooms appear in nature; he suggests that the fruit-body initials are present, but lack of moisture prevents elongation of the stipe and expansion of the pileus. As conditions in which the cultures were kept were dry, this could have added to other factors which prevented the development of the fruit-body initials.

In M. rotula long hair-like structures, resembling the rhizomorphs of M. androsaceus, were produced at the tips of which the hyphae ended in claviform cells not unlike the cells of the epicutis of the cap of the fructification. The presence of the claviform cells suggested that these structures might be fruit-bodies in which the pileus for some reason or other had failed to expand. Buller (1909) states that in Lentinus lepideus 'a fruit body begins its existence in light or darkness as a tiny papilla. If developed in the dark the papilla grows out as a finger-like stipe which is completely indifferent to geotropic stimuli.' He goes on to say that it attains great length without showing signs of a pileus. If, however, it is exposed to light of sufficient intensity the pileus expands. Cultures bearing these structures were exposed to brilliant sun-light, but no expansion of the pileus took place. Kühner (1933) describes the early

stages in formation of fruit-bodies in M. rotula, which do not resemble the early stages seen for the structures in question, and so the structures do not appear to be sterile fruit-bodies. Furthermore, the cultures of M. rotula on which they were found were kept under the same conditions as those under which the cultures of M. androsaceus were grown, which did produce fruit-body initials. If the initials on M. androsaceus were unable to progress beyond the dome-shaped stage before withering, it is hardly likely that those on M. rotula would progress very much further.

The alternative suggestion is that these structures are rhizomorphs. Not only did they resemble the rhizomorphs of M. androsaceus, but they exhibited similar qualities; i.e. they responded negatively to gravity but positively to light of sufficient intensity; some became branched, and as on the rhizomorphs of M. androsaceus, tufts of hyphae were seen growing from the older ones. They were produced on the inoculum or on any area of the mycelium which had become damaged. Alexopoulos (1962) states that 'rhizomorphs are resistant to adverse conditions and remain dormant until favourable conditions return'; the rhizomorphs on M. androsaceus are formed where conditions are adverse,

as innumerated above, as are the structures on M. rotula. They are then in all probability rhizomorphs. Rhizomorphs have been mentioned in the literature occurring in growths of M. rotula, but these are of the Armillaria mellea type (boot-lace); these have been seen by the present author, as already stated, on the sides of the container in which the culture was grown, but to his knowledge this is the first mention made of the androsaceus-type of rhizomorph in connection with M. rotula.

The structure of the nucleus of the vegetative mycelium as seen in the living condition under phase-contrast, agrees with that of other fungi as seen by other authors, and with the description given by Macdonald (1949 a) for M. androsaceus. The granules referred to by Macdonald between the two nuclei are the granules of the cytoplasm that lies between the two nuclei.

Granules called 'granula' by Girbardt (1955) and 'Woronin bodies' by Buller (1933) have been mentioned in connection with many fungi. As already stated, they have reacted positively to tests for sites of oxidative-reductive activity, in Marasmius androsaceus and M. rotula. Novikoff (1961) points out that a positive reaction to indophenol oxidase and tetrazolium reagents is misleading,

as the indophenol blue and formazan diffuse, probably from mitochondria where they are formed, and dissolve in the 'refractile granules'. Besides being dissolved by acetic acid, and being extremely plastic, these structures have been seen to give positive reactions to the above mentioned reagents even in cells where filiform mitochondria have not been seen. It is thus difficult for precise distinction to be made between them and the 'true mitochondria', and until electron microscope studies of their internal structure have been made, no definite pronouncement can be made on them.

# SECTION III.

## SECTION III (A)

## THE FRUCTIFICATION

### Materials and Methods.

A. Collection of Materials.

The fructifications of Marasmius androsaceus were collected from mid-September to late October. They were found growing on the needles of Pinus nigra, which had fallen among the Pleurozium schreberi covering the ground between the service road and a plantation of mixed pine on Forestry Commission land at Tentsmuir, Fife, in the region of NO 499268 (National Grid Reference).

Some fructifications were fixed immediately, and others were kept in damp moss and taken back to the laboratory if they were to be treated in the living condition before fixation.

Fructifications of M. rotula were found in Mid-October, growing among Bryum sp., on the bark of an elm on the shores of the Lake of Menteith in Stirlingshire, in the general area of NN 5600 (N.G.R.). The bark bearing the moss and fructifications was removed and taken to the laboratory, where it was kept in a jar of water placed under a bell jar, under normal laboratory conditions. The bark was so placed in the jar that only

a small portion was submerged under water; it thus acted as a wick to keep the moss damp. The culture produced fructifications for a period of over six months.

B. Preparation of Material for Examination.

1. Fixatives.

It was decided that as wide a selection of stains as was possible should be used, (a) to effect a comparison between division figures stained by chromatin specific stains, e.g. Feulgen, and those stained by non-specific stains, and (b) to stain as many structures in the division figure as was possible. To this end a variety of fixatives was tried, as 'certain fixatives favour the action of basic dyes, others the action of acid dyes; others again allow the easy colouration by both.' (Baker, 1958). These fixatives can be grouped under three headings and are listed below. Their advantages and disadvantages as fixatives in general are dealt with by Baker, and are discussed in the text in conjunction with the author's experience of them.

(a) Osmic fixatives.

Osmium tetroxide vapours.

Fructifications were exposed to the vapours of a 2%

solution of osmic acid for from 5 minutes to half an hour. They were mordanted for 5 - 15 hours in a 2% solution of iron alum prior to staining with haematoxylin, and for 15 minutes to one hour in a 1% solution of chromic acid prior to staining with crystal violet.

Flemming 1882. (Darlington and La Cour, 1960).

On account of the small size of the fructifications and the thinness of the pileus flesh, they were fixed in their entirety, for periods ranging from 15 minutes to one hour. Material was washed with distilled water before being stained.

Baker discusses the effects on cells of the constituent solutions of fixative mixtures - primary fixatives - more fully than he does the effects of the mixtures themselves; he states, however, that 'each component of a fixative mixture so far as was possible compensates for a defect in another.' It was found, however, with the material used, that the fixative mixture often gave the defect of one or other of its constituent parts without apparent compensation by the others.

According to Baker, osmium tetroxide vapour leaves the protoplasm homogeneous, readily stainable by basic dyes but scarcely by acid dyes: chromic acid, on the

other hand causes a coarse coagulation of the cytoplasm leaving wide meshes, but renders it acidophil. Both reagents preserve the shape of the nucleus faithfully, though with chromic acid the nuclear sap is coarsely coagulated. Chromosomes are well shown after fixation with chromic acid whereas with osmic acid they are not.

With osmic acid vapour it was found by the author that the nuclei appeared to be well preserved, but on account of the fact that the basiphil cytoplasm and nuclear sap became stained, particularly after haematoxylin, great difficulty was experienced in interpreting nuclear figures. With Flemming's solution the cytoplasm became coarsely granular, and difficulty was experienced in distinguishing between nuclear material and cytoplasmic granules in the neighbourhood of the nucleus.

(b) Formalin fixatives.

Karpechenko 1927. (Darlington and La Cour, 1960).

Material was fixed in the fixative mixture for 15 minutes to one hour, and washed with distilled water before being stained.

Sanfelice 1940. (Darlington and La Cour, 1960).

Material was again fixed for periods from 15 minutes to one hour, and was washed with distilled water before being stained.

Baker lists formaldehyde as a fixative which does not fix the ground cytoplasm homogeneously, but leads to granulation; the cytoplasm is rendered very acid (basiphil) and retains little affinity for acid dyes; chromatin is strongly coloured by basic dyes; the shape of the nucleus is well preserved, though there is a tendency for the nuclear sap to be granular; the nucleus is less clearly seen than before fixation, and mitochondria are preserved though these may become moniliform. With both solutions the author found that the cytoplasm of the material became coarsened, particularly with Sanfelice, and extremely granular. This, coupled with the fact that basic dyes were readily taken up, and that mitochondria were preserved, made it extremely difficult to distinguish with any certainty and to interpret the nuclear figures seen.

(c) Alcohol fixatives.

Acetic alcohol. (Darlington and La Cour, 1960).

Material was fixed in the solution for 10 minutes to one hour. "Cytoplasm is rendered rather strongly acidophil, though it will also take basic dyes. The chromatin of interphase nuclei colours rather feebly with basic dyes, and scarcely with acid ones (probably because it is

represented only by DNA, the protein constituent having dissolved away). Metaphase and anaphase chromosomes colour strongly with basic dyes", writes Baker. He further states that the cytoplasm is poorly represented; mitochondria are not seen, this being a characteristic of acetic acid fixation. The shape of the nucleus is fairly well retained, but the nuclear sap seems not to be fixed and there is only a coarse reticulum within the interphase nucleus, with a swollen, often vacuolate, nucleolus. The mitotic spindle appears fibrous. As regards ethanol, he states that mitochondria are destroyed, a coarse coagulum is produced throughout the cytoplasm, and appears also in the nucleus, and the nucleolus is shrunken. It was found by the author that any length of time within the given range gave satisfactory results with most stains. The cytoplasm was more homogeneously fixed than with any other fixative mixture; the cytoplasm was not strongly basiphil; nuclear sap was well preserved, and mitochondria were destroyed. The latter fact proved an asset, for as Baker states "In studies of chromosomes, it is generally best to use a fixative that will either destroy mitochondria or allow them to be destroyed by subsequent treatment, for

otherwise they will obscure the view." As this fixative was found to be the most satisfactory it was used throughout the investigation on the fructifications. Material fixed was stored in 70% alcohol, and was kept either in the laboratory at room temperature, or under refrigeration conditions at a temperature of 5°C. Whether kept at room temperature or at 5°C., it was found that the material reacted to the stains up to a period of four months after fixation.

2. Stains.

Seven stains were chosen on their reputed ability to stain various structures of the dividing nucleus and of the division apparatus in other basidiomycetes.

The stains chosen were Haematoxylin, Crystal Violet, HCl-Geimsa, Aceto-carmine, Aceto-orcein, Feulgen's reagent, and Fast Green. Two points must be borne in mind, (a) that the success of the stain to a large extent depended on the fixative used, and (b) all material was prepared as temporary squashes, and as such, where differentiation was required, difficulty was experienced as the material tended to be washed off.

Haematoxylin was prepared according to the formula used for Heidenhain's method (Gurr, 1957). When ferric

salts and haematin are mixed together an insoluble lake tends to be deposited; to obviate this fact the Heidenhain technique makes use of the two bath method where the material is mordanted first in the ferric salt then in the 'ripened' (half oxidised) haematoxylin. Any excess of dye is removed by soaking the sections in the mordant for a second time. "Almost anything in the cell can be revealed by careful differentiation." (Baker, 1960).

Bits of the fructification were left in both solutions for equal periods ranging from half an hour to 24 hours. On removal from the stain, squashes were made and examined. In all cases the tissues were densely stained. The bits of fructification were then placed in the mordant and at 15 minute intervals a squash was made to see whether or not differentiation had reached the required stage. This method proved unsuccessful and time consuming. One of the snags is explained by Baker (1960) when he says that differentiation is made difficult by the fact that the extraction by the mordant seems to go faster and faster towards the end.

An attempt was made to fix the basidia obtained in the squashes on to glycerin-albumen-smeared cover-slips, which cover-slips were floated off and the staining

carried out on the cover-slip. It was found, however, that the cytoplasm took up so much of the stain (in spite of bleaching) that it was useless as differentiation proved such a problem. Coupled with this is the fact that where the cytoplasm became granular, interpretation of nuclear figures was rendered difficult.

Crystal Violet is recommended for use on smears and sections after aqueous fixatives (Darlington and La Cour, 1960). The solutions were prepared after Newton's method (Darlington and La Cour, 1960). Fructifications were stained for 10 minutes in the crystal violet solution, rinsed, left in potassium iodide-iodine solution for 40 seconds, passed through 95% alcohol and absolute alcohol for 5 seconds each, then placed in clove oil for 30 seconds, then through xylene and mounted in canada balsam. It was found that differentiation was difficult and as interpretation was difficult the method was abandoned.

In the HCl-Geimsa method, Gurr's Improved Geimsa Stain R.66 was used. Fructifications were hydrolysed in 1/N HCl at 60°C. for 10 minutes. Before being used the stain was diluted 1 drop per ml. of distilled water buffered to pH 7.2 (using Gurr's buffer tablets). The fructifications were stained in the above solution for

40 minutes then rinsed in distilled water. The chromatim was stained in all preparations, but on account of the fact that there was a lack of background staining, photography even with a filter was rendered difficult. Although this method gave fair results, it was abandoned as two other methods described below were preferred.

Aceto-carmine was prepared according to the method given in Gurr's formulary. Fructifications were stained in the solution for 10-30 minutes, then squashed in a drop of the solution on a slide. The chromatin was only faintly stained, and the method was abandoned.

Aceto-orcein was prepared according to Gurr's formulary also. Fructifications were stained in the solution for 10 minutes. A small piece of the gill was then placed in a drop of the stain on a slide and tapped gently with the blunt end of an inoculating needle handle until a 'mush' was obtained. A cover-slip was placed on the preparation, and the slide with the coverslip in position was placed between layers of filter paper and pressure was applied, making sure that this did not cause sideways movement of the coverslip. The coverslip was ringed with glycerin jelly, and preparations made in this way kept for weeks. On examination it was found that the

chromatin was deeply stained, the nucleolus showed as a very faintly stained sphere in a clear area of the nucleus. The cytoplasm took up the stain, but this was advantageous in the early stages of meiosis as it helped to demark the nuclear area. When it was thought necessary to differentiate the cytoplasm, the slide was warmed over the flame from a spirit lamp before the squash between the layers of filter paper was made. This had two effects: (a) it flattened the cells giving better squashes, and (b) it differentiated the cytoplasm and appeared to intensify the stain in the chromatin. On occasions the tissue was hydrolysed in 1/N HCl at 60°C. for 6 minutes, before staining. The only advantage appears to have been a softening of the tissues which facilitated separation of the cells one from the other.

Feulgen's reagent was prepared according to the method given in Gurr's formulary (Gurr, 1957). Bits of the fructification were washed in water, then allowed to stand in cold 1/N HCl for 1 minute (Gurr, 1957). They were then hydrolysed in 1/N HCl at 60°C. for 6 minutes. The tissues were then stained in the Feulgen's reagent for 3 - 24 hours. In all cases similar results were obtained. On removal from the stain the tissues were drained of excess stain and bleached in three baths of

sulphurous acid for 5 minutes each. Squashes were then made in water, the technique being similar to that used in the aceto-orcein squashes. Alternatively the tissue on removal from the stain was placed in 45% acetic acid. A bit of the tissue was placed on a slide in a drop of 45% acetic-acid and tapped to a much. A coverslip was then lowered over the preparation and the slide gently warmed over a flame. In both cases the chromatin was stained, though not as intensely as with aceto-orcein, but the cytoplasm was devoid of colour, and was very refractive. To overcome this difficulty, the tissue, after being removed from the sulphurous acid bath, was taken through the alcohols and stained with fast green in 95% alcohol. This stained the cytoplasm, but resulted in so great a degree of shrinkage of the tissue, that the method was abandoned.

Both Feulgen and Aceto-orcein gave satisfactory results with acetic-acid fixation, so these two stains were used for the investigation. The chromatin stained by both these stains showed similar configurations, but as the staining was more intense with aceto-orcein, this stain was mainly used; the Feulgen being chromatin specific, was used when it was necessary to determine whether or not

a structure was chromatinic.

c. Microscopy and Photomicrography.

Stained preparations were examined with the aid of a Watson Bactil Binocular Research microscope, fitted with a 2 mm. apochromatic oil immersion objective (N.A. 1.37; x 84), compensating eyepieces (x 7) and an Universal No.1 sub-stage condenser 10 mm. (N.A. 1.0); a green sub-stage filter (546mμ) was used. For phase contrast observation a Watson phase contrast objective Para 2 mm. oil immersion (N.A. 1.28; x 100) was used, with a Kodak No. 74 Wratten filter (540mμ). The light source was a Horizontal ribbon lamp (6V. 108W.) controlled by a resistor and transformer from 240v. A.C.

Photographs were taken with a Watson eyepiece camera for 35 mm. roll film, using a compensating eyepiece (x 10) and either the apochromatic oil immersion objective or the phase contrast objective. Ilford Pan F roll film was used, and was developed with Ilford ID-11 developer, and printed on Kodak photographic paper, Bromide single weight, extra hard No. 4. The scale is given with the photographs, and the illumination used (whether bright light or phase contrast) is included in the captions.

Drawings of the tissues of the fructifications were

made with the aid of a Watson Abbe Camera lucida, using a (x 10) compensating eyepiece. Other drawings were made free hand at the side of the microscope, using an eyepiece micrometer to get as close an approximation to the scale as was possible.

D. Observations.

1. Tissues of the Fructification.

Free-hand vertical sections of the fructifications of Marasmius androsaceus and M. rotula were cut, supported between blocks of carrot. The pileus of M. androsaceus is about 136 - 140 μ in depth, and consists of an outer covering or 'epithelium' of long cylindrical cells, formed by large hyphae (8 - 10 μ in diameter) which run transversely across the surface of the cap. The outer surfaces of these hyphae are made irregular by numerous, long, branched, finger-like outgrowths (Plate XIV; Plate XV, fig. 1). These are the cells to which Kühner (1933) no doubt refers as being 'herisses en brosse' which he observed on 'le revetement de leur chapeau'. Beneath the epithelium the flesh of the pileus is formed of loosely interwoven thin-walled hyphae with diameters ranging from 5 μ - 10 μ (Plate XVI; PLATE XI, fig. 2). The gills,

which are not all equal in length, are wedge-shaped in vertical section; the longest one observed (1.18 mm.) was 281.8 μ at the proximal end and 82μ at the distal or free end. The gills are very clearly divided into three layers or zones; (a) the trama, (b) the sub-hymenium, and (c) the hymenium. The trama, the central region of the gill, consists of thin-walled hyphae (5μ - 10μ) which are loosely interwoven in the same manner as are those of the pileus; the trama appears to be a downgrowth of the hyphae of the pileus so that there is no definite junction between the gills and the pileus. The outer elements of the trama are somewhat more closely packed together than are those of the innermost region, and run roughly parallel to each other and to the sides of the gills. Their distal ends turn out at right angles to the long axis of the section and become divided into the small, closely packed, irregularly shaped cells of the sub-hymenium (Plate XVII). The small size of the cells of the sub-hymenium is due to the fact that clamps are formed in close succession to each other in this region. The tips of the hyphae grow out at right angles to the sides of the gills to form the hymenium. The hymenium covers not only the surfaces of the gills, but all parts of the under surface of the pileus

not occupied by gills; the effect is thus one continuous deeply pleated hymenial surface. There are two types of elements to be seen in the hymenium; (a)fusiform elements, and (b) claviform elements. There is no apparent sequence of the two types of elements along the surface of the gills, but they are found intermixed with each other. The fusoid elements are 30 - 35$\mu$ in length by 8 - 10$\mu$ in breadth, and are seen under phase to possess a large nucleus (5 - 8$\mu$). The nuclear area is clearly distinguishable from the surrounding cytoplasm by virtue of the fact that it appears optically empty. Within it is a prominent nucleolus, and strands of chromatin reminiscent of early meiotic prophase. More mention is made of these elements later in this section.

The claviform elements are easily recognisable as maturing and mature basidia; all stages of development are seen:- basidia with developing sterigmata, basidia with young spores, basidia with mature spores, basidia from which the spores have been discharged. There is no gradation of basidia as regards maturity from the distal end to the proximal end as found in some agarics, e.g. the Coprinus- or Inaequihymeniferous-Type (Buller, 1933), but basidia with immature spores are found among

those with mature spores and even among those from which the spores have already been discharged.

The edge of the gills, that is the distal or free ends, lack basidia, in the place of which elements are found from which branched finger-like outgrowths project (Kühner's 'herisses en brosse') such as those of the epithelium.

In M. rotula the pileus is 140μ deep. As is the case in M. androsaceus there is an epithelium covering the cap, but here the cells are regular, giving rise to a cellular layer the elements of which are club-shaped. Some of these cells show fingerlike outgrowths, but others are devoid of any appendages (Plate XV, figs. 3 & 4; Plate XVIII). The flesh of the pileus consists of interwoven hyphae ( 4 - 10μ in diameter) but more loosely interwoven than those of M. androsaceus. The gills are wedge-shaped in vertical section and are all equal in depth (1.121 mm.). The proximal ends measure 320μ in width, while at the distal end they are 80μ. The gills show the three typical zones: trama, sub-hymenium and hymenium. The trama is much wider than that of M. androsaceus, the hyphae being less closely interwoven, giving rise to large interhyphal spaces. The other elements of the trama are not very closely packed and do not run

either parallel to each other or to the sides of the gills as do those of M. androsaceus (Plate XIX). The sub-hymenium occupies a much wider band than it does in M. androsaceus, its cells being longer and less branched. Here too, the hymenium covers not only the surface of the gills but the entire under-surface of the pileus, and consists of both claviform and fusiform elements. The fusiform elements are not cystidia according to Kühner (1933), who writes "we noticed a long time ago that the hymenium of M. rotula and other species of the genus Marasmius include fusoid elements, with reduced conical tips, which could be taken for small cystidia which are not prominent. We have seen these again in M. androsaceus, and the cytological study we have made of these two species has shown us that they were unquestionably young basidia or basidioles and not cystidia."[1] A further study of these elements appears in a later section.

The edge of the gills is covered with elements which are 'herisses en brosse' as are those of M. androsaceus.

2. Nuclear phenomena in the somatic cells.

The cells of the pileus, both epithelial and cortical,

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1 Author's translation from Kühner (1933).

and those of the trama all seem to be formed as are those in the vegetative hyphae, by the production of cross-walls at the time of clamp connection formation. The cells are all binucleate, the nuclei being in a resting state. The term 'resting nucleus' is used to describe all nuclei not in the process of division. The resting nucleus, which stains very feebly with Feulgen and only slightly more intensely with aceto-orcein, is 2 - 4 $\mu$ in diameter. It is characterised by having a clear nuclear sap, which shows no affinity for stains, a nucleolus which is Feulgen negative but shows as a faintly stained body after staining with aceto-orcein, and a feebly stained thread of chromatin having along its length deeply stained swollen heterochromatic segments which give it a beaded appearance and the nucleus a granular look. Although it was impossible to count the number of 'beads' even in the most favourable preparations, it appears that their number bears no relationship to the number of chromosomes, about which more is said later in this section.

The great range in size of the resting nucleus is accounted for by the fact that the term covers both migratory and non-migratory nuclei. The larger nuclei are non-migratory and are found in the pileus and trama;

in these nuclei the chromatin strand is very loosely coiled and the nucleus is found outside the periphery of the chromatin strand to which it is attached by a faint thread, presumably an end of the strand (Plate XX, fig.1). The coiled strand may show either a simple open loop, or two smaller more closely coiled loops. This type of nucleus is also found in the cells of the sub-hymenium that are situated nearest to the trama.

The tips of the hyphae are considered here to be elements of the sub-hymneium until such time as they have been cut off from the cells of the sub-hymenium as young basidia, by the cross-walls of the last clamp formed within the sub-hymeniu, hereafter referred to as the 'ultimate clamp'. It is within these hyphal tips that the migratory nuclei are best seen. These nuclei are more compact than are the non-migratory ones, and are 2 - 2.5 $\mu$ in diameter. Their nuclear sap is clear and in the centre of the nucleus is the feebly stained nucleolus. The chromatin strand is much shorter than that of the non-migratory type, probably due to greater condensation. The 'beads' stain more deeply, as indeed does the rest of the strand. The strand is closely appressed to the nucleolus which it partially encircles (Plate XX, fig. 2).

In neither type of nucleus described has a nuclear membrane been seen, regardless of the stain used.

3. Division of the nucleus in the 'ultimate clamp'.

Although the division of the nucleus in the 'ultimate clamp' of Marasmius androsaceus and M. rotula is fundamentally the same, there are certain differences and separate treatment of the process in the two species is given here for the sake of clarity. The description which follows immediately refers solely to M. androsaceus.

The tips of the hyphae in the sub-hymenial layer grow into the hymenial layer and, after reaching a length of 10 - 15 $\mu$ a clamp begins to form; this takes place between 10 - 15 $\mu$ along the hypha from the tip. As in the vegetative hyphae the clamp is formed by an outgrowth and backwards bending of an excrescence from the hypha. The clamp increases in size until it is large enough to accommodate one of the two nuclei. While clamp formation is proceeding, the nuclei enter the earliest observed stage of division. The nucleolus of what was hitherto a migratory nucleus is no longer centrally placed; it is seen at the end of a highly condensed, deeply stained chromatin component, which is no longer a single strand,

but is seen as a double structure, as though the single strand were bent backwards upon itself. (Plate XX, fig. 3). The nucleus is slightly elongated and measures between 3 - 3.5 μ in length. One nucleus passes into the neck of the clamp, the other remaining within the hypha proper. The nucleolus becomes fainter and more indistinct, while the chromatin mass stains more deeply and assumes an angular shape, 2 - 2.5 x 2 - 2.5 μ. (Plate XX, fig. 4). The double chromatin next increases the diameter of its loop, either remaining quite angular, (Plate XXI, figs. 1, 2 and 3), or losing its angular appearance and assuming the form of a double loop (Plate XXI, fig. 4). The strand, which has maintained its beaded appearance throughout, now shows what appears to be a shortening in some areas of the intervals between the beads along the strand, so that groups of heterochromatic areas are seen (Plate XXI, figs. 1, 2, 3 and 4). The nucleus then undergoes a great extension in length, reaching between 6 - 6.5 μ; the chromatin strand may appear either as a complete ring, or as one long strand doubled upon itself. The strand, whether as an entire ring or having a break within it, appears to become twisted in a figure of eight, which doubles over on itself (Plate XXII, figs. 1a & b; 2a & b; Plate XXIV, fig. 1). This leads in each nucleus to the

formation of two small rings lying one above the other. Great contraction of the figures so formed then takes place (Plate XXIII, figs.1, 2, 3 & 4). A break occurs in the rings and the arms extend linearly, giving two rods of chromatin which may appear either crossed upon each other (Plate XXIV, fig. 2) or may be seen as two separate entities (Plate XXIV, fig. 4). These two bodies resemble two chromosomes (cf. p.101). The two rods of chromatin are 2 - 2.5 μ long and still show a beaded appearance. The beaded segments are much fewer in number than before, about four per chromatin rod, and thus give the rod a knobbly, rather than a smooth and regular, outline. The nuclear area is no longer seen, and the chromatin rods lie free in the surrounding cytoplasm (Plate XXV, fig. 1).

Lateral movement of halves of both chromatin rods next takes place, giving the impression that each rod was a telocentric chromosome, the daughter chromatids of which lay 180° apart from each other. The resulting 'anaphase' figures resemble two chromosomes moving towards each 'pole'. The outlines of the chromosomes are not entire but often show a constriction in their mid-region (Plate XXV, figs. 2, 3 & 4). Chromatin bridges are seen and these persist (Plate XXVI, figs. 1, 2, 3a & 3b ; Plate XXVII, figs. 1a & b, & 2a & b), even into 'late anaphase' and early

'telophase', when the chromatin of each daughter nucleus is seen to round off into four 'chromosomes' (Plate XXVIII, figs. 1a & b, 2a & b). A nucleolus is reorganised in each daughter nucleus, each nucleus remaining in a highly contracted form for some time, the chromatin being represented by four deeply staining bodies. Cross-walls are formed across the neck of the clamp and across the main hypha in the region in which division of the nucleus took place. The two nuclei within the hyphal tip, now the young basidium, become migratory and move towards the tip (Plate XXIX, figs. 1 & 2).

When the clamp fuses with the main hypha, the daughter nucleus within the clamp may move into the main hypha and form, along with the nucleus already there, the pair of resting nuclei of the cell. Alternatively a branch may grow out from the clamp, into which branch these nuclei pass. Another clamp is then formed on the branch, cutting off a new basidium.

4. Development of the basidium.

At the beginning of its development, before fusion of the nuclei has taken place, the basidium is cylindrical. It increases in length and may show a few small vacuoles. The two nuclei show a conspicuous nucleolus, at the side

of which the chromatin component (no longer seen as four bodies as in late 'telophase' but as a granular mass) is situated (Plate XXIX, fig. 2). As the basidium increases in size, so do the nuclei, which eventually show a very clear nuclear area which is distinct from the cytoplasm that it seems reasonable to suppose that they are separated by a membrane. In this area a prominent nucleolus is seen, to which is attached a strand (sometimes double) of beaded chromatin (Plate XXX, b; Plate XXXI, fig.1). The nuclei approach each other until a very narrow band of cytoplasm lies between them. This cytoplasmic barrier gets progressively narrower until only a very fine layer of cytoplasm exists between the two nuclei (Plate XXX b; Plate XXXI, fig.1). The barrier breaks down and the nuclear areas of the two nuclei become one. The nucleoli approach each other and fuse together, forming at first an oval body which eventually becomes spherical (Plate XXX c). The chromatin strands of both nuclei become intermingled and soon appear as one long thread along the length of which numerous, deeply stained, chromatin beads are seen. The strand may be loosely coiled, or may appear as several threads greatly twisted (Plate XXX a; Plate XXXI, fig. 2).

It will be remembered that mention was made earlier (p.65) of two types of elements within the hymenium layer, viz. fusiform elements and claviform elements. It appears as though the early development of both these elements is the same up to the point of fusion of the nuclei. After fusion, which takes place round about the middle of the element, the resulting nucleus is diploid and is the only diploid nucleus of the life cycle of the fungus. In those elements which become claviform, the nucleus increases in size (5-6 x 8-8.5 $\mu$) and moves towards the tip. The element itself, as if to accommodate the expanding nucleus, increases in size, and the upper half, the distal end, assumes the characteristic club-shape of the mature basidium (Plate XXX, fig. a). The fusiform elements remain cylindrical, the nucleus within them remaining in the middle of the element. Within the nuclear area which is elongated and thus eliptical rather than oval (3-3.5 x 7.5-8 $\mu$) the nucleolus is quite clearly seen, along with what appear to be chromosomes in meiotic prophase, two of which are attached to the nucleolus. The nuclei appear to remain in this state for a considerable time, as though they were in 'resting prophase' (Plate XXXI, fig. 3).

5. The division of the nucleus in the basidium.

The diploid nucleus of the basidium undergoes a meiotic division and gives rise to the four nuclei which migrate into the spores which are ultimately formed on the basidium. Leptotene, as known in higher plants, is either very brief or is completely absent; this stage has never been seen in any of the hundreds of basidia examined. The earliest change observed from the nucleus with the prominent nucleolus and greatly twisted chromatin strand seen after fusion, is a stage analagous to that of zygotene in higher plants. Four pairs of greatly elongated chromsomes are seen, one pair of which is attached to the nucleolus. The chromosomes are seen as thin threads which show a granular appearance; this gives the impression of a 'string of unequal beads unequally strung together' (Darlington, 1958). The beads,[1] the chromomeres and heterochromatic knobs, on one chromosome are seen to pair exactly with similar beads on the homologous

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[1] The term "beads" has been used here instead of either chromomere or heterochromatic knobs.
Swanson (1957) states that the term 'chromomere' covers a wide range of morphologically recognisable structures, even though there is every likelihood that they are structurally quite dissimilar. The large knobs found in maize are not chromomeres in the usual sense since they are heterochromatic rather than euchromatic. "Beads" thus avoids confusion of terms.

chromosome (Plate XXXII, figs. 1a & b). Of the four chromosomes present, two are longer than the others, the longer of the two long chromosomes has been designated Chromosome I, the shorter Chromosome II; of the two shorter chromosomes, the longer has been designated Chromosome III, the shorter (the shortest of the complement) Chromosome IV. Some time after this stage the nuclear "membrane" breaks down and a clear nuclear area is no longer seen. The bivalents are seen to lie freely in the cytoplasm. The nucleolus by this time has become indistinct and has probably disappeared. The bivalents are very highly condensed and have been reduced to less than half their zygotene lengths. The relative lengths seem to be somewhat retained, however, as chromosomes I, II, III, and IV can still be recognised as such. Chromosome II, by virtue of the fact that it bears a satellite, and chromosome IV on account of its small size (about 1 $\mu$) are particularly recognisable (Plate XXXII, figs. 3 & 4; Plate XXXIII, figs. 1 & 2). At no time could centromeres be recognised. The bivalents were long enough to show an arrangement of large beads which lie close to each other; these beads are fewer in number than those seen in zygotene. The inter chromomeric

regions are now very short. The four-stranded stage of some pachytene bivalents in higher plants has never been seen; the bivalents often appear as one rather thick chromosome, and only in places where synapsis appears to have been incomplete, or where what probably is a chiasma is seen (Plate XXXIII, fig. 2) can the double nature of the bivalents be recognised. The bivalents although lying free in the cytoplasm show an orientation, being all aligned parallel to each other (except for the smallest chromosome in some cases) and either parallel to or slightly at an angle to the short axis of the basidium (Plate XXXII, figs. 3 & 4; Plate XXXIII, figs. 1 & 2). Diplotene such as is known in higher plants is not seen, the bivalents each appearing as a unit. In what may be called diakinesis, the bivalents are seen to have lost their beaded appearance, and to have become highly contracted and to be very deeply stained. They are seen to be connected one to the other by a faintly stained thread (Plate XXXIII, fig. 3). The orientation of the bivalents parallel to each other and to the short axis of the basidium is still evident. A premetaphase in which the bivalents lose their parallel alignment and become arranged in a ring still connected to each other, takes place. In some preparations at this

stage, the duality of the bivalents can be seen (Plate XXXIII, fig. 4). Metaphase is probably a very short phase, judging by the scarcity of figures seen. A spindle in the classical sense with fibres has never been seen, neither have centromeres been seen at any stage.

Anaphase separation results in the movement of four chromosomes to each pole. The chromosomes are still connected to each other by faintly stained threads, parts of which persist between the two groups of chromosomes forming 'bridges'. These are evident whether material is stained with Feulgen or aceto-orcein. The chromosomes move at different rates towards the poles, and the smallest chromosomes are often seen lagging behind on the 'bridges' (Plate XXXIV, figs. 1a & b; 2a & b; Plate XXXV, fig. 1). At this stage the outline of the chromsomes is smooth, and in favourable preparations characteristic chromosomes, such as satellited chromosome II, can be distinguished (Plate XXXIV, figs. 1a & b). The 'bridges' persist into late anaphase and early telophase (Plate XXXV, figs. 2, 3 & 4). The four chromosomes round off and in late telophasic figures the nuclei are seen as consisting of a ring of four chromatin bodies, all of which appear to be roughly of the same size (Plate XXXVI, figs. 1 & 2; Plate XXXV, figs. 3 & 4).

After the first division the two daughter nuclei become reorganised. The chromosomes lose their affinity for stain, and two small clear areas are seen in the cytoplasm, representing the nucleus in each of which is embedded a nucleolus. The chromatin makes its reappearance as a long looped strand attached to the nucleolus (Plate XXXVI, fig.3). As is the case in the division of the nucleus in the ultimate clamp, the nucleolus becomes indistinct and disappears quite early in the process of division. The chromatin shows an increasing ability to take up stains, and is soon seen as a double strand, beaded in the same manner as the nucleus in the ultimate clamp (Plate XXXVII, figs. 1 & 2). The nucleus undergoes great extension in length and the double strand opens out so that the two component strands are widely separated from each other except at one or both ends at which they may be joined. The strand becomes twisted to give a figure of eight, the two loops of which fold over on each other (Plate XXXVIII, figs. 1 a & b, 2, & 3). The rings appear to break (Plate XXXIX, figs. 1 & 2), and their arms extend linearly to give two rods of chromatin (Plate XXXIX, figs. 3& 4 ; Plate XL, figs. 1 & 2). As in the division in the ultimate clamp the rods of chromatin show

a beaded appearance. The rods lie freely in the cytoplasm, as a nuclear area is not seen. Later a movement of halves of both chromatin rods now takes place (Plate XLI, figs. 1, 2, 3 & 4) to give 'anaphase figures'. The daughter groups are connected by chromatin bridges (Plate XLII, figs. 1, 2 & 3) which persist into late 'anaphase' and early 'telophase' (Plate XLIII, figs. 1 a & b), at which stage the chromatin is seen to round off into four 'chromosomes' (Plate XLII, fig.4).

The four nuclei formed as a result of the above described division become reorganised and are seen to consist of a nucleolus to which a strand of chromatin is attached (Plate XLII, figs. 2 & 3). The nuclei soon assume the form of the non-migratory type of nucleus (Plate XLIV, fig. 1). While the nuclei are in the resting state, sterigmata are formed on the basidium. These first appear as small bumps on the rounded tip of the basidium; they increase in length and become horn-like, being conical with very acutely pointed tips. When they have reached a length of 6-8 $\mu$, the spores begin to form as small rounded swellings on the tips of the sterigmata. The nucleus in the meantime has become highly contracted as it prepares to move into the spore. It is not to be

thought that the contracted state here is similar to that recognised in migratory nuclei. In migratory nuclei a nucleolus is obviously present, whereas in the nuclei about to move into the spores it is impossible to see a nucleolus. The chromatin is heavily stained and is seen as a strand showing two very tight coils, so that it resembles two small circles lying one on the other. In this state, one, two, three or all of the nuclei may undergo a second 'mitotic division', so that the number of nuclei seen within a basidium at this stage may be anything from 4 - 8 (Plate XLIV, fig. 2). The spores increase in size becoming pip-shaped, and of the order of 3 - 3.5 x 7.5 - 9$\mu$. The nuclei undergo a further change in shape; the chromatin becomes bent into 'hair-pin shaped' threads, the apices of which are directed towards the sterigmata. They move through the sterigmata into the spores, where they assume the highly contracted circular appearance seen prior to the hair-pin stage (Plate XLIV, fig. 3). They are next seen as resting nuclei (Plate XLIV, fig.4) each of which has a nucleolus. As there are four sterigmata per basidium and hence four spores, the spores may be uni-nucleate or bi-nucleate, depending on whether or not the four nuclei resulting

from the meiotic division undergo another division. A spore in which the nucleus appeared to be dividing was seen on one occasion.

6. The division of the nucleus in the 'ultimate clamp' of M. rotula.

The division of the nuclei during the formation of the 'ultimate clamp' in M. rotula resembles that of M. androsaceus in general, and thus only the differences which do occur in the configurations seen are pointed out here.

The resting nucleus closely resembles that of M. androsaceus. The early stages of division are similar to those of the nucleus in M. androsaceus; the nucleolus disappears quite early in the process of division, and only the chromatin strand is seen, but the double nature of the strand is not evident here. On the ring of chromatin four deeply stained bodies are seen (Plate XLV, fig.1); at the corresponding stage in M. androsaceus the nucleus appeared to be quite angular but showed no such definite bodies as are seen in M. rotula. The stages following resemble in the main those seen in M. androsaceus (Plate XLV, fig. 2); a break appears in the figure formed

and the arms open out linearly, to give two rods of chromatin which show a beaded appearance (Plate XLV, fig. 3). 'Anaphase' separation is similar to that of M. androsaceus (Plate XLV, fig. 4; Plate XLVI, fig.1). At 'telophase' the four 'chromosomes' are, however, much more clearly delimited than are those of M. androsaceus (Plate XLVI, fig. 2).

7. The development of the basidium in M. rotula.

As in M. androsaceus the young basidium in M. rotula is formed from the outgrowths of tips of hyphae of the sub-hymenium after the 'ultimate clamp' has been formed. Unlike the case in M. androsaceus, however, the nuclei which go to form the fusion nucleus are reorganised very soon after 'telophase' and fuse fairly near to the site of clamp formation, even before the backwards bending clamp has fused with the main hypha, and while the nucleus which remained in the clamp after division still exhibits a late 'telophasic' appearance (Plate XLVI, fig. 2).

On account of the precocious fusion of the nuclei they do not show clear nuclear area containing a nucleolus with attached chromatin strand seen in the nuclei of M. androsaceus prior to fusion. Instead there is seen a nucleolus at one side of which is the granular chromatin.

The nuclei approach each other at the nucleolar ends and fuse, giving first a dumb-bell shaped body (Plate XLVI, fig. 2), then an oval nucleus. The nuclear area of the fusion nucleus is somewhat smaller than that of M. androsaceus, being 4 - 5 x 5 - 6μ. On account of the early fusion the nucleus goes into an early prophase, and is already in that stage by the time that the basidium has attained its full size.

There are in the hymenium the two types of elements that have been described in M. androsaceus, fusiform and claviform elements. The fusiform elements are more acutely pointed at their tips than are their counterparts in M. androsaceus, but their nuclei exhibit a behaviour similar to that in the latter species (Plate XLVI, fig. 3).

8. The division of the nucleus in the basidium.

As mentioned above the nucleus goes into prophase before the basidium has matured fully, and here what can be considered as a leptotene is seen, closely approximating to that occurring in higher plants, but not observed in M. androsaceus. A tangled mass of many long, beaded, chromosomes is seen, which fills the entire nuclear area (Plate XLVII, fig. 1). Zygotene follows leptotene,

during which pairing of the chromosomes in intimate association begins, and here for the first time a count of the chromosomes can be made. Four pairs of chromosomes are seen, one of which as in M. androsaceus is attached to the nucleolus. From zygotene to early pachytene the nuclear membrane appears to break down and the nucleolus to disappear, for in pachytene the bivalents are seen to lie freely in the cytoplasm. (Plate XLVII, figs. 2 & 3a). The bivalents show the same orientation parallel to each other and roughly parallel to the short axis of the basidium as that seen in M. androsaceus at the same stage.

Late pachytene shows that synapsis may be incomplete in some of the bivalents (Plate XLVIII, figs. 1a, 1b & 2). At this stage the chromosome morphology of the complement can most readily be seen. As in M. androsaceus there are four chromosomes which were designated Chromosomes I, II, III and IV (Plate XLVIII, fig. 3).

Chromosome I in late pachytene has an average length of 4.25 $\mu$ (maximum 5.4 $\mu$; minimum 4 $\mu$). Eight beads (chromomeres and heterochromatic knobs) were always obvious, which in conjunction with its length made the chromosome easily recognisable in preparations.

Chromosome II was always identified by its satellite.

Nearly as long as Chromosome I, it consists of four large beads one of which is separated from the others by a long 'inter-chromomeric region' on which a small bead is seen. The average length in late pachytene was just over 3 $\mu$.

Chromosome III is very much shorter than Chromosomes I and II, and has two large beads at one end and two smaller ones at the other.

Chromosome IV which was measured only with the greatest difficulty on many occasions is on the average 1 $\mu$ long, and consists largely of two beads.

Longitudinal separation of the paired chromosomes is never seen, hence the duality of the individual chromosomes was not observed. The homologues remain in close relationship with each other and relational coiling of the chromosomes of the bivalents appears to take place. The coiling appears to be of the plectonemic nature found in some prophase chromatids in higher plants, as the whole length of the bivalents appear to be thrown into large coils. (Plate XLIX, figs. 1 & 2). In diakinesis, with contraction apparently at its maximum, the duality of the bivalents is seen (Plate XLIX, figs. 3 & 4).

A premetaphase in which the chromosomes lose their sharp outlines and no longer show as double structures now

takes place. At the same time they are seen to be joined to each other by a faintly stained thread (Plate L, fig.1).

The spindle has never been seen but in metaphase the bivalents, all attached to each other, become aligned on the 'metaphase plate'.

The movement of the chromosomes to the poles constitutes anaphase as in higher plants. In favourable preparations four chromosomes can be seen going to each pole. The chromosomes are all joined to each other, as are the two groups, by a thread which persists, forming a bridge as was seen in M. androsaceus (Plate L, fig. 2).

In late anaphase to early telophase, the four chromosomes are seen to form a ring (Plate L, figs. 3& 4; Plate LI, fig. 1 & 2), sometimes still showing a bridge between the two groups.

Telophase is followed by an interphase during which the nuclei are reorganised. In prophase II the nuclei are seen as double strands of chromatin, which show a beaded appearance, the beads of one strand matching those of the other (Plate LI, fig. 3). The strands open out so that the two parts are separated from each other, except at one or both ends. They become twisted to form a figure of eight, the loops of which double over on each

other (Plate LI, fig. 4). A break in the figure so formed and a linear extension of the arms gives rise to two rods of chromatin per nucleus. Anaphase separation takes place as in M. androsaceus and in the ultimate clamp in both species. The daughter groups are connected by chromatin bridges which persist for some time, and in 'telophase' each is seen to consist of four rounded chromosomes (Plate LII, figs. 1 & 2). The nuclei become reorganised and soon assume the non-migratory resting state. Four sterigmata develop on the basidium, the development being similar to that in M. androsaceus. (Plate LII, figs. 3a & b). The nuclei become contracted, the nucleoli become indistinct and, as in M. androsaceus, either one, two, three or all four nuclei may undergo a second 'mitotic' division. When the spores have reached a size of the order of 3 x 7-9 μ, the nuclei migrate through the sterigmata and take up their position in the distal ends of the spores. Prior to migration the nuclei assume the hair-pin shape appearance seen in M. androsaceus. The mechanism which accounts for the movement of the nuclei into the sterigmata has not been seen. Marie[1] has recorded

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1 Heim (1954)

centrosomes which position themselves at the sites of sterigmata formation and guide the nuceli; Kühner[1] has recorded a 'kinoplasmic' differentiation into fibrils, in the basidium of Psathyrella pennata, and Vokes has recorded centrosomes in Coprinus atramentus. Once in the spore, the nuclei assume the resting state. Nuclei have been seen which appeared to be undergoing a division in the spore and binucleate spores have been observed.

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[1]Heim (1954)

## SECTION III (B).

Kennedy and Burnett (1956) state that there are four mechanisms which can result in the production of spores both homokaryotic and heterokaryotic for mating type by a single fruit body. In describing one such mechanism they wrote, "In certain Basidiomycetes with four-spored basidia, an additional mitosis after meiosis results in the occurrence of eight nucleate basidia. Two nuclei migrate into each basidiospore and they may carry the same or different mating type factors, that is, the basidiospores will be homokaryotic or heterokaryotic for mating type, respectively." In order to find out whether or not this is true of Marasmius androsaceus and M. rotula, where an additional division of the nucleus in the basidium is sometimes found, spores from the fructifications were germinated and matings made between the monokaryotic cultures obtained.

Materials and methods.

The fructifications were collected from the same sources as the fructifications used for the investigation dealt with in Section III (A).

Fructifications of Marasmius androsaceus and M. rotula were suspended on wire hooks over malt-agar (2% Difco-bacto

agar; 2.5% malt extract) coated slides which lay on glass triangles on layers of water-saturated filter paper in deep Petri-dishes. After half an hour the slides were examined microscopically and it was found that spores had been deposited on the agar surface. The deposit was thinnest around the periphery of the area which it occupied. The agar around single spores in the peripheral regions was scored with a chisel-pointed inoculating needle and the block of agar with spore was lifted from the slide, the whole operation being carried out under low power (x10) of the compound microscope. The blocks of agar with single spores were used to inoculate malt-agar plates which were incubated at 22°C. It was found that the spores germinated overnight, having on the day following inoculation, germ-tubes ranging from 30μ - 40μ (Plate LIII, fig. 1).

The cultures had grown sufficiently in six days to allow sub-cultures to be made. The monospore cultures were examined microscopically, and from a batch of 25 spores of M. androsaceus and 35 of M. rotula plated out, one culture of M. androsaceus and 3 of M. rotula possessed clamp connections and binucleate cells.

Matings were made between 15 of the cultures of M. androsaceus (Cultures a - o); similar matings were made between 14 cultures of M. rotula (Nos. 1-9 and 11-15).

Results.

The results obtained from the matings of M. androsaceus revealed the fungus to be tetrapolar. The results obtained from the matings of M. rotula also revealed that fungus to be tetrapolar, but one culture behaved as though it possessed two incompatible nuclei. The matings were repeated thrice, and on all occasions the same results were obtained. The results are shown in tabular form in Plate LIV.

The culture referred to above as behaving as though it possessed two types of nuclei, as regards mating type, was examined microscopically under phase contrast. The hyphae showed monokaryotic characteristics in that they were branched and lacked clamps, but in some of the cells two nuclei were seen (Plate LIII, fig. 2).

From the table on Plate LIV, it will be seen that the nuclei can be divided into four types, $A_1B_1$; $A_2B_2$; $A_1B_2$; $A_2B_1$. Culture No. 6 showed matings consistent with both types $A_2B_1$ and $A_1B_1$.

DISCUSSION.

Savile (1939) reported the existence in some species of Uredinales and also in a species of Mycena of two types of resting nuclei which he named 'expanded', the nucleus being a non-stainable body containing a stainable sphere, and 'unexpanded', where the nucleus is compact and densely stained. Evans (1959) suggests that the unexpanded and expanded states are stages in the development of the resting nucleus, "the unexpanded condition representing nuclei which have recently undergone mitosis and the expanded type being the mature resting nucleus". He further states that the developmental sequence is found in the cultivated mushroom, the larger granular nuclei being the expanded type. He found, however, two kinds of small dense nuclei, one of which conformed to Savile's unexpanded type while the other was somewhat larger though not as large as the expanded type. The latter type which he describes as mature and densely staining, and which he believes does not owe its compactness to the after effects of recent mitosis, he names 'homogeneous', to avoid confusion with the unexpanded type. The nuclei of Puccinia helianthi 'do assume two principal quite different forms which, without implying acceptance of

all Savile's connotations, may be described as expanded and unexpanded, as the terms seem descriptively apposite.' (Craigie, 1959).

In the present work, what is apparently the counterpart of Savile's unexpanded type of resting nucleus has not been included in the description of resting nuclei, as it appears to the author that these densely compacted nuclei which show no nucleoli and are found only immediately after division, are late telophasic nuclei, which have not yet become reorganised. The resting nuclei seen fall, however, into two types, migratory and non-migratory. The migratory nuclei, such as those found in the sub-hymenium and in the young basidium before fusion of the nuclei takes place, possess nucleoli around which the chromatin is tightly appressed, whereas the non-migratory nuclei such as those found in the trama and cells of the pileus, have non-central nucleoli, wide loops of chromatin strands, and are much larger than the migratory type. From an intensive study of nuclei in rust fungi, Savile (1959) concluded that only nuclei in the unexpanded state were able to migrate; he states, however, that he did not observe any migrating nuclei in the thallus hyphae. He was of the opinion that migrating pycniospore nuclei

seldom or never divided until they reached the margin of the protoaceium. If, however, pycniospore nuclei or their progeny migrate in their unexpanded state to the protoaecia, the detection of at least a few of these migratory nuclei should not be difficult. As it is believed by the present author that the 'unexpanded' types referred to are late telophasic nuclei, and not the same as the migratory type referred to in this work, the terms 'unexpanded' and expanded' though popular in work of this kind have not been used.

Whether the cells contain migratory or non-migratory nuclei they are always binucleate. Colson (1935) and Evans (1959) report that the cells of the fructification in the cultivated mushroom are multinucleate, but that the number of nuclei per cell decreases as the gills are approached; the cells of the trama of the gills are often binucleate, while those of the sub-hymenium and hymenium are always binucleate. Hirmer (1920) suggests that this last condition is arrived at by the non-division of one or more of the nuclei in the multi-nucleate cells which are undergoing division. Evans (1959) suggests that the important fact is that cell division is independent of nuclear division. If one or more cross-walls are formed

across a cell, this will be an effective method of reduction of nuclear number, particularly if this is not coincident with nuclear division. The binucleate condition in M. androsaceus and M. rotula is believed to be maintained by the regular production of clamps at the time of nuclear division. Morphological evidence in the cells of pileus and trama suggests the presence of clamps; these, however, are very small in comparison with the diameters of the hyphae and may easily be overlooked. Their presence is obvious in both the sub-hymenium and at the bases of the basidia.

If there is any genetical importance attached to the nuclear fusion and consequent meiosis in the basidium, any genetical difference between the conjugate nuclei of the vegetative hyphae must be maintained. Lambert (1938) and Kligman (1943) show that in the cultivated mushroom segregation occurs at meiosis, and it has been inferred from the presence of chromatin bridges at anaphase separation that the nuclei involved in the premeiotic binucleate condition are of different genetic constitution. The genetic balance which exists therefore is maintained in the fructification of M. androsaceus and M. rotula as a result of the production of clamps at the time of nuclear division as in the vegetative hyphae.

Other workers have not recorded the division of the nucleus at the time of clamp connection formation within the fructification owing to its small size. Kühner (1933), working on the cytology of M. androsaceus and M. rotula, ignored it, focusing his attention on the division in the basidium of which he said "nous n'insisterons pas sur les phénomènes intimes des deux division successives que subit le noyau de fusion dans la basside, la numeration des chromosomes étant d'autant plus délicate que les élèments hyméniens de M. androsaceus et M. rotula sont de taille réduite." Heim (1954) also ignored these divisions. The question as to whether the division is mitotic or amitotic is applicable here as it is to the division in the vegetative hyphae.

The division of the nucleus in the ultimate clamp cannot be said to follow the stages of classical mitosis faithfully. Chromosomes are not resolved as separate entities during any stage of division. The 'chromosomes' are all joined together on one long strand; furthermore, they show a beaded appearance, unknown in the chromosomes of classical mitosis, though typical of meiotic chromosomes. It is now clear that the individual beads are not individual chromosomes as thought by Heim and other

workers, because their number is greater than that of the chromosome complement or even greater than twice the chromosome complement. Further, as division proceeds and the strand contracts, these beads are seen to become grouped into larger heavily stained sections of the strand. Spindle formation is not evident, but a metaphase plate is in all probability present, upon which the double ring of chromatin, formed as a consequence of the formation of the figure of eight, becomes aligned. Centromeres have, however, never been seen.

If the same names were to be applied to the stages of division of the nucleus in the ultimate clamp as are applied to those of classical mitosis, prophase includes a larger number of events. At the initiation of prophase reduplication of the chromatin strand will have been complete, having taken place in interphase. It is believed that the double strand seen in the early stages of division represents the two sets of four 'chromatids' strung together end to end, the strands being joined to each other at one end or at both. Longitudinal splitting of the original strand must then have taken place very early in the process of division, to account for this structure. The 'chromosomes' elongate, hence the great

stretching of the strand that is seen, and each presents a beaded appearance such as is seen in meiotic chromosomes in higher plants. Contraction of the 'chromosomes' follows, during which the beaded appearance becomes less obvious, and larger sections of stained material are seen along the strand. The figure of eight formation, which follows, may not always present the perfect appearance of an eight and may constitute a 'prometaphase'. By the time the double rings so formed are aligned on the 'metaphase plate', 'metaphase' is reached.

What is implied above is that there are four chromosomes in the complement; this fact is based on the count at diplotene in meiosis, and on the fact that in telophase in both meiosis and 'mitosis' in the ultimate clamp, four chromosomes are always seen. The chromosomes are all strung together linearly and reduplicate as a unit. The sequence of events in division is interpreted as follows: Very early in 'prophase' longitudinal splitting of the reduplicated chromosomes occurs, giving rise to two linear strands of four chromatids each, these strands being joined together at one or at both ends. The strands of chromatids elongate, the chromatids showing a beaded appearance. They then contract and the beaded appearance is largely lost. The strands display a sigmoid

curve, each crossing on the other and giving a figure of eight. These stages are represented diagramatically in Plate LV, figs. 1 - 3. The loops of the figure of eight double over on each other giving rise to two rings, one on the other (Plate LV, figs. 5 & 6). If the chromosomes are numbered 1 to 4 on the strand, and crossing of the strand took place between chromosomes 2 and 3 on each strand, then on the upper ring will be found chromosomes 1 and 2 of one strand and chromosomes 1 and 2 of the other; on the other ring, chromosomes 3 and 4 of each strand will be found. Between metaphase and anaphase, the points at which the strands are connected - at the free ends of chromosomes 1 and 4 if both ends are connected - break, and the contracted rods of four chromosomes become stretched linearly on the 'spindle', parallel to the long axis of the spindle, i.e. from pole to pole (Plate LV, figs. 6 a & b). The order of chromosomes along the rods would be from left to right 1, 2, 3 and 4 on the upper rod, and 4, 3, 2 and 1 on the lower rod. The picture seen at this stage resembles, as mentioned before, two chromosomes and as the spindle is not seen, one may be tempted to suppose that each rod constitutes a nucleus, particularly as each would possess a full complement of chromosomes. 'Anaphase' separation

shows that a portion of each rod goes to each pole. The evidence presented strongly suggests that the junction between chromosomes 2 and 3 in each rod breaks down, so that chromosomes 3 and 4 of the upper rod in the diagram and chromosomes 2 and 1 of the lower move towards one pole, while the other four chromosomes move towards the other pole. Each nucleus thus has a full complement. (Plate LV, figs. 7 & 8).

Dowding and Weijer (1962) working on species of Neurospora and Gelasinospora came to the conclusion that the chromosomes appeared to be arranged in a linear series on a filament. To them the following types of nuclei constituted consecutive stages in mitosis. "(1) a network within a spherical membrane; (2) an elongate thread free of the membrane; (3) a narrow thread; (4) a thread longitudinally split; (5) two separate daughter threads; (6) a shortened and thickened filament with distinct chromosomes; (7) a similar filament coiled within a membrane." While the earlier stages which they describe resemble those seen by the author in M. androsaceus and M. rotula, the series observed after the recognition of the double strand differs appreciably, in that each single strand has been interpreted in the case of Neurospora and

Gelasinospora as representing a single nucleus, whereas in the species of Marasmius examined, the observations now recorded lead to the conclusion that, if the results of the twistt in the formation of the figure of eight are being correctly interpreted, each nucleus consists of a part of each of the strands. Furthermore, in their diagrams, Dowding and Weijer show the strands aligned on the metaphase plate parallel to the equatorial plane, unlike those of M. androsaceus and M. rotula where the alignment is roughly parallel to the polar plane; this necessarily results in the differences in the anaphase figures seen.

Kühner (1933) states that the fusiform elements seen in the hymenium of members of the genus Marasmius are not cystidia but young basidia or basidioles. The early stages of development of these elements resemble those of the claviform elements, but whereas the nucleus in the claviform element moves towards the tip of the basidium, that of the fusiform element remains at the site of which fusion of the nuclei occurred. The long tangled chromatin thread seen in the fusion nucleus of the claviform element of M. androsaceus is not seen in the fusiform element where the nucleus appears to reach and remain at an early

stage of prophase. It is known that the members of the genus Marasmius possess the ability to revive after periods of desiccation and to produce spores within hours of revival (Rea, 1922); if these fusiform elements are immature basidia, it is possible that the nuclei remain in prolonged prophase, and later produce the second crop of spores, the first crop being those produced by the claviform elements. If this is the case, the spores produced on revival of the fructification after desiccation, are produced on these elements.

Writing on meiosis in the basidium, Heim (1954) states that the different stages of the meiotic prophase should be 'peu marqués' and should take place rapidly if one were to judge by the rare pictures found between the quiescent nucleus and the end of prophase when the bivalents are formed. She continues that no figure shows satisfactorily the arrangement of 'partenaries qui doivent s'associer' during the meiotic cycle. In M. androsaceus, although prophase stages as early as those in M. rotula were not observed, stages sufficiently early to permit the observation of homologous chromosomes in close association before synapsis, were obtained. Similar stages were obtained for M. rotula. Heim records that

Kniep[1] saw double filaments in Armillaria mellea, and Bauch[1] in Nidularia piriformis, while Wakayama showed coupled chromosomes in different species. The stages of prophase are not as clearly marked as those in meiosis in higher plants, but the chromosomes are clearly resolved, and successful counts have been made at diakinesis. The count of n = 4 in both species of Marasmius disagrees with that of Heim who found n = 8.

Of prophase in the basidium of mushrooms in general, made from a study of several species of different genera, including M. androsaceus and M. rotula, Heim states that the division starts by an accentuation of the chromocentres which become more chromatic by a type of contraction and by their progressive separation from the filaments which bound them. This massing together continues until the end of prophase, without there being the least suspicion of duality, so confused are the coupled elements. She further says that when the 'gemini' are completely separated from their anastomoses they are very condensed, of small size and granular, or in short rods. While agreeing with the statement that the duality of the

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[1]Heim (1954)

individual chromosomes is not evident, the author cannot agree that a massing together of the coupled elements takes place, for the bivalents of both M. androsaceus and M. rotula always lie in the cytoplasm after the dissolution of the nuclear membrane, which takes place fairly early in the process, and shows a characteristic orientation, parallel to each other and to the short axis of the basidium. Each bivalent is so clear as a separate entity, that in some preparations chiasmata are seen.

A point which has not been mentioned by Heim is the fact that the chromosomes become joined to each other by a thread faintly stained with either aceto-orcein or Feulgen. This phenomenon is known to exist in certain Hemiptera in the animal kingdom, for example Mecistorhinus melanoleusus (Schrader, 1946) where shortly before the breakdown of the nuclear membrane the chromosomes, still not fully condensed, tend to form a more or less circular chain in the equator. Some of the components may be in actual contact with each other while others may be connected by Feulgen positive bridges or show no connection. This is assumed to be due to the loss of the power of mutual repulsion, coupled with the fact that the

chromosomes remain in close contact with the nuclear wall, which has constricted in the mid-region due to elongation of the nucleus. Schrader states that it is impossible to decide whether the bridges represent viscous connections, which persist after a former contact, or are indicative of a 'reaching out' of the chromosomes to each other. In M. androsaceus and M. rotula the nuclear membrane has already disappeared when the connections are formed, and no evidence of former contact of the chromosomes is seen for one to suppose that the connections are due to the persistent viscous connections of former contact. There is apparently an intimate association of the four chromosomes (in the somatic nuclei they are linearly arranged to form a filament) which is lost during early prophase; the connections seen possibly re-establish the association before the bivalents become aligned on the metaphase plate so that at anaphase the chromosomes move in their associated order, and enter telophase in their correct associations.

In both M. androsaceus and M. rotula division I is followed by an interphase when the nucleolus is reorganised. Wakayama in his study recognised this interphase only in Mycena haematopus, in the other species the second division follows the first immediately, before complete reorganisation

of the nuclei takes place.

Heim (1954), referring to the second division, states that the prophase phenomena must also take place very rapidly and that the reduced size of the nucleus does not permit the following of the sequence of the phases, "de même qu'on ne peut pas suivre la disjonction des chromosomes à la metaphase." She speaks, however, of a grouping of the small chromosomes in the centre of the nucleus at metaphase, these chromosomes "issus de la contraction sur eux-même de chromocentres et des filaments qui les relient", divide and produce beautiful pictures of small granules which separate in equal lots. Marie[1] writes that the 'caryosomes' which encrust the linin reticulum, are transformed immediately into two short fat chromosomes without passing through the stages of proto-chromosomes; in other words there is no prophase in the second division of the basidial nucleus. Kühner[1] in Psathyrella and Chou-Chung Hwang (1934) in several members of the genus Coprinus mention chromatic corpuscles which finally unite into two metaphase chromosomes. Kühner[1] in Sebacina gloeocystidiata notes an indeterminable number

[1]Heim (1954)

of chromosomes of which he claims there are two at metaphase. These reports led to the fact that n = 2 was for a long time attributed to many of the Basidiomycetes.

In M. androsaceus and M. rotula, division II follows along the lines of the division of the somatic nuclei in the ultimate clamp, and it appears that the two chromosomes referred to by the above mentioned authors are the two metaphase rods which the present author interprets as being the highly contracted strands consisting of four chromatids each. If one examines the description of the nucleus given by various workers, it is seen that the majority refer to granules or 'chromocentres' or 'prochromosomes' which are small, stain deeply and, according to Heim, their number is equal to, or approaches that of, the chromosomes. These granules are joined by filaments, which stain feebly with Feulgen, while the granules themselves stain deep violet. These granules, which Marie calls 'protochromosomes', are said to all fuse in the centre of the nucleus to form two chromosomes at metaphase. Swanson (1957) points out that bodies other than the nucleolus may be found in the interphase nucleus of some species. These are often several in

number and the name prochromosomes was applied to them because they were thought to be the precursors of the chromosomes, structures into which the chromatin resolved itself during division. 'It is now generally recognised that the prochromosomes or chromocentres as some authors have labelled them, are specialised portions of the chromatin which unlike the remainder of the chromatin stain deeply during interphase.' They are readily seen in the vegetative cells of many plants such as the bryophyte Pellia, tomato and Impatiens, as well as in early meiosis in higher plants in general.

The protochromosomes of Marie, the chromocentres of Heim and the granules of the other workers are believed by the present author to be the beads which he has seen which give the beaded appearance to the double strand of chromatin, and which are the deeply staining heteropycnotic areas. Since the filaments stain feebly they may easily be overlooked, and the events which lead to the 'massing together of the chromosomes' (Heim) into two chromosomes recorded by Kühner, Wakayama and Marie, at metaphase would, if unobserved, lead to the belief that prophase is non-existent in the second division of meiosis in the basidium.

The present work offers an explanation for the first time of the nature of the 'two chromosomes' which appear at metaphase from many granules. It demonstrates the successive stages leading to the formation of the two metaphase rods each consisting of a complement of four chromosomes.

The spindle has never been seen in M. androsaceus nor in M. rotula during the present work. Heim writes that it is not apparent, that is to say, it neither shows fibrils (which aspect appears especially in preparations that have undergone treatment with fixatives rich in acetic acid) nor cytoplasmic radiations or asters, nor centrosomes. Judging by the movement of chromosomes to the poles, the spindle is, however, always aligned parallel to the short axis of the basidium in division I, and either parallel to or oblique to the short axis at the second division.

The second mitotic division which some or all of the basidial nuclei undergo, has been observed in other genera by other workers. Kühner (1933) was unable to find basidia with eight nuclei in M. androsaceus and M. rotula, but having observed nuclei within basidia upon which nucleated spores were borne, came to the conclusion

that nuclei divide frequently in their passage through the sterigmata, one daughter nucleus going into the spore, the other moving back into the basidium. He states that the reason for their additional division completely escapes him as half the number of nuclei formed are bound to degenerate; he was convinced that the nuclei which return to the basidium after intersterigmatic division never go back into the spores and are not used for a second generation of spores.

The second mitotic division undergone by the nuclei does take place in the basidium and not necessarily in the sterigmata as Kühner alleges. Division has even been seen to take place in the spore. The phenomenon is not common to all the basidia and it is possible that if the division cycle is well timed and regulated, division would occur in the basidium instead of in the germinating spore, if the development of the sterigmata is for some reason or other retarded. This, of course, leads to the question of whether or not this results in cases of amphithallism. The mating experiments carried out showed that one culture contained two types of nuclei carrying different mating factors. The term heterokaryotic is given as meaning 'the condition of having two or more

genetically different nuclei' (Ainsworth and Bisby, 1961); as such the culture referred to above is heterokaryotic. Although the culture is heterokaryotic it does not produce clamp connections. Buller (1931) states that "if two mycelia which do not react sexually happen to unite, no association takes place between their nuclei." Nuclei in association with each other - conjugate nuclei - divide conjugately and the clamp connection is the mechanism whereby the separation into two daughter cells of sister nuclei arising from the division is ensured. Since the two types of nuclei do not react sexually, i.e. they are not compatible, association into conjugate pairs are not set up, hence the absence of clamp connections on the hyphae of the culture. Marasmius rotula, having produced homokaryotic and heterokaryotic mycelia as regards mating type factors, from the spores of the same fruit body, is therefore amphithallic. A careful investigation on the subject in Marasmius androsaceus and M. rotula would be worth while.

The mechanism which accounts for the movement of the highly contracted hair-pin shaped nuclei into the sterigmata is not seen, and the author is inclined to agree with Ritchie[1] that Marie's centrosomes, Kühner's 'kinoplasmic' differentiation, and Vokes' centrosomes may be artefacts due to fixation.

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[1]Heim (1954)

# SECTION IV

## THE VEGETATIVE HYPHAE.

### 1. Materials and Methods

Organisms.

The cultures of Marasmius androsaceus and M. rotula used for making preparations for the present study were obtained from the stocks kept by the author and used for the live observations dealt with in Section II.

Methods.

Observations were made on preparations obtained by one of three procedures, differing in the manner of handling the fungus prior to fixation and staining.

Method I. Agar film.

No. 1 cover-slips (2 x $\frac{7}{8}$") were coated with thin layer of malt-agar (2% Difco-bacto agar, 2.5% malt extract) in the manner in which the cover-slips were prepared for the live observations of the fungus dealt with in Section II. The coated cover-slips were autoclaved, then inoculated and incubated at 22°C. for three days. At the end of the three days, the mycelium had grown sufficiently to cover a large area of the cover-slip. The cover-slips were immersed in acetic-alcohol (1:3) and left for 10-12 minutes. They were then stored in 70% alcohol. The agar film, which

was now hard and opaque, was carefully removed from the cover-slip using a scalpel, and was floated in a 4% solution of formalin on glass slide which had previously been coated with a thin layer of glycerin-albumen; the slide was placed in an oven at 60°C. and left for 2 hours, at the end of which time the dried slide was ready for further treatment.

Method II. Cellophane technique.

The advantages of the cellophane technique for various purposes were first seen by Flemming and Smith (1944) and later emphasised by Carmichael (1956). Discs of cellophane (dialysis grade) were sandwiched between layers of wet filter paper, placed in a covered Petri-dish and autoclaved. The sterilised discs were spread over the surfaces of malt-agar plates and were inoculated, five pieces of widely spaced inoculum to each plate. The plates were incubated at 22°C. for three days. Cultures grew on the cellophane which was peeled from the agar and cut into five pieces, each with a mycelium on it. The cultures were fixed in acetic-alcohol (1:3) and stored in 70% alcohol. It was found that the mycelium could be removed quite easily from the cellophane in the case of Marasmius androsaceus, and thus it was removed and floated on 4% formalin on glycerin

albumen slides and allowed to dry in an oven for 2 hours at 60°C. In the case of M. rotula the cellophane was floated inoculated side downwards on the formalin and treatment thereafter was similar to that for agar film.

Method III. Agar blocks.

Malt agar plates were inoculated with mycelial blocks and incubated at 22°C. for three days. At the end of this time the medium was cut into around each mycelium and the whole block removed. The cultures were fixed in acetic-alcohol, then stored in 70% alcohol, or fixed over the vapour of a 2% solution of osmic acid and hardened in 2% chromic acid for 1 hour. The blocks were washed in distilled water and fixed on the slide in the manner described above for Methods I and II.

Stains.

The seven stains referred to in Section III were tried and successful results were obtained with Feulgen, counterstained with Fast Green, and to a less extent with haematoxylin.

Feulgen. Slides bearing the mycelium, covered with agar-film, agar block or cellophane, depending on the method employed, were rinsed in cold 1/N HCl for 1 minute (Gurr, 1957) then hydrolysed in 1/N HCl at 60°C. for 6 minutes;

during hydrolysis the agar or cellophane came off, leaving the slide free of anything but mycelium. The slides were placed in the stain and left for 3 hours; on removal from the stain the slides were dried of excess stain and placed in three baths of sulphurous acid for 5 minutes each. They were rinsed in distilled water, taken up through the alcohols from 10% - 95% at 10% intervals, and placed in Fast Green in 95% alcohol for 3 seconds. They were rinsed in 95% alcohol and mounted in Euparal.

Haematoxylin. The slides were prepared in the same manner as those mentioned above, up to the point of hydrolysis. The agar blocks were removed by soaking the slides in water at 60°C. for 6 minutes. Material was mordanted in iron alum solution for 5 hours, rinsed with water and allowed to remain in haematoxylin for 5 hours. The slides were rinsed in distilled water, and differentiated in iron alum under the microscope. They were rinsed in running water for 5 minutes (Gurr, 1957), taken through the alcohols as in the case of the Feulgen stained slides, and mounted in Euparal.

Microscopy and Photomicrography.

Stained preparations were examined with the aid of a Baker Series 4 BW Research Microscope, fitted with a 2 mm fluorite oil immersion objective (N.A. 1.30; x 100) compensating eyepieces x 8 and a Trilux sub-stage condenser. The microscope was fitted on to a Baker Projectolux II fitted with a lamp 48W, 6V, a pre-focus cap, and a reostat; this served as the microscope illuminant.

Photographs were taken with the equipment used in Sections II and III and processed in like manner. Drawings were made free hand at the side of the microscope. The scale of the photographs is given with the captions.

Results

It was found that satisfactory results were obtained with any of the three methods employed. In cultures grown on cellophane the diameter of the hyphae was less than the diameter of the hyphae grown on agar. The hyphae on the whole were narrow compared with those in the fructification, hence the nuclear figures seen were much smaller and more difficult to photograph. Drawings are therefore given along with most of the photographs.

The resting nucleus.

The resting nucleus where seen in osmium-fixed haematoxylin stained hyphae, consists of a deeply stained central nucleolus, surrounded by a clear area which showed no marked affinity for the stain. The nuclei in the terminal cells were often spindle shaped, whereas in the sub-terminal cells they were spherical.

In acetic-alcohol fixed Feulgen and Fast Green stained hyphae, the nucleus consisted of a central 'cavity' which was Feulgen-negative and stained only weakly with Fast Green, surrounded by a halo of Feulgen-positive granules, connected to each other in some preparations by a faintly stained Feulgen-positive thread. In the sub-terminal cells the nuclei were spherical, but in the terminal cell they were spindle-shaped (Plate LVI, fig.1), and often elongated. Where elongated, the nucleus was seen as consisting of two fine threads connecting a series of Feulgen-positive granules spread out in the cytoplasm along an area of the hypha (Plate LVI, figs. 2a and 2b). The connecting threads were Feulgen positive; the nucleolus was not stained, even by Fast Green.

Division of the nucleus.

In haematoxylin preparations it was impossible to find nuclei in the region of the backwards bending clamp. This is on account of the fact that the nucleoli were the landmarks by which haematoxylin stained nuclei were recognised, and the nucleoli have disappeared during division. In the vicinity of developing clamps, i.e. before backwards bending of the tip takes place, the nucleolus was the prominent feature of the nucleus and appeared to fill the hypha.

In Feulgen stained preparations figures of the chromatin component of the nucleus, similar to those seen in the ultimate clamp were obtained, and are depicted in Plates LVII - LXV.

## DISCUSSION.

Stained preparations show the nucleus to consist of a central Feulgen-negative body, the nucleolus, which is stained intensely by haematoxylin, surrounded by a halo of Feulgen-positive granules often seen connected together by a faintly Feulgen-positive thread. If the structure of the nucleus as seen under phase contrast in live observations is recalled, (Page 37), it will be remembered

that a central spherical-oval grey body (black under good phase) was seen surrounded by a clear halo; on a comparison of the stained preparations with the structures seen in live observations it is seen that the central grey body is the nucleolus, and the surrounding clear halo the matrix containing the Feulgen-positive chromatin component of the nucleus.

It will also be remembered from live observations that the clear halo was seen to change its shape continually, often elongating, sometimes to lengths as great as 25 μ (Plate VIII, fig. 4); in stained preparations the nucleus in its elongated state is seen to consist of Feulgen-positive granules connected by faintly stained threads (Plate LVI, figs. 2 & 3). This phenomenon was reported by Macdonald (1949 a) who mentioned Feulgen-positive granules in the cytoplasm of the terminal cells in the hyphae of M. androsaceus. The phenomenon is common in both M. androsaceus and M. rotula, but has been observed in both species only in the terminal cells of the hyphae. It will be remembered that prior to nuclear division the nuclei in the hyphae were seen to elongate and contract; the elongated nuclei seen in stained preparations were always in the part of a hypha in which clamp formation was imminent, i.e. they were never seen

near to clamps that had just completed their formation. This elongation, seen so regularly in live observations before nuclear division and encountered in stained preparations only in nuclei 'likely to divide', i.e. positioned approximately in areas of the hyphae where one would find nuclei in which division was imminent, is probably a pre-division change which the nucleus undergoes, or it may even be the initial stage of division.

The division of the nucleus in the vegetative hypha resembles that of the nucleus of the ultimate clamp. As was pointed out in the discussion following Section III (Page 98), the division of the nucleus in the ultimate clamp does not follow the scheme of classical mitosis faithfully; one of the great differences between the former (division in the ultimate clamp) and the latter (classical mitosis) is the beaded appearance of the chromatin strand of the nucleus in the ultimate clamp, unknown in mitosis but common in meiosis prophase as described in all organisms. 'Mitosis in pre-meiotic cells has not been extensively studied, but in Maize it appears that the prophase chromosomes are more extended than in ordinary somatic cells' (Rhoades, 1961). The divisions in the ultimate clamp are pre-meiotic somatic divisions in

the fructification and one may be tempted to assume that the case which occurs in maize as mentioned above is applicable here. This idea must be ruled out when it is realised that the pre-meiotic somatic divisions are the same as those of the vegetative hyphae and therefore are normal for the organism.

The scheme of mitosis taken as the norm requires: (a) the duplication of chromosome substance; (b) the 'condensation' of the chromosomes, accompanied by the disappearance of the nucleolus; (c) the movement of sister chromosomes to opposite poles (Mazia, 1961). Apart from mitotic division nuclei may undergo endomitosis where the chromosomes undergo a normal cycle of duplication condensation and reassembly into an interphase nucleus without movement of the sister chromosomes to opposite poles, although they do separate visibly. Amitosis, another variant which is widespread and successful in some ciliates and suctorian protozoa, takes place by an elongation of the nucleus and constriction at a point corresponding with the plane of division which is later seen, and division into halves which separate and move apart. (Mazia, 1961). The division seen in the ultimate clamp and in the vegetative hyphae cannot be said to be endomitotic, as a separation of 'sister chromosomes' to

different poles does occur. The elaborate figures through which the nucleus goes and the disappearance of the nucleolus (as seen in live observations and haematoxylin stained preparations) rule out a simple amitotic elongation and constriction of the nucleus. The elongation of the nucleus followed by constriction and division into two daughter cells seen by Robinow (1957 a & b), Bakerspigel (1958, 1959 a & b) and others, involves the nucleolus, which is not the case in Marasmius androsaceus and M. rotula, and this leads one to assume that there are widely differing mechanisms of division in the fungi. This is borne out by work recently published by Robinow (1963) on Basidiobolus ranarum, in which the nucleus "divides by an ordinary form of mitosis," during which the nucleolus becomes rearranged to form the mitotic spindle. This, writes Robinow "is in reality the most remarkable and least suspected" property of the fungus as "there are indications that vegetative nuclei divide, as a rule, not by an ordinary but by a modified form of mitosis which involves neither an obvious spindle nor a metaphase plate." Does the division seen in Marasmius androsaceus and M. rotula then satisfy the scheme of mitosis, the criterion of which is the separation of sister chromosomes at anaphase?

It will be remembered from the hypothesis depicted in Plate LII that the double strand of chromatin undergoes a twist into a figure of eight, thereby forming two rings which on opening out linearly give rise to two chromatin rods, each of which contains a full chromosome complement. These rods undergo an anaphase separation in which half of each goes to each pole resulting in a four chromosome nucleus at each pole. Such a division, though not faithful to the classical scheme of mitosis, does satisfy the criteria of mitosis, and the division in the ultimate clamp is as that in the vegetative hyphae, a mitotic division.

## SUMMARY.

i) Nuclear division in the fungi, particularly in the basidiomycetes, is briefly reviewed, and three questions arising out of this have been posed: (a) How do nuclei divide as seen in live observations? (b) How do nuclei of vegetative hyphae divide as seen from fixed and stained preparations? (c) How closely do meiotic divisions I and II in the basidium resemble meiosis in higher plants?

ii) The classification and affinities of Marasmius androsaceus and M. rotula are briefly discussed, and work carried out on the organisms has been described.

iii) Live observations using phase contrast microscopy were carried out on the organisms, and the mycelia in culture were described. From live observations it was found that (a) growth takes place at the tip of the hypha, and is uninterrupted during the production of clamp connection on the hypha, (b) there is an apparent correlation between the tip of the hypha and the fore nucleus which is usually

approximately 120 $\mu$ from the tip, (c) during nuclear division the central grey body of the nucleus disappears, and the clear area only is seen, which eventually disappears too, (d) the filiform mitochondria change their shape constantly, and form a spherical body behind the septum across the main hypha prior to the fusion of clamp with the main hypha, after which they revert to their filiform shape, and (e) the granules within the cytoplasm give positive reaction to the Nadi reagent and Tetrazolium chloride, suggesting they might be mitochondria. These findings are discussed in the light of findings of other workers on other basidiomycetes.

iv) The cytology of the basidia has been studied, using the squash technique after staining with Aceto-orcein or Feulgen and Fast Green. The anatomy of the fructifications is described; the findings were in the main (a) there are two types of elements in the hymenium, which agrees with Kühner's findings; (b) the cells of the fructification are binucleate, this condition being maintained by the production of clamps at the time of somatic nuclear division;

(c) the chromosomes of the nucleus appear to be arranged linearly on a strand, which divides as a unit; (d) the fusion nucleus in the basidium divides meiotically, but the bivalents become joined one to the other during diakinensis, and afterwards behave as a unit; (e) division II of meiosis resembles the division in the ultimate clamp; (f) some or all of the nuclei resulting from the meiotic division may undergo an additional 'mitotic' division giving rise to from 4 to 8 nuclei within the basidium which may lead to amphithallism; (g) the species are both tetrapolar. These findings are discussed and compared with findings of other authors in other basidiomycetes, and an explanation of the 'two metaphase chromosomes' which has puzzled many workers, is offered.

(v) The division of the nuclei in the vegetative hyphae is studied from fixed and stained preparations, using Feulgen and Fast Green. The division is found to be the same as that of the nuclei in the 'ultimate clamp' and the division II of meiosis: this, in brief, is:- 1) The beaded double strand of four chromosomes (doubled during replication)

splits, and the nucleolus disappears. 2) The sister strands open apart from each other, remaining joined at one or both ends. 3) The ring so formed expands and undergoes a twist into a figure of eight. 4) The loops of the figure bend over on each other to form a double ring of beaded chromatin. 5) A break appears in the rings and the arms open out linearly. 6) Anaphase separation takes place, during which bridges of chromatin are seen. 7) At telophase four chromosomes are seen which form a circle before the nucleus is reorganised.

A case for the justification of calling the division a mitotic division though different from classical mitosis is presented.

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## APPENDIX I.

One may say that all truly collariate species have insititious stipe and in the young stages hymeniform epicutis broom cells.

A. Lamellae without collarium.

B. Stipe with strigose or fibrillose base, neither insititious nor practically absent.

C. Epicuticular layer of the pileus formed by broom cells which are arranged hymeniformly; hyphae amyloid, rarely inamyloid.....SICCI

C. Epicuticular layer of the pileus formed by smooth (or sometimes slightly nodose) cells which are usually arranged hymeniformly, more rarely ascendant to erect and somewhat scattered and interrupted by stretches of exposed hypodermium; hyphae amyloid or inamyloid.

D. Hyphae completely inamyloid.....ALLIACEI

D. Hyphae weakly and slowly to strongly and immediately amyloid.....GLOBULARES

B. Stipe insititious or practically absent, rarely finely fibrillose and then connected with small white rhizomorphs.

E. Epicutis of pileus formed by irregular broom cells, not hymeniform; either centrally stipitate and predominantly boreal (tropical species with distinctly amyloid trama and without pleurocystidia), or laterally to strongly eccentrically stipitate.

F. Stipe central; cystidia none....ANDROSACEI

F. Stipe not central, or absent; cystidia conspicuous, fusoid; trama of pileus amyloid.....FUSICYSTIDES.

E. Epicutis of the pileus hymeniform.

G. Hyphae inamyloid.

H. Habit pleurotoid; stipe strongly reduced or none.....APUS

H. Habit mycenoid.

I. Pleurocystidia distinct, fusoid to ampullaceous; pileus usually white (at least white in marginal zone when young); stipe white (or at least white in apical zone and pale colored below), not shining; epicuticular elements usually smooth, globose, rarely appendiculate and then appendages not setulose, cells not typical broom cells, not dendrophysoid, not pigmented.... .....EPIPHYLLI

I. Not combining these characters.. .....HYGROMETRICI

G. Hyphae amyloid.

J. Habit pleurotoid.....NEOSESSILES

J. Habit mycenoid or collybioid (see Epiphylli).

A. Lamellae with collarium.

K. Stipe insititious, central; epicuticular bodies "en brosse," or dendrophysoid; habit mycenoid; stipes sometimes ramified.....ROTULAE

K. Not combining the characters enumerated above (see "B").

Singer (1958)

# A STUDY ON

# MARASMIUS ANDROSACEUS FR.

# and

# MARASMIUS ROTULA (Scop.) FR.

by

Edgar Julian Duncan, B.Sc.

Volume II.

A thesis submitted to the University of St. Andrews for the degree of Doctor of Philosophy.

Department of Botany,
University of St. Andrews.

July, 1963.

# PLATE I.

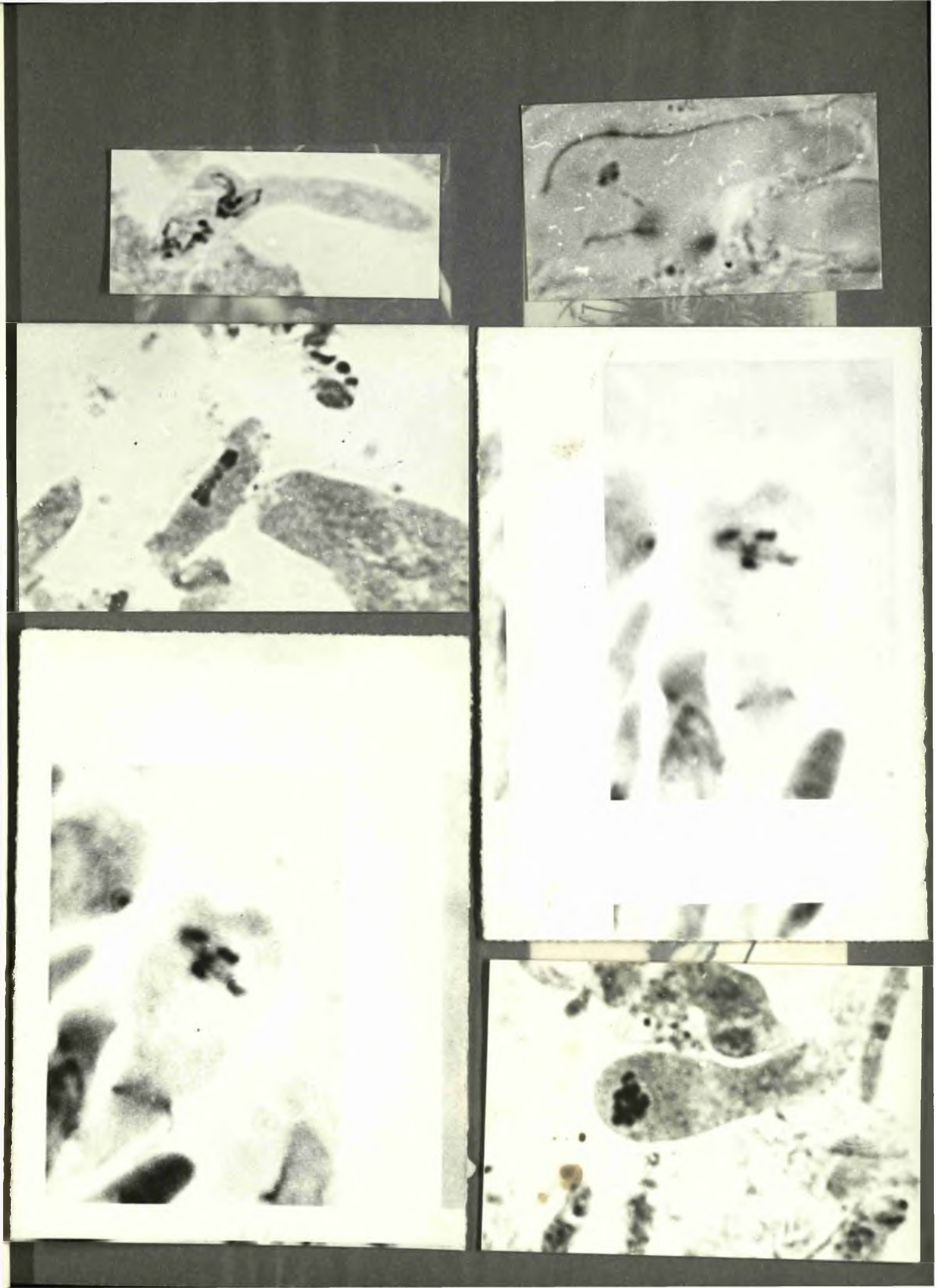
Fructifications of *Marasmius androsaceus* in nature.

Fig. 1. On fallen pine needles among *Pleurozium schreberi* x 3/2.

Fig. 2. As above x ½.

Fig. 3. On pine needles.

Fig. 4. On a shoot of *Calluna vulgaris* x 1.




1


2


3


4

PLATE II.

Graph showing the rates of growth of Petri-dish cultures, *Marasmius androsaceus* and *M. rotula* on malt agar, maintained at 22°C. plotted as diameter of mycelium in mm. against time in days.

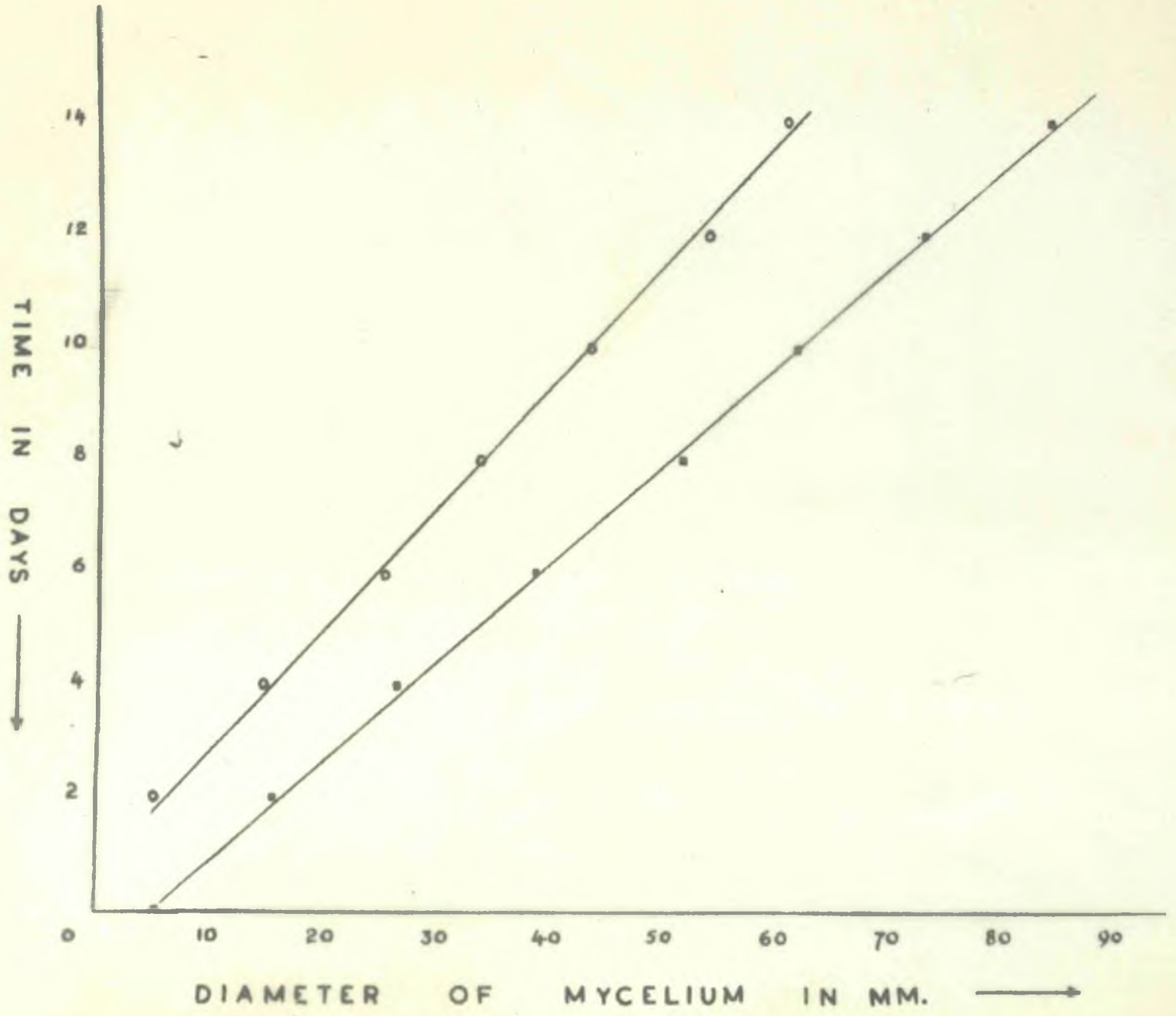

TIME IN DAYS
14
12
10
8
6
4
2
0
10
20
30
40
50
60
70
80
90
DIAMETER OF MYCELIUM IN MM.

PLATE II.

Graph showing the rates of growth of Petri-dish cultures, *Marasmius androsaceus* and *M. rotula* on malt agar, maintained at 22°C. plotted as diameter of mycelium in m/m. against time in days.

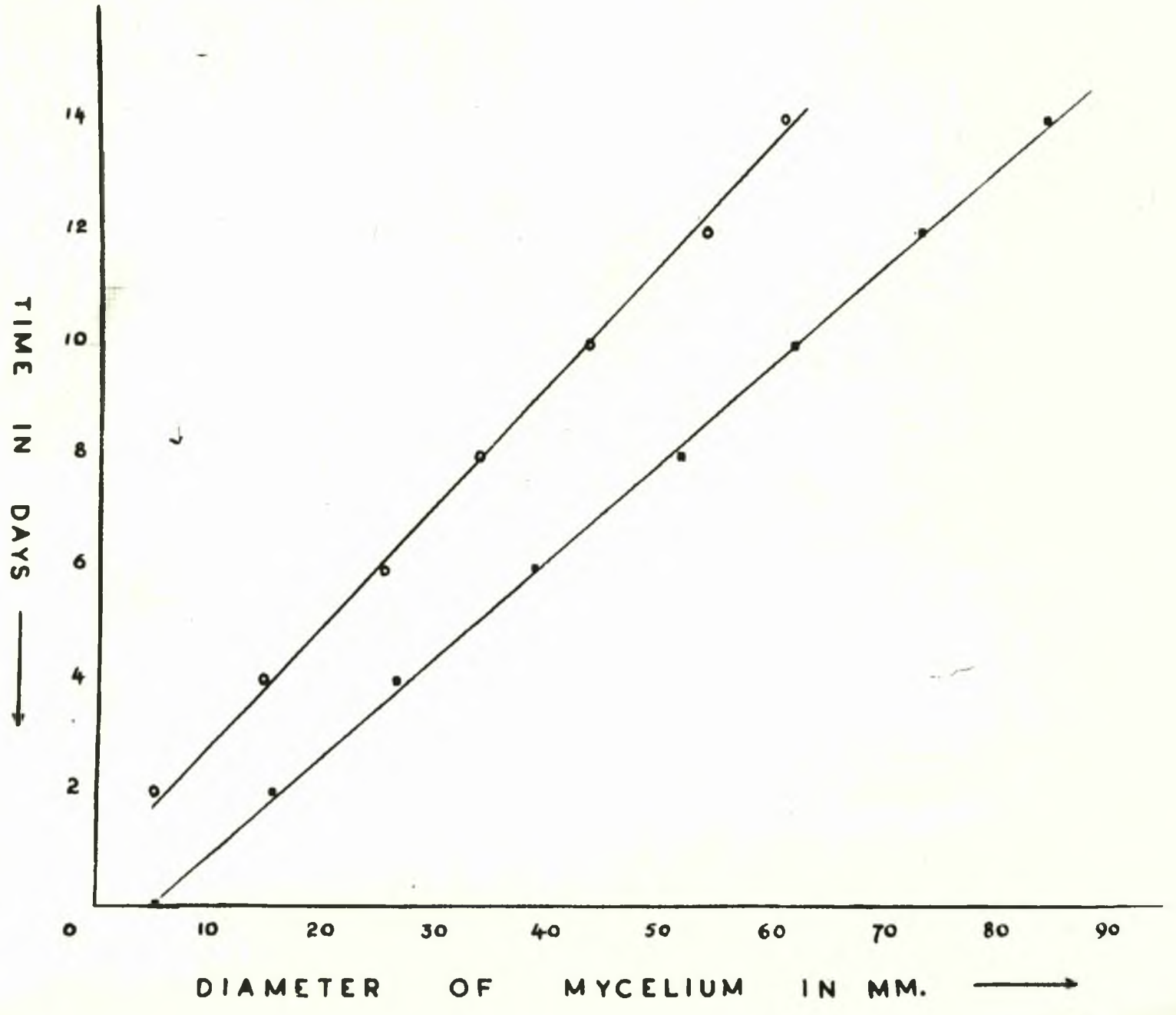
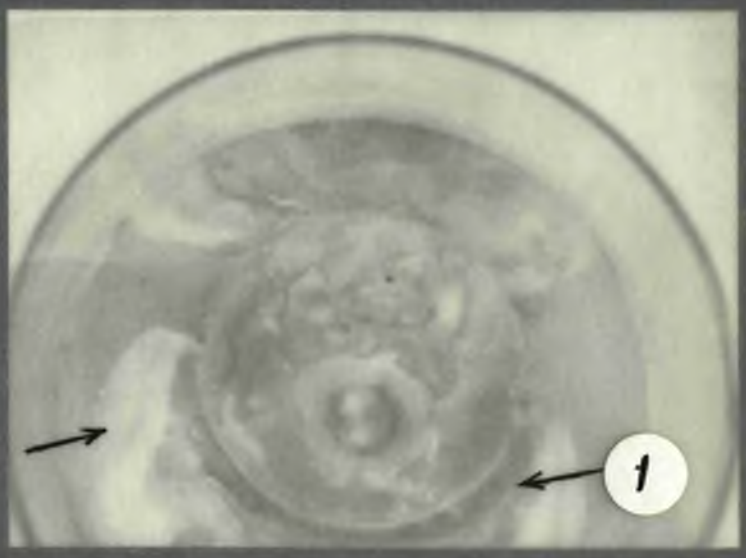
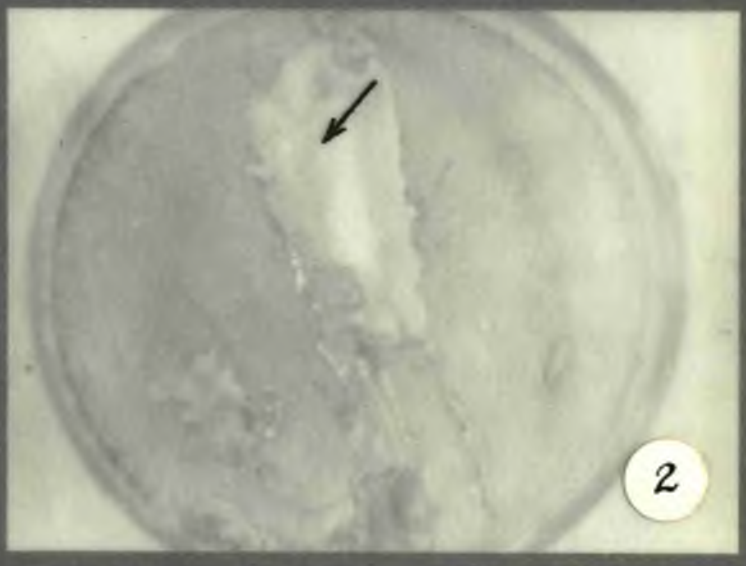

TIME IN DAYS
14
12
10
8
6
4
2
0
10
20
30
40
50
60
70
80
90
DIAMETER OF MYCELIUM IN MM.

PLATE III.

Figs. 1 and 2. Petri dish cultures of *Marasmius rotula* showing the wrinkled skin-like appearance of the fungus on the agar surface. Arrows mark areas which have been overgrown with white tufts of hyphae.


1


2

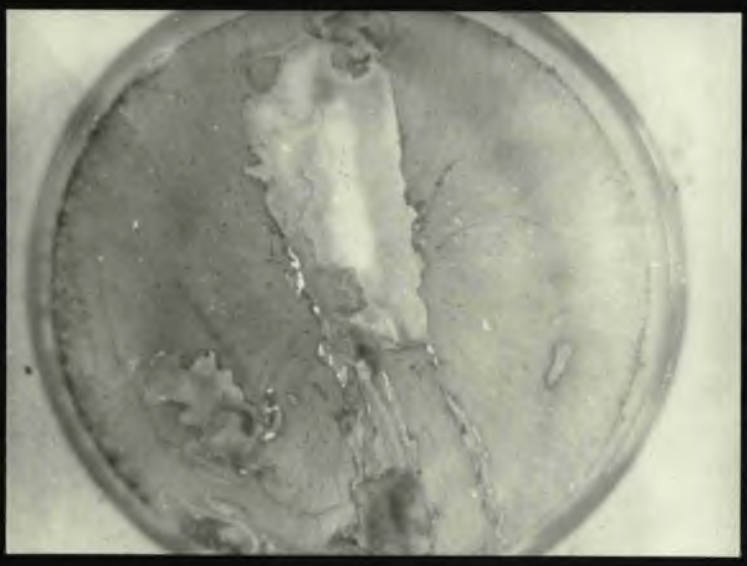


PLATE IV.

Structures seen on cultures of *Marasmius rotula*, resembling the rhizomorphs of *M. androsaceus*.

Fig. 1. On a petri dish culture of *M. rotula* x 1.

Fig. 2. Central portion of one of the structures showing long unbranched hyphae twisted as the strands of a thread. (Phase contrast).

Fig. 3. Claviform cells at the tips of the hyphae at the distal ends of the structures (Phase contrast).

Fig. 4. Basal or proximal portion of one of the structures showing loosely twisted hyphae, covered in parts by plate-like cells. (Phase contrast).


1


100μ
2


5μ
3


100μ
4

PLATE IV.

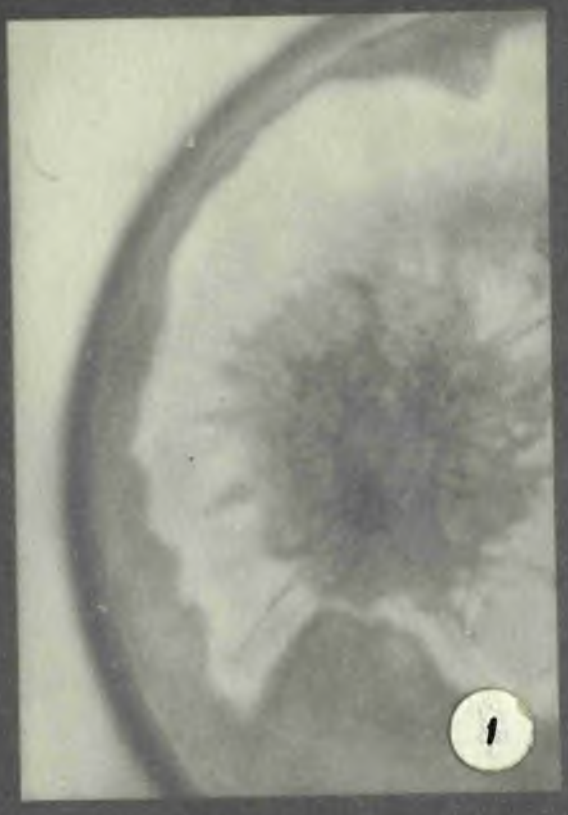
Structures seen on cultures of Marasmius rotula, resembling the rhizomorphs of M. androsaceus.

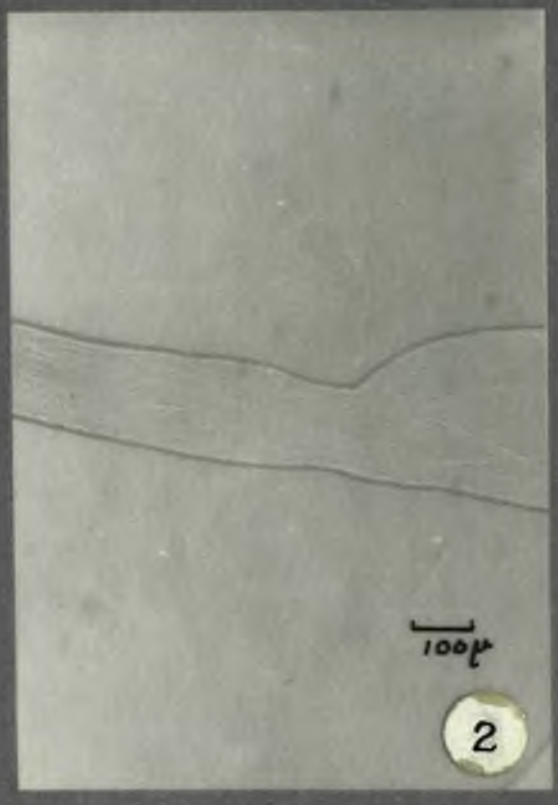
Fig. 1. On a petri dish culture of M. rotula x 1.

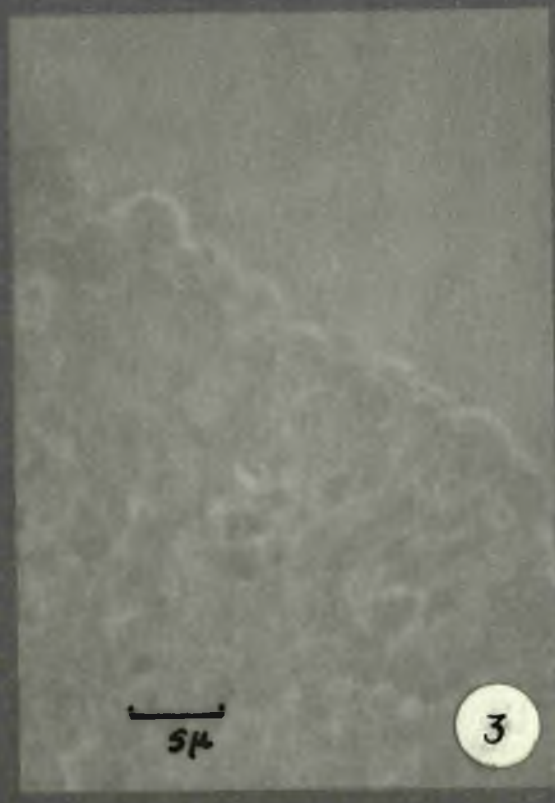
Fig. 2. Central portion of one of the structures showing long unbranched hyphae twisted as the strands of a thread. (Phase contrast).

Fig. 3. Claviform cells at the tips of the hyphae at the distal ends of the structures (Phase contrast).

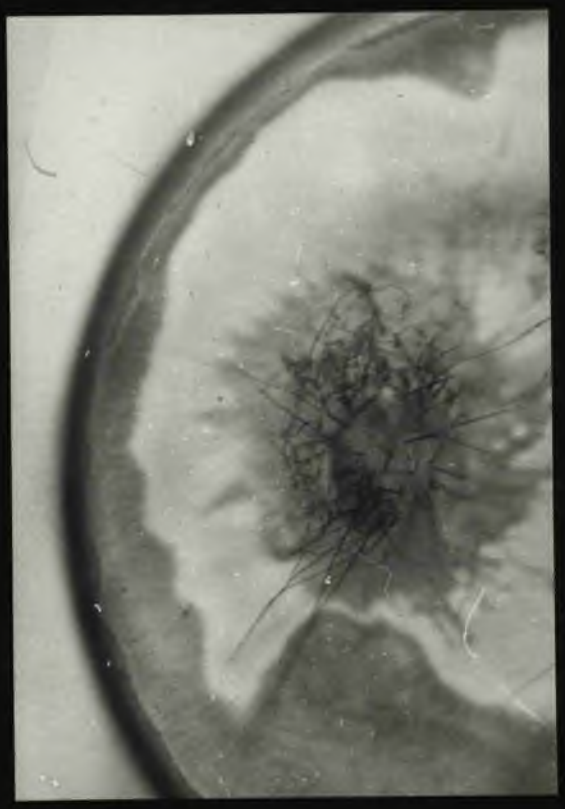
Fig. 4. Basal or proximal portion of one of the structures showing loosely twisted hyphae, covered in parts by plate-like cells. (Phase contrast).

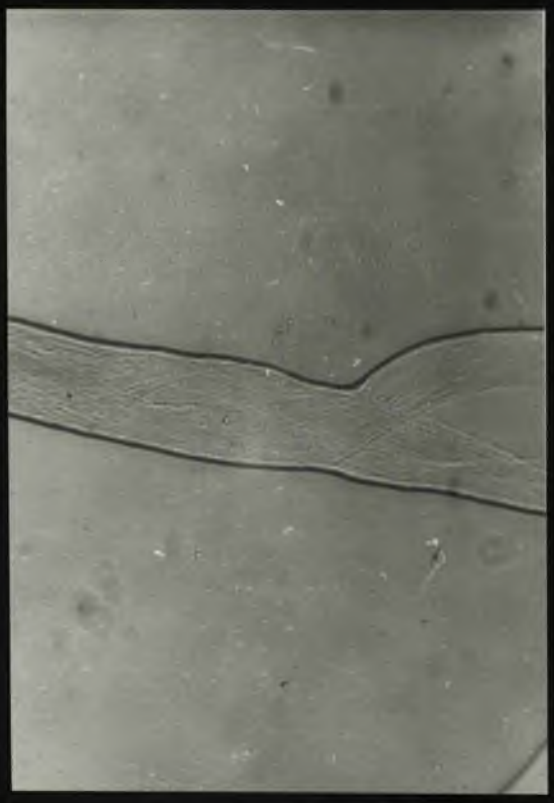

1

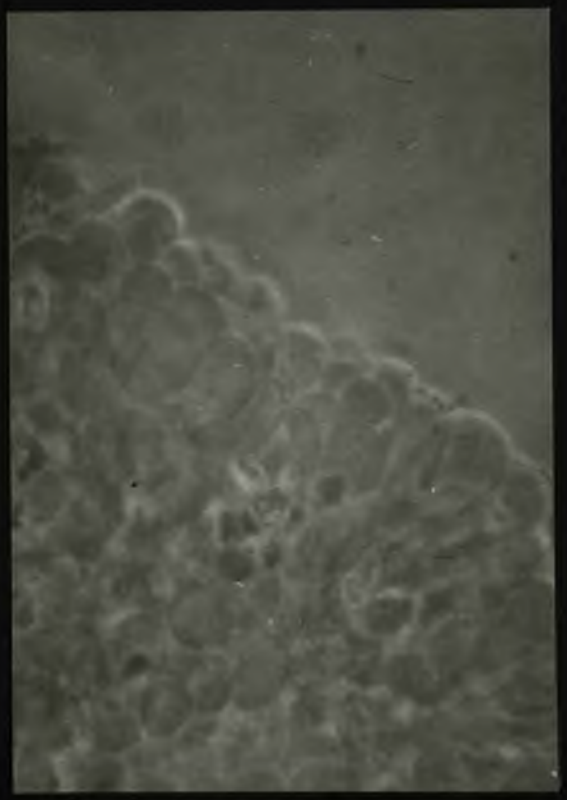

100μ
2


5μ
3


100μ
4







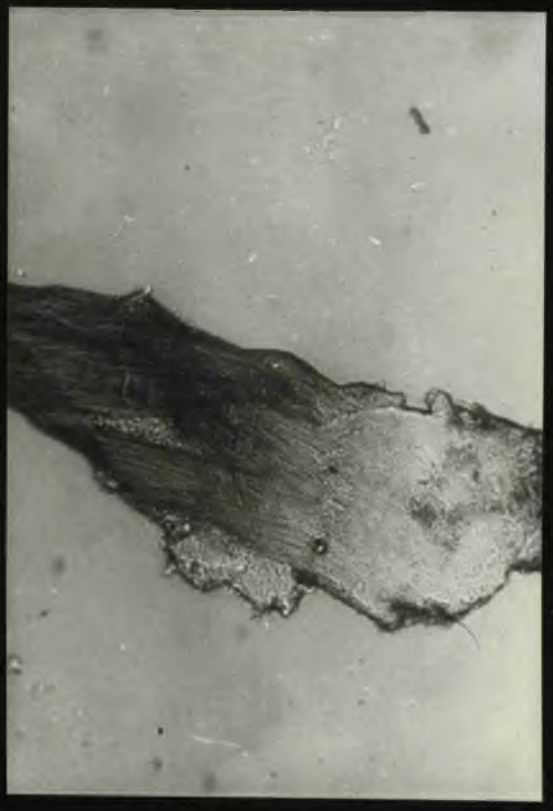
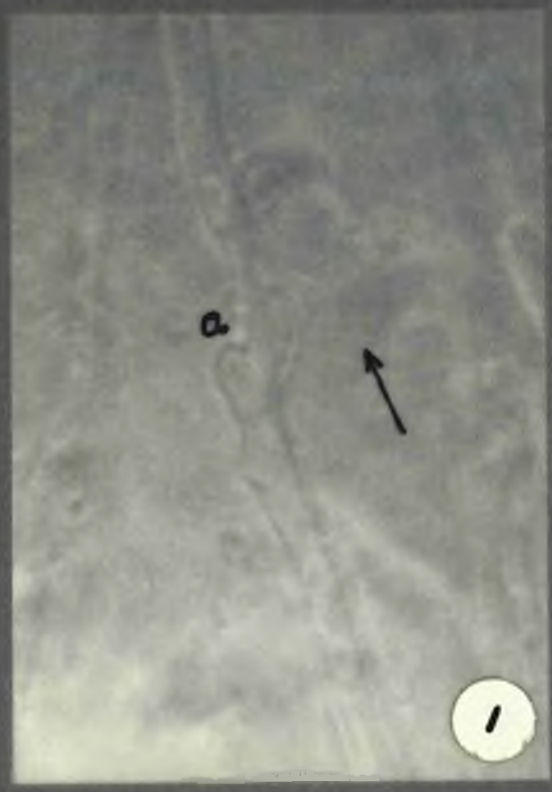


PLATE V.

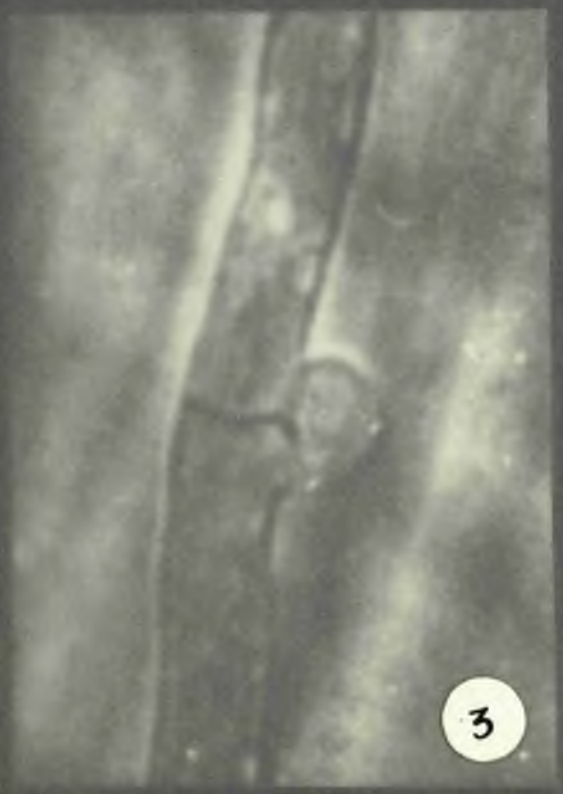
Fig.1. Living hyphae of M. androsaceus showing sub-terminal branch through which growth of hypha was resumed after arrestation of growth at tip (a); arrows indicate direction of growth (Phase contrast).

Fig.2. One of the various shapes taken on by filiform mitochondria (arrow) in M. androsaceus. (Phase contrast).

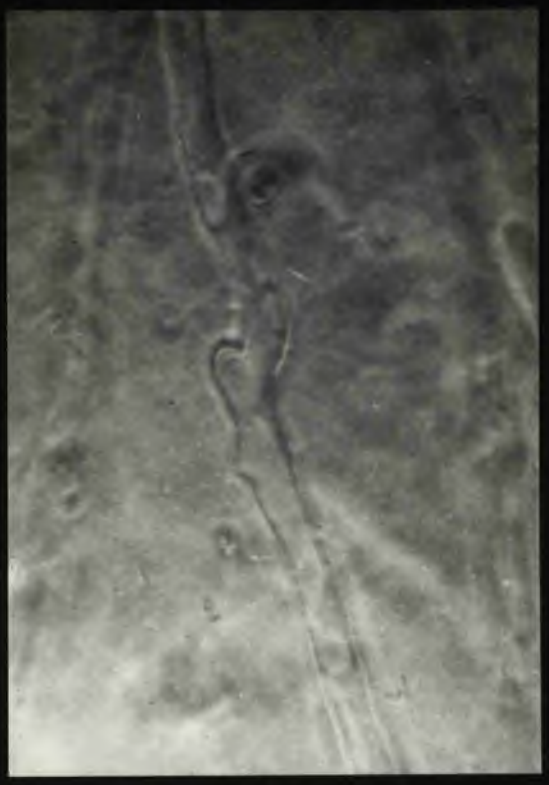
Figs. 3 & 4. Mitochondria reverting to thread like form from dumb-bell shaped body formed during fusion of clamp with main hypha in M. androsaceus. (Phase contrast).

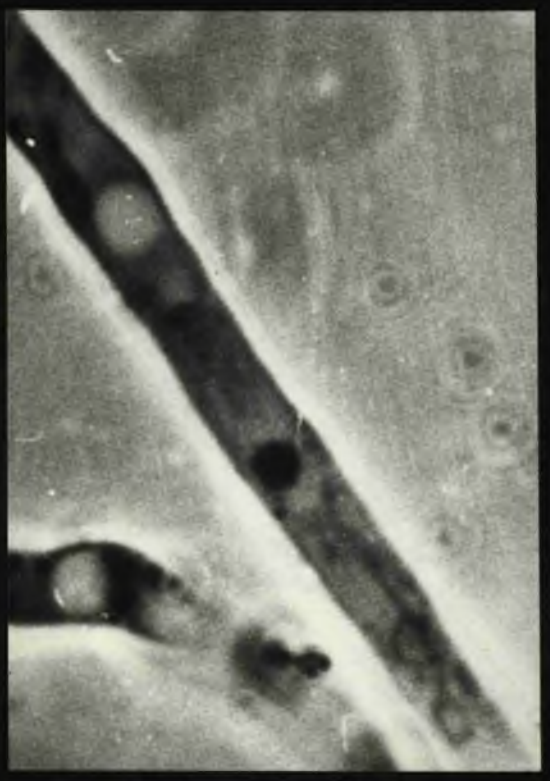

a
1

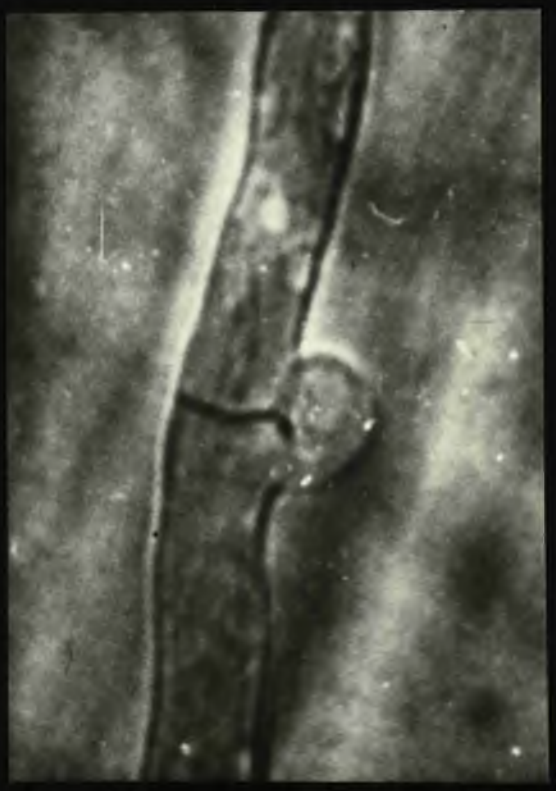

2


3


5μ
4







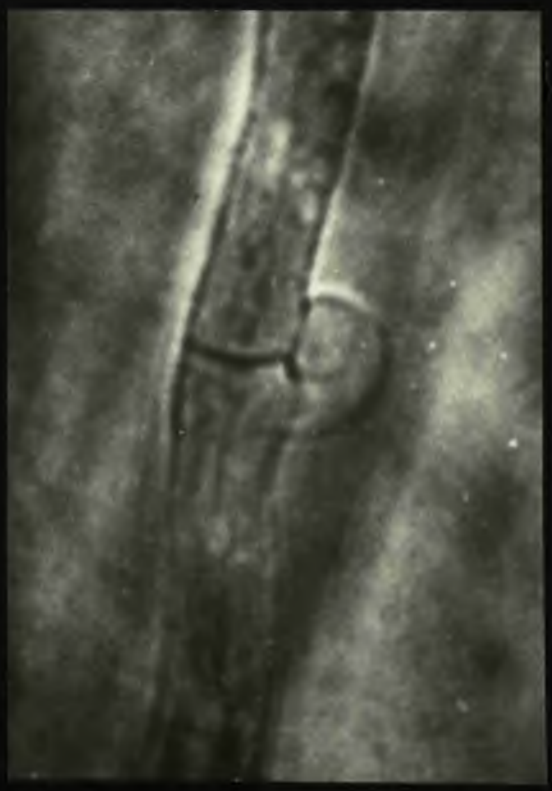


PLATE VI.

Changes in shape of spherical body formed by mitochondria at time of fusion of clamp with main hypha.

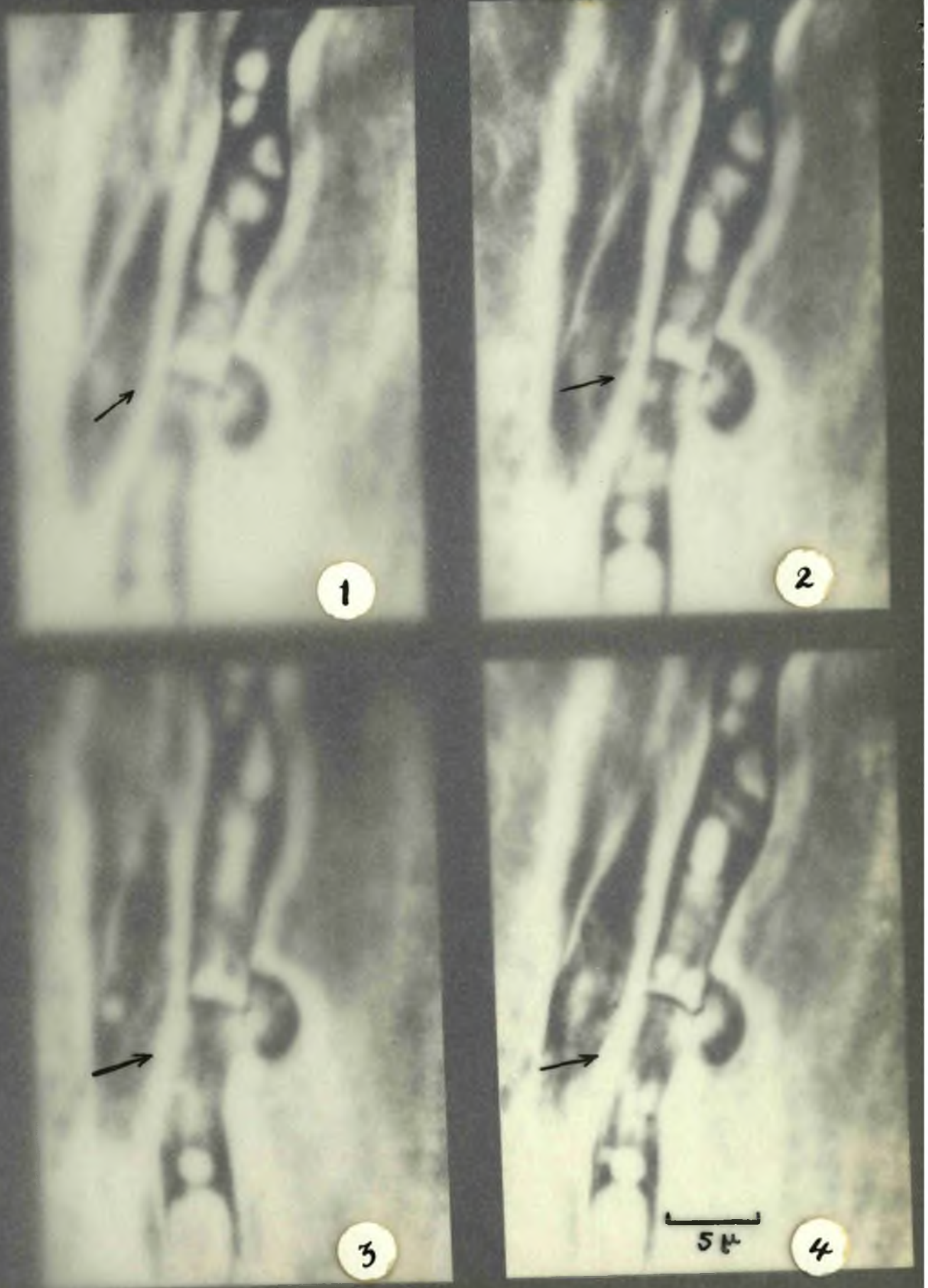
Fig. 1. Spherical body (arrow) seen approaching septum.

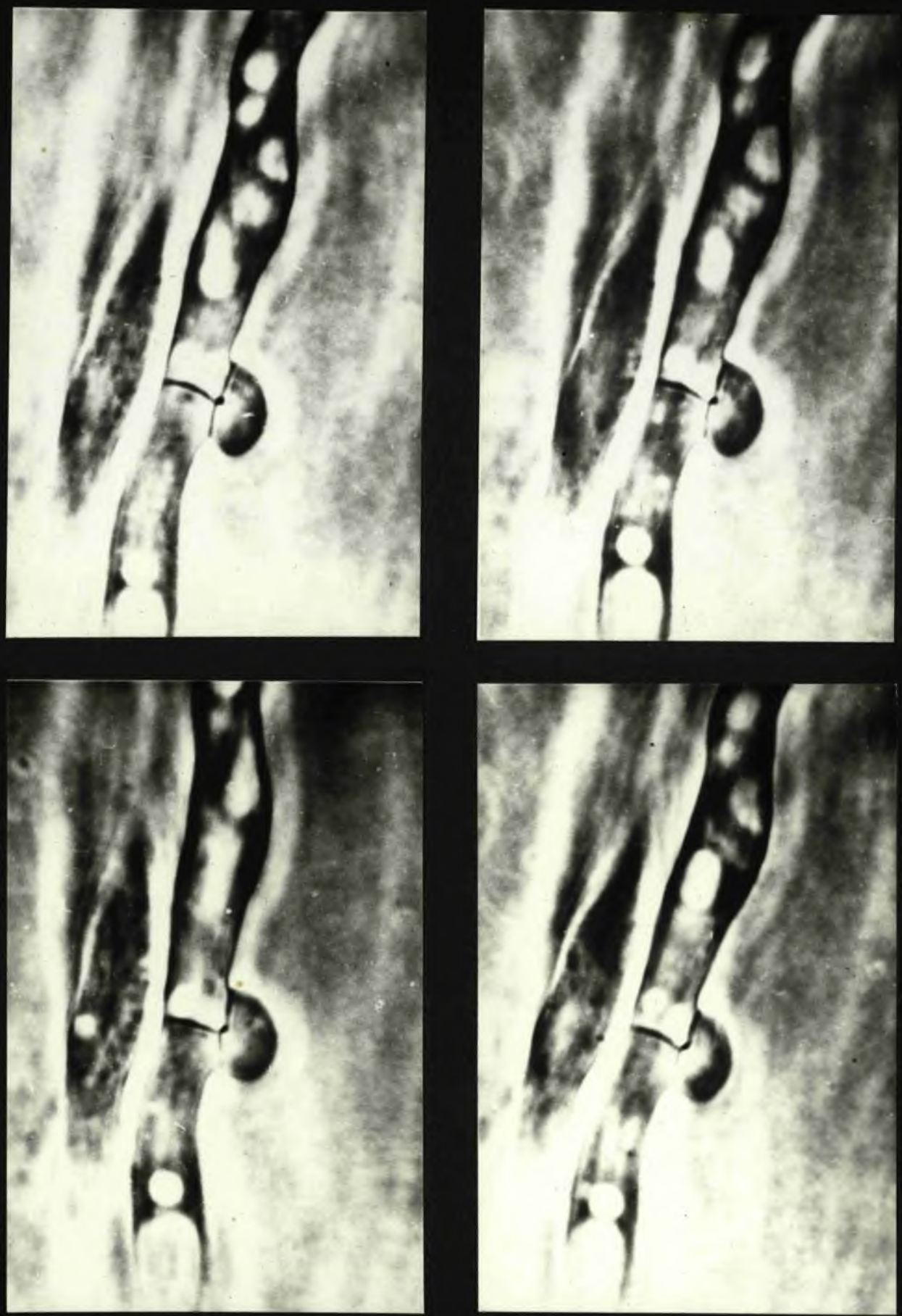
Fig. 2. Spherical body (arrow) at septum.

Fig. 3. Mitochondria (arrow) revert from spherical body to filiform shape (arrow); fusion of clamp and main hypha begun.

Fig. 4. Filiform mitochondria move away from area of fusion; fusion almost complete.

(Phase contrast).


1
2
3
4
5μ



# PLATE VII.

Camera lucida drawings showing the series of changes undergone by the mitochondria in the distal end of the subterminal cell, prior to fusion of clamp with the main hypha.

(a) Three small spherical bodies, one having a tail, are seen (2.25 p.m.)

(b) The three bodies fuse, tail still present (2.27 p.m.)

(c) Tail disappears; body divided into two smaller units (2.28 p.m.)

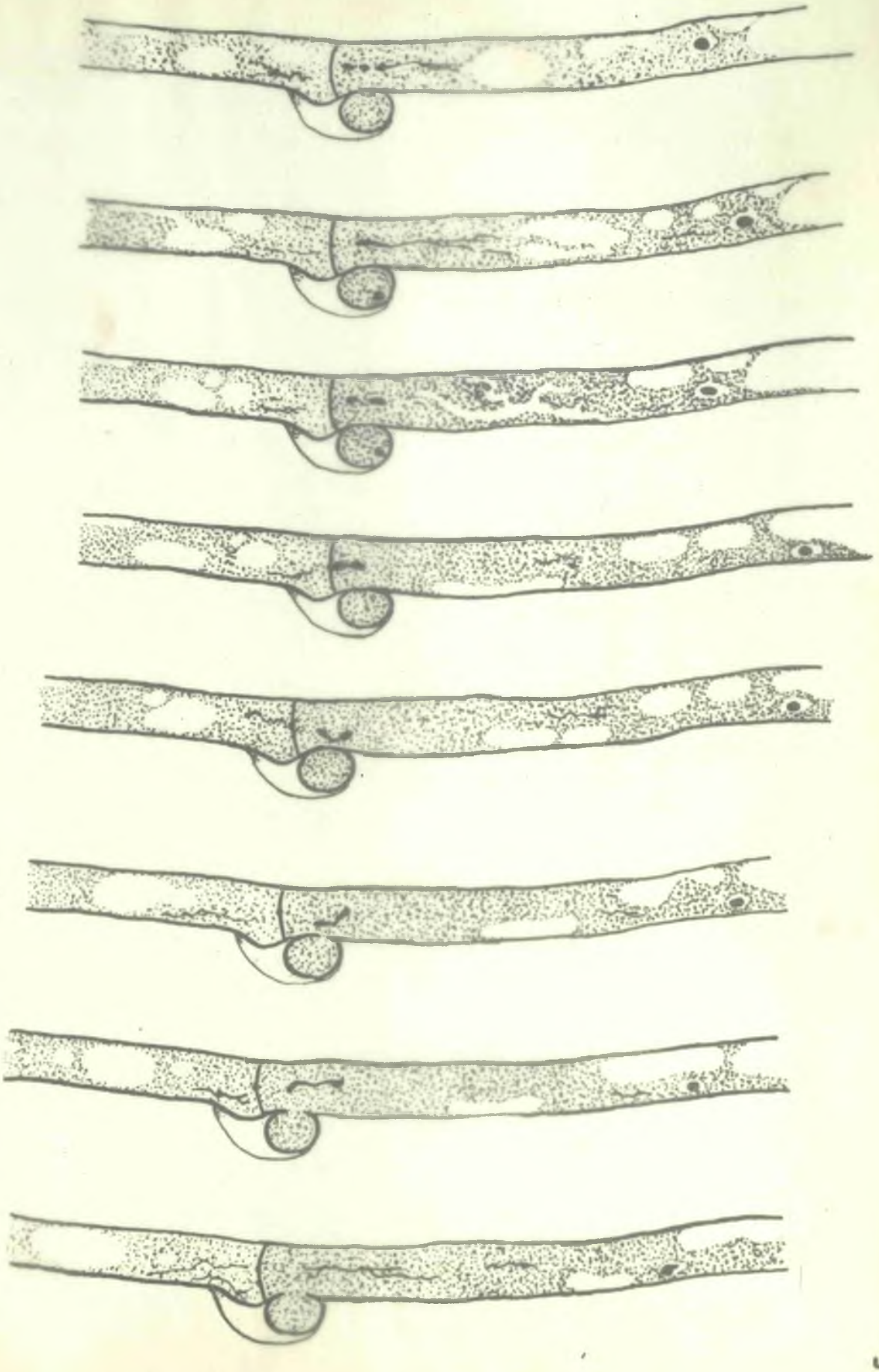
(d) Two bodies join to form a dumb-bell shaped structure, which approaches the septum (2.29 p.m.)

(e) Dumb-bell shaped structure moves to the point of fusion of clamp with hypha (2.30 p.m.)

(f) Fusion of clamp with hypha begins; dumb-bell shaped body moves away (2.33 p.m.)

(g) Dumb-bell shaped body becomes filiform and moves further from the point of fusion (2.37 p.m.)

(h) Mitochondria revert to typical filiform shape (2.40 p.m.)


5μ

## PLATE VII.

Camera lucida drawings showing the series of changes undergone by the mitochondria in the distal end of the subterminal cell, prior to fusion of clamp with the main hypha.

(a) Three small spherical bodies, one having a tail, are seen (2.25 p.m.)

(b) The three bodies fuse, tail still present (2.27 p.m.)

(c) Tail disappears; body divided into two smaller units (2.28 p.m.)

(d) Two bodies join to form a dumb-bell shaped structure, which approaches the septum (2.29 p.m.)

(e) Dumb-bell shaped structure moves to the point of fusion of clamp with hypha (2.30 p.m.)

(f) Fusion of clamp with hypha begins; dumb-bell shaped body moves away (2.33 p.m.)

(g) Dumb-bell shaped body becomes filiform and moves further from the point of fusion (2.37 p.m.)

(h) Mitochondria revert to typical filiform shape (2.40 p.m.)

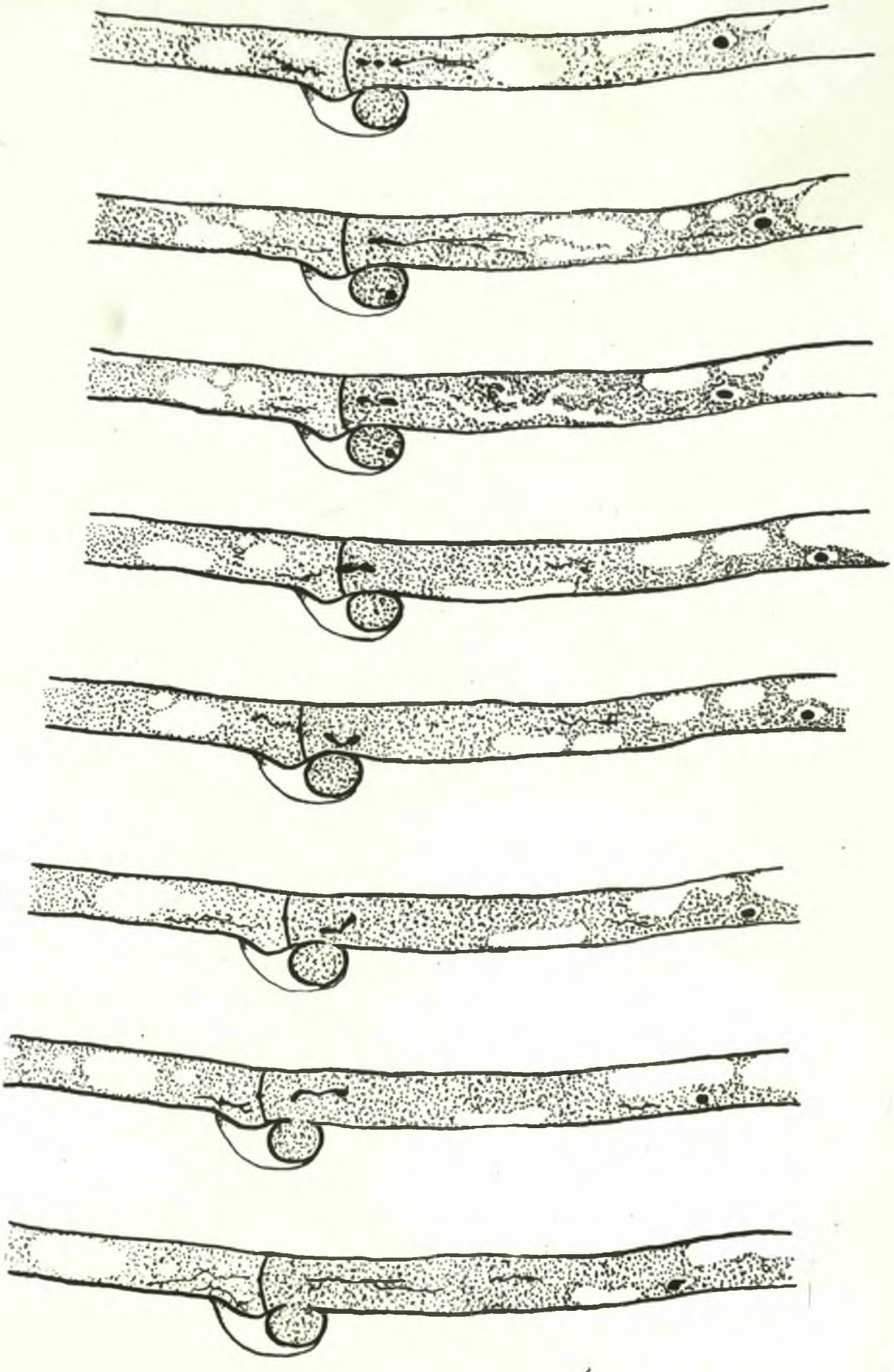

5μ

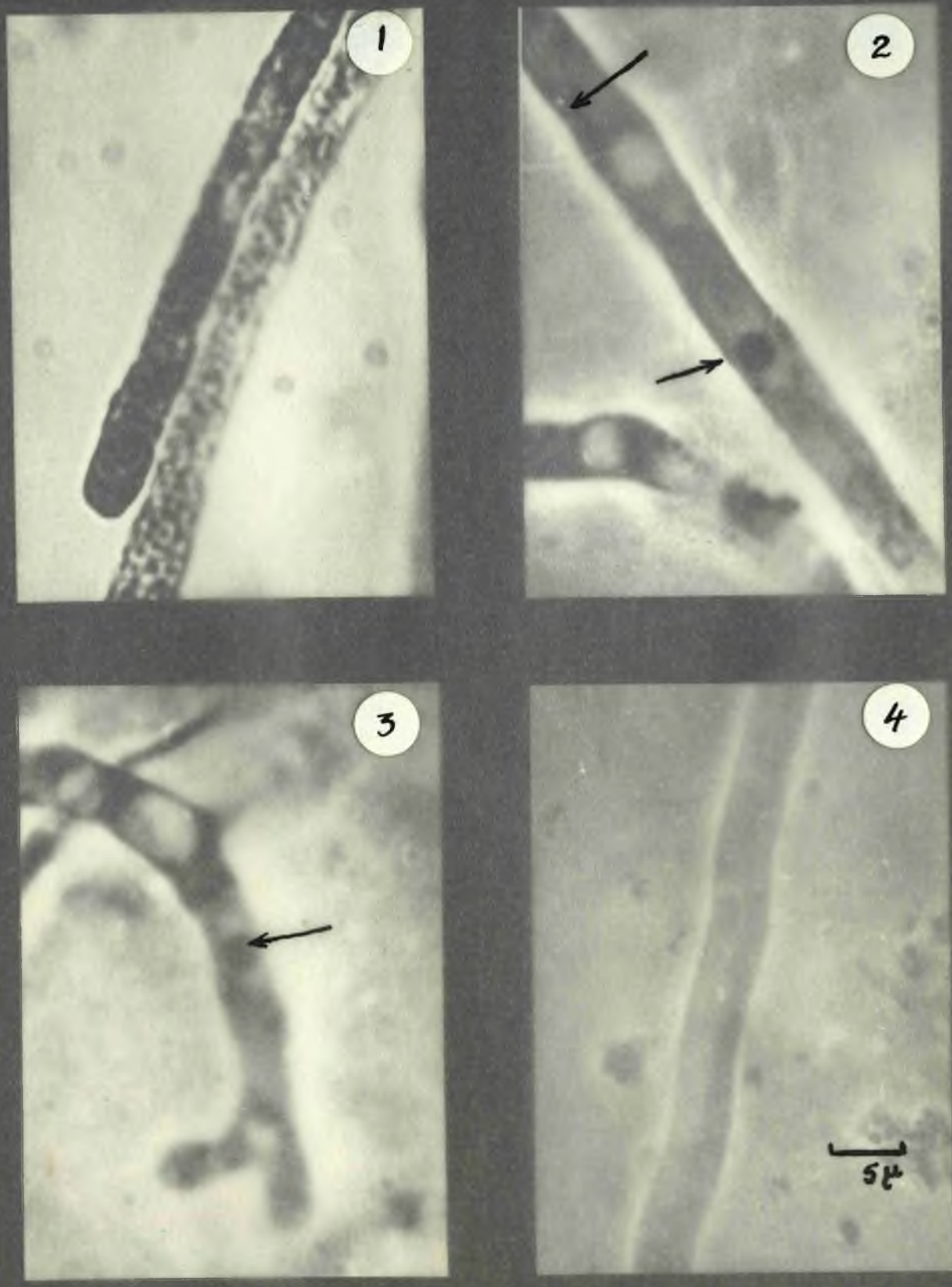
PLATE VIII.

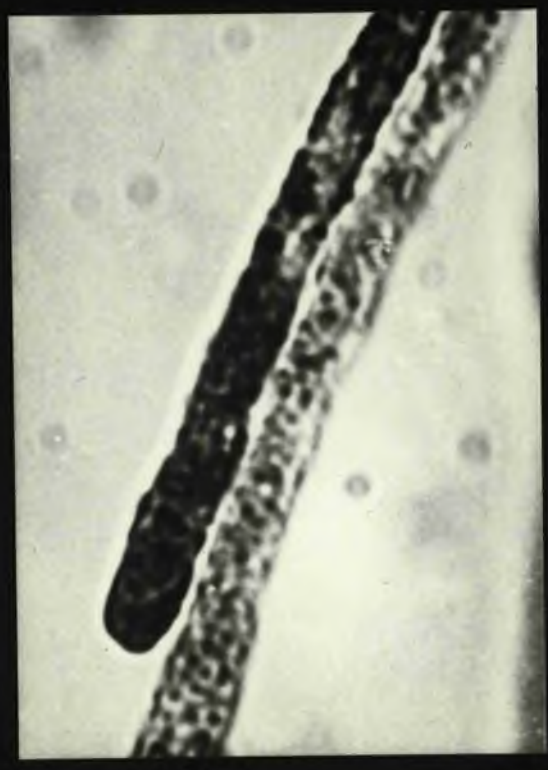
Fig. 1. Terminal cells of hyphae of M. androsaceus showing granules surrounded by bluish-purple halo of indophenol blue. (Bright light).

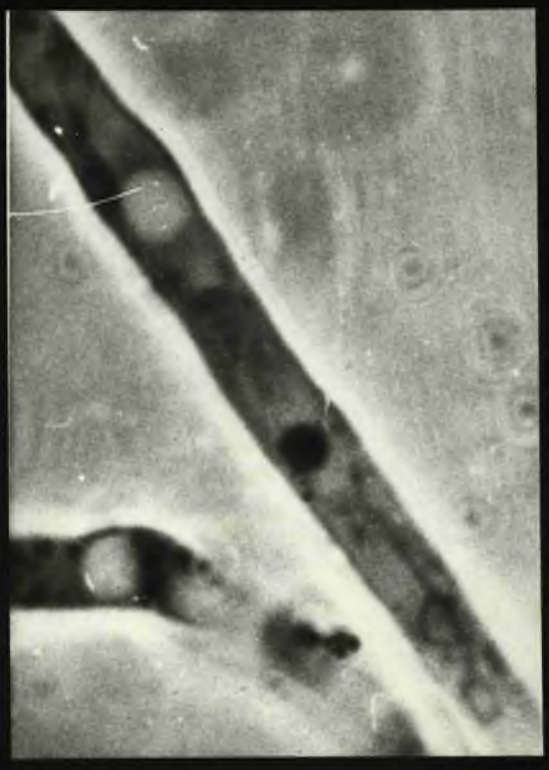
Fig. 2. Hypha of dikaryophase mycelium of M. androsaceus showing nuclei (arrows) consisting of dark central bodies surrounded by optically clear areas (Phase contrast).

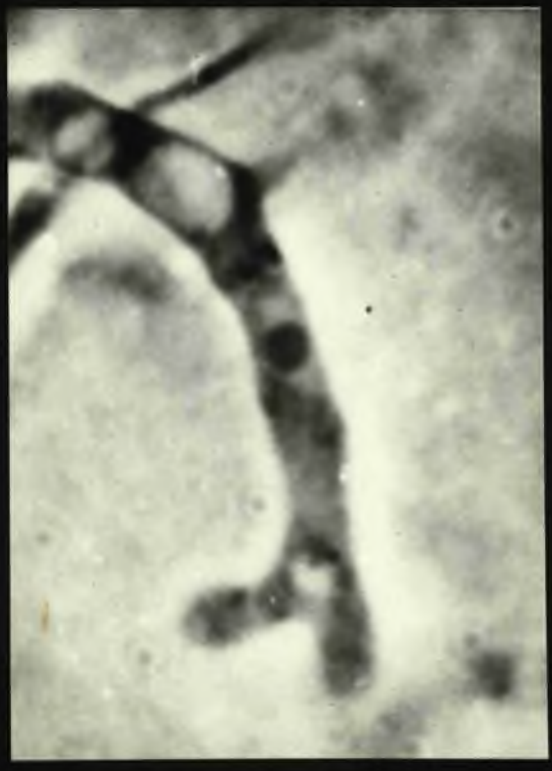
Fig. 3. Hypha of monokaryophase mycelium of M. rotula showing nucleus (arrow) consisting of dark central body surrounded by optically clear area (Phase contrast).

Fig. 4. Hypha of dikaryophase mycelium of M. rotula showing nucleus elongated to 25 (Phase contrast).


1
2
3
4
5μ







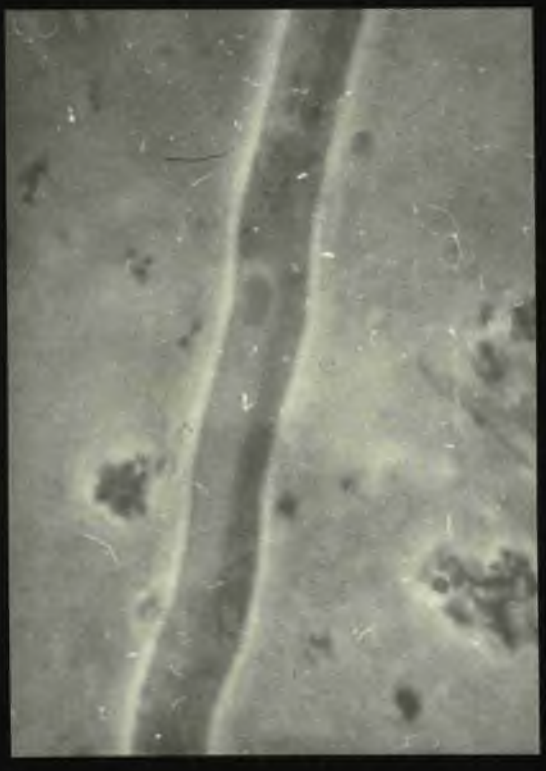
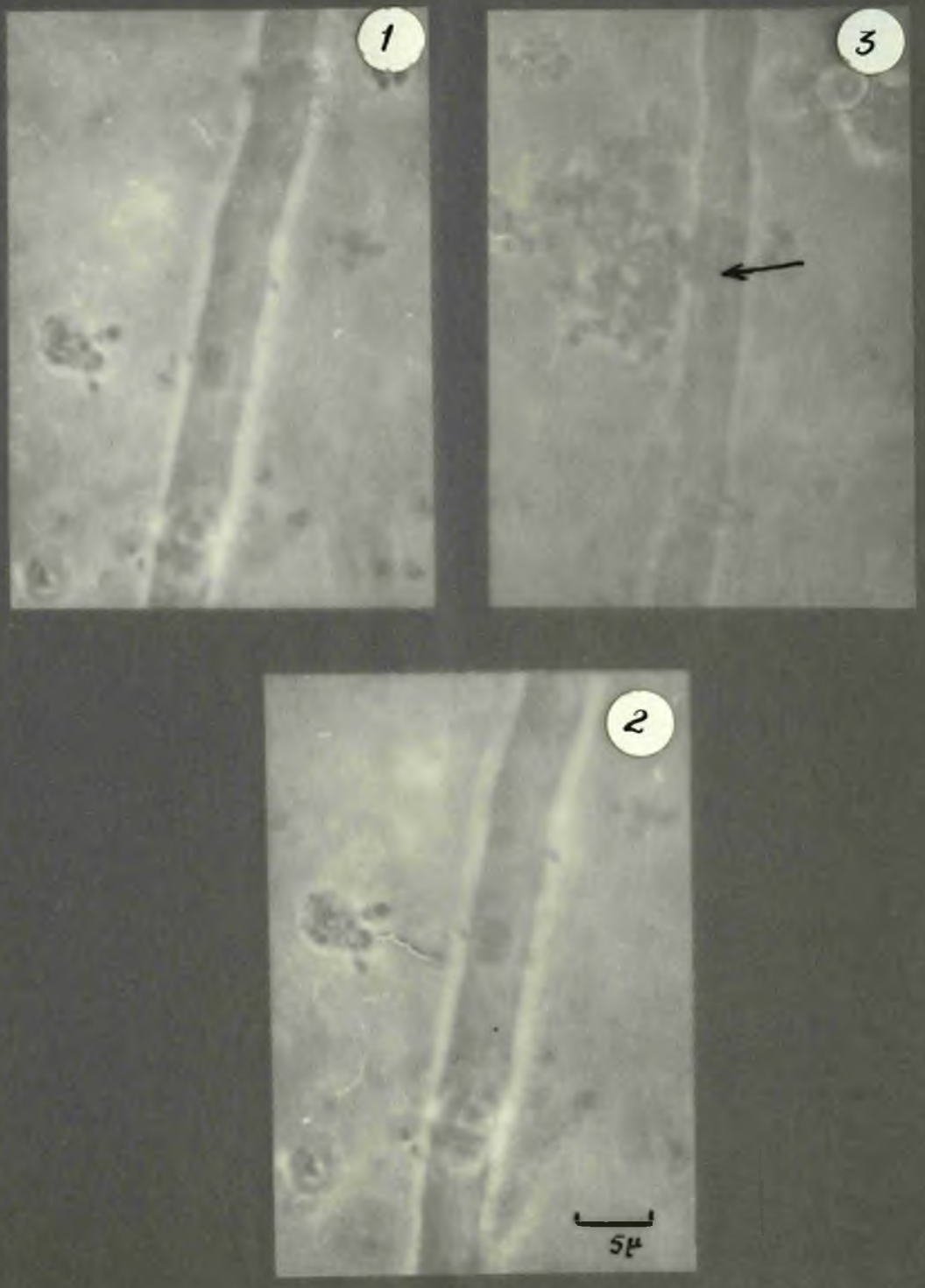
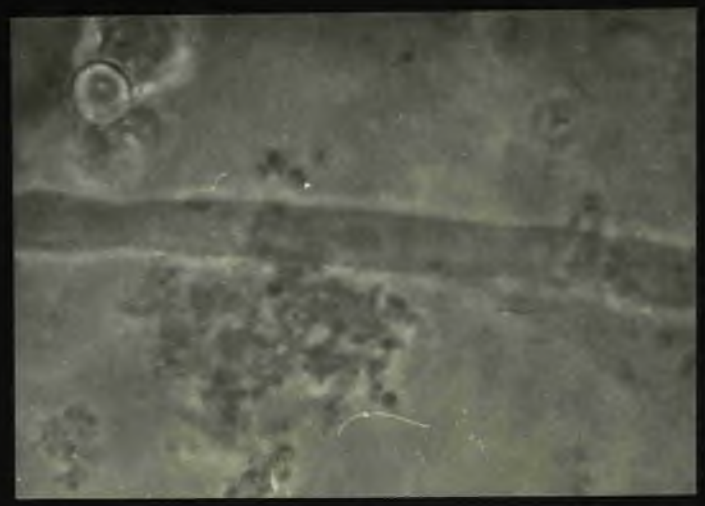
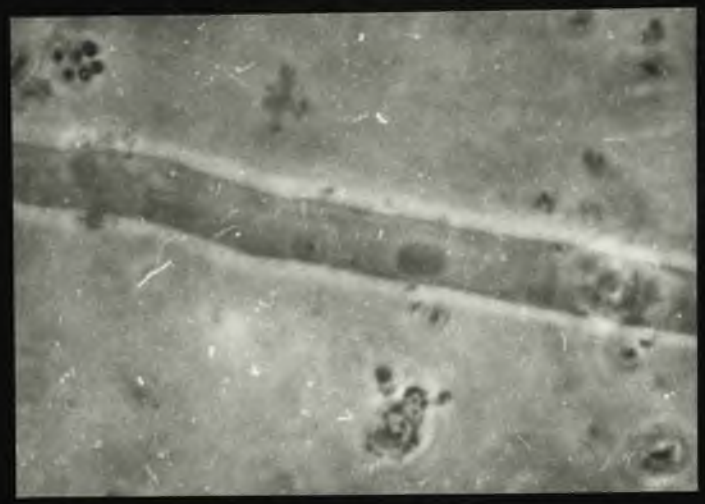


PLATE IX.

Figs. 1, 2 & 3. Nuclei of hyphae of dikaryophase mycelium of *M. rotula* showing various shapes. Central body in fig. 3 prolonged into beak (arrow). (Phase contrast).


1
3
2
5μ





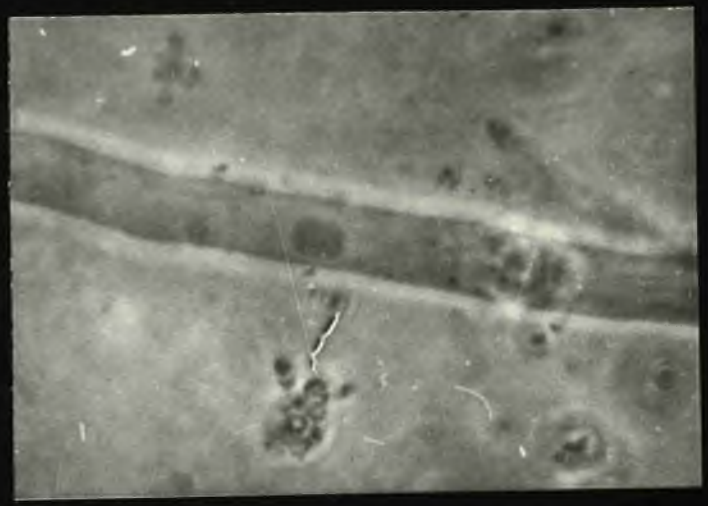
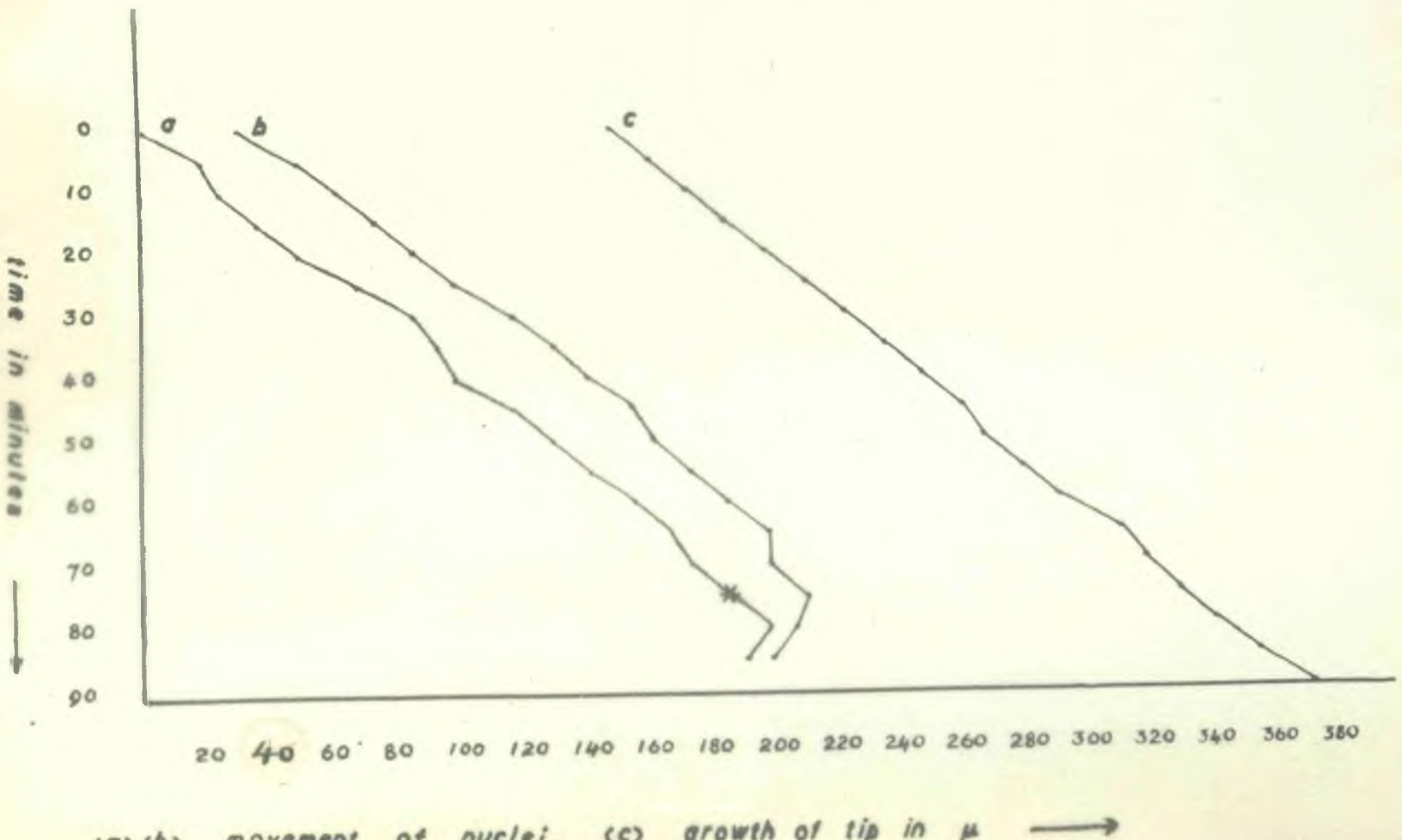


PLATE X.

Graph to show the relationship between the growth of the tip of the hypha and nuclear movement in M. androsaceus.

The movement of the hind nucleus (a) and the fore nucleus (b) and growth of the hyphal tip (c) are plotted as forward movement in  against time in minutes.

* marks the position and time of appearance of clamp excrescence.



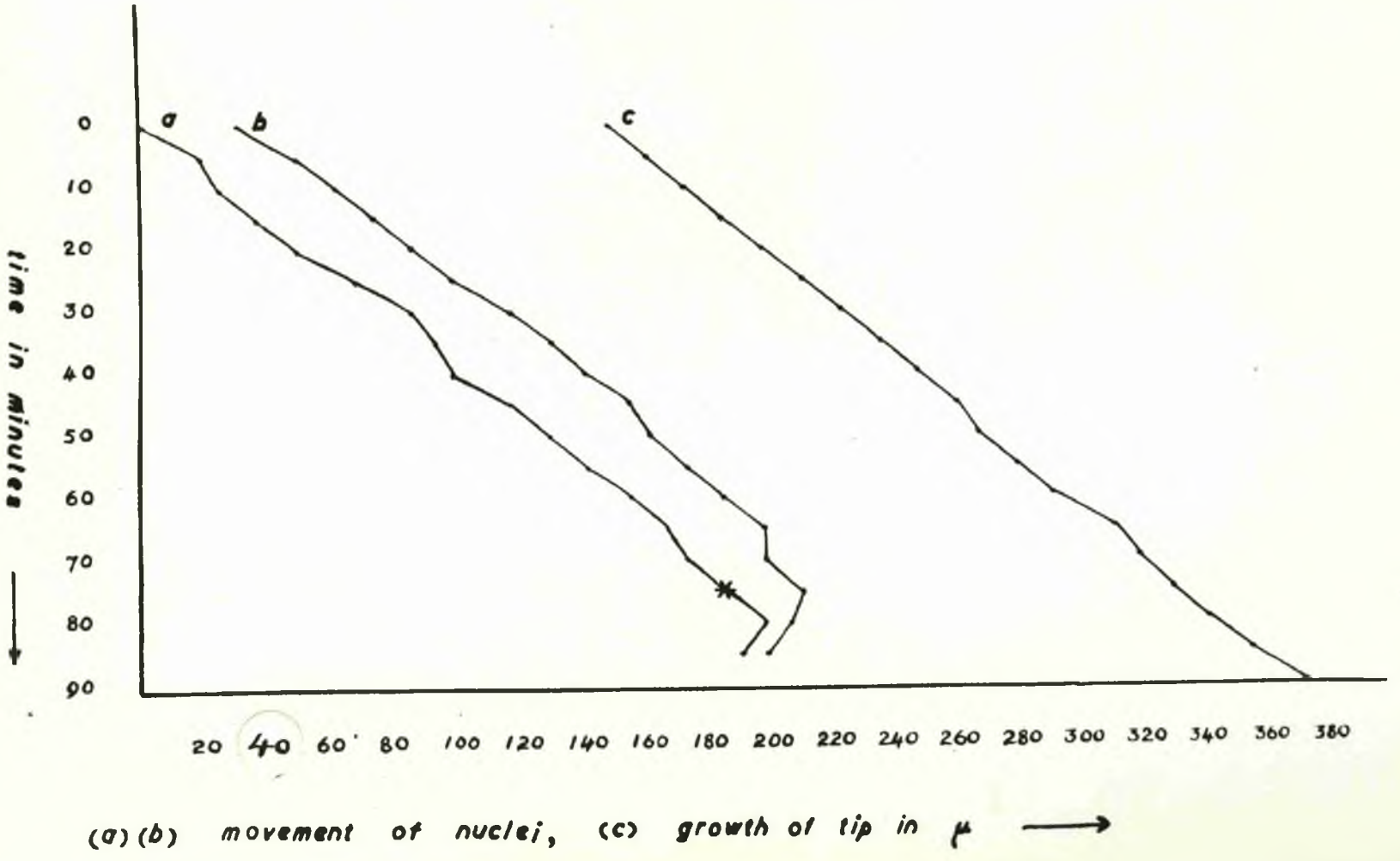

(a) (b) movement of nuclei, (c) growth of tip in μ ⟶

PLATE X.

Graph to show the relationship between the growth of the tip of the hypha and nuclear movement in M. androsaceus.

The movement of the hind nucleus (a) and the fore nucleus (b) and growth of the hyphal tip (c) are plotted as forward movement in against time in minutes.

* marks the position and time of appearance of clamp excrescence.




(a) (b) movement of nuclei, (c) growth of tip in μ ⟶

# PLATE XI.

Graph to show the relationship between the growth of the tip of the hypha and nuclear movement in M. androsaceus.

The movement of the hind nucleus (a) and the fore nucleus (b) and growth of the hyphal tip (c) are plotted as forward movement in against time in minutes.

Arrow shows the position at which the nuclei ceased moving for a short period; tip grows on during this period, unaffected by behaviour of nuclei.

* makrs the position and time of appearance of clamp excrescence. The nuclei move backwards to the vicinity of the clamp, disappear from view during division. 8 - 10 minutes later three of the four nuclei resulting from the conjugate division are seen.

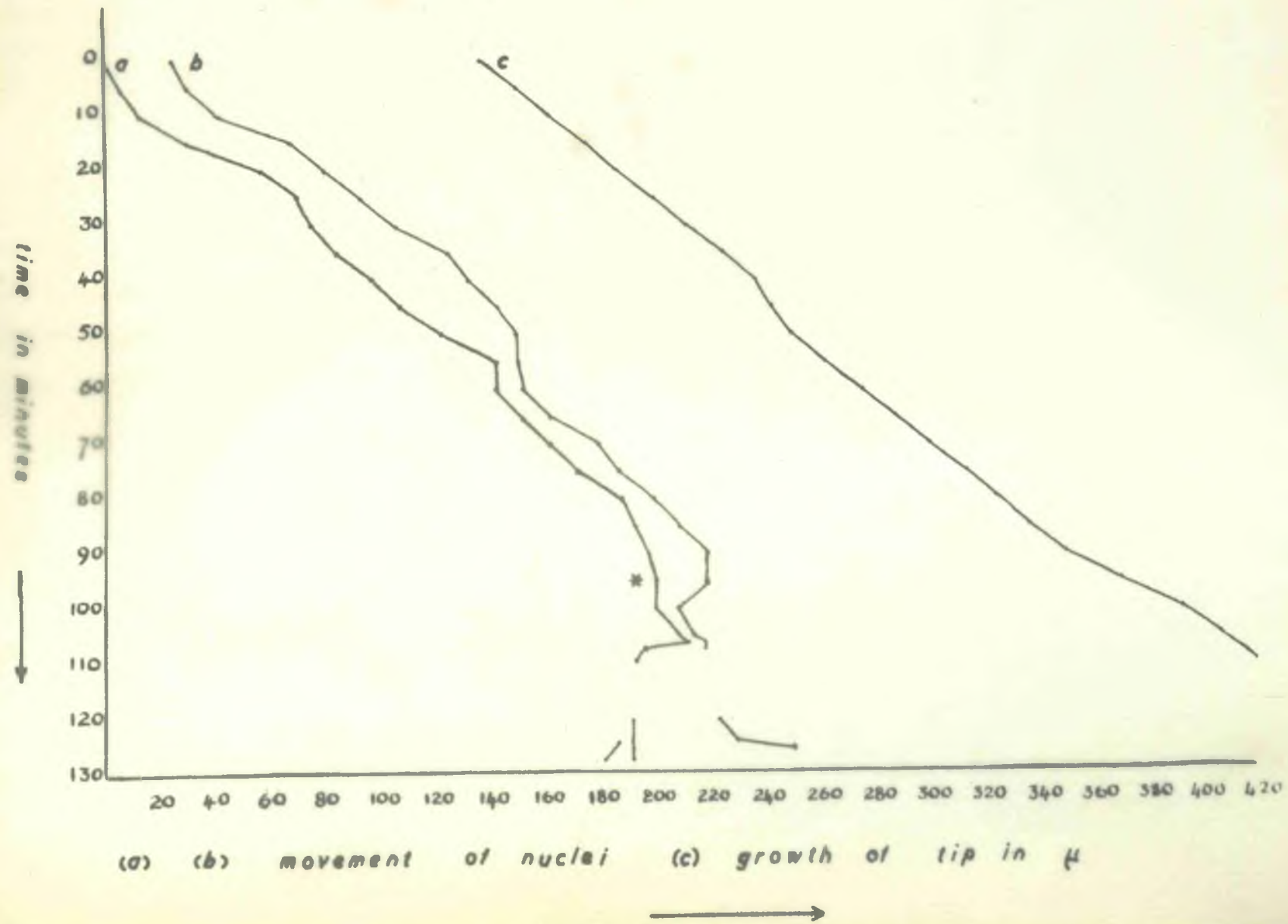

a
b
c
0
10
20
30
40
50
60
70
80
90
100
110
120
130
time in minutes
20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420
(a) (b) movement of nuclei (c) growth of tip in μ

PLATE XI.

Graph to show the relationship between the growth of the tip of the hypha and nuclear movement in M. androsaceus.

The movement of the hind nucleus (a) and the fore nucleus (b) and growth of the hyphal tip (c) are plotted as forward movement in against time in minutes.

Arrow shows the position at which the nuclei ceased moving for a short period; tip grows on during this period, unaffected by behaviour of nuclei.

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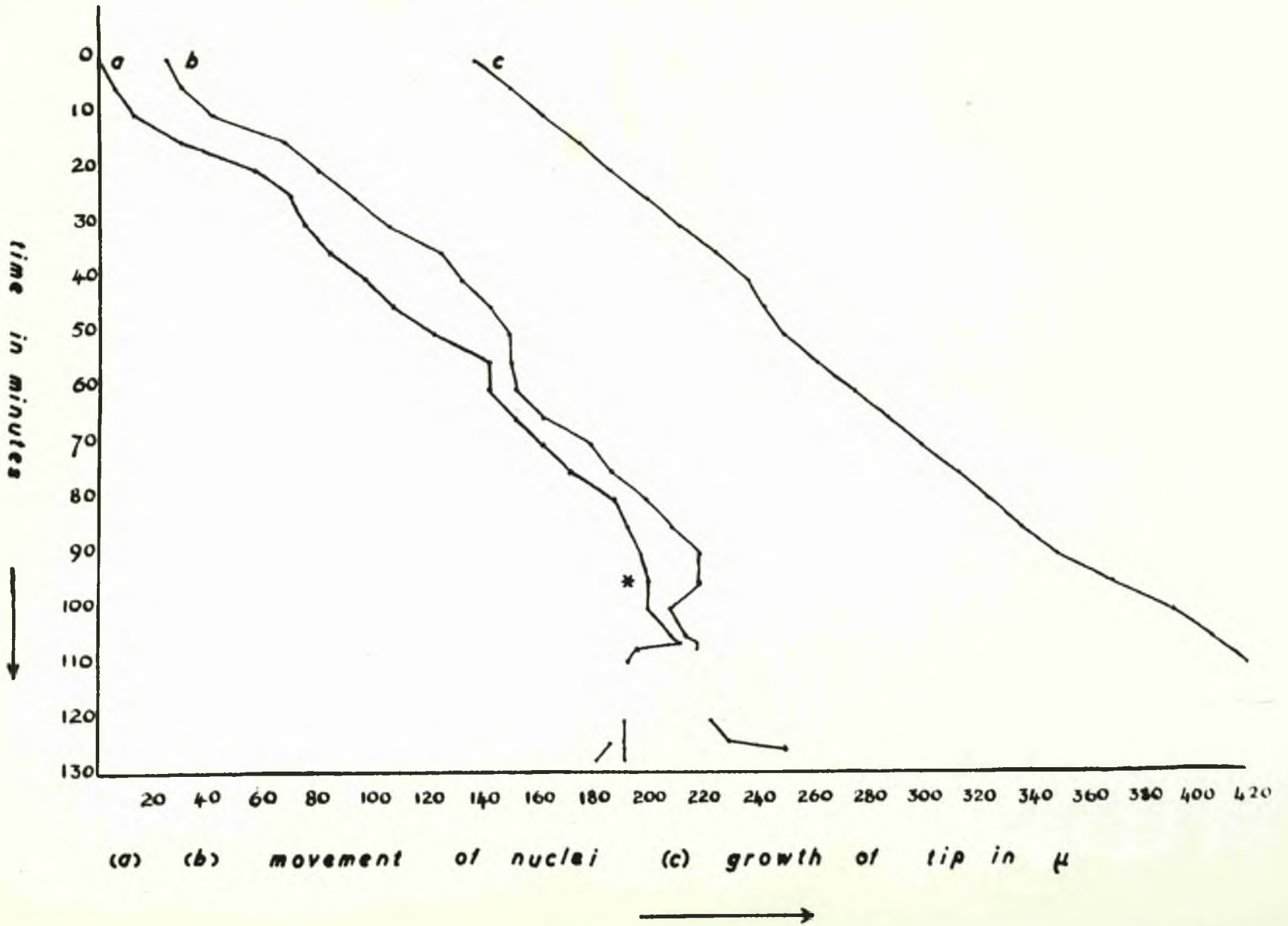

a
b
c
0
10
20
30
40
50
60
70
80
90
100
110
120
130
time in minutes
20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420
(a) (b) movement of nuclei (c) growth of tip in μ

# PLATE XII.

Wall formation in the hypha of *M. androsaceus*.

(a) Hypha with clamp before commencement of wall formation.

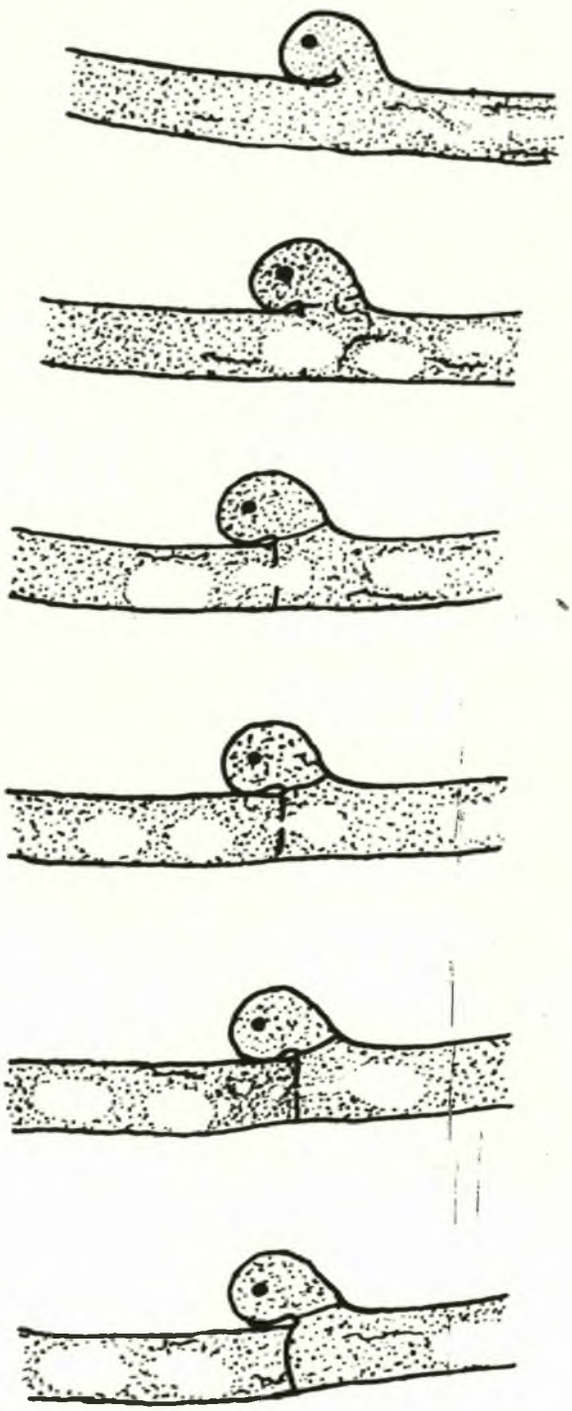
(b) Forming wall seen in optical view as granules on either wall of the hypha.

(c) The wall seen closing inwards like an iris diaphragm, with vacuole passing through central pore.

(d) Central pore much smaller, vacuoles are constricted as they pass through.

(e) Central pore very small about 0.5$\mu$; vacuoles pass through by forming a finger-like projection to pore from which small vacuoles are budded off on the other side of the septum.

(f) Wall formation complete.



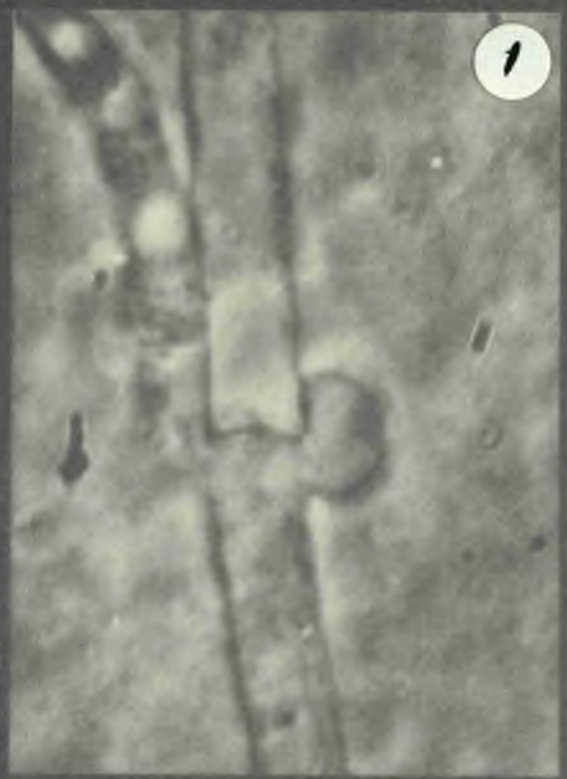
5μ

PLATE XIII.

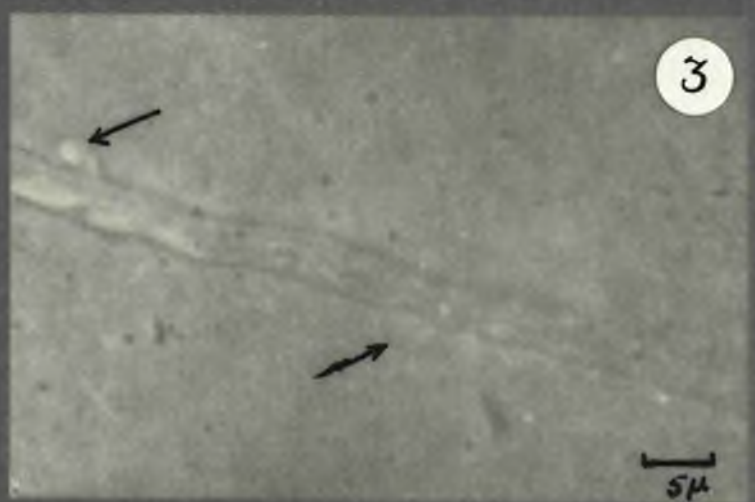
Fig. 1. Hypha showing septum with central thickening formed by dolipore and parenthosomes (M. rotula). (Phase contrast).

Fig. 2. Hypha from which a secondary projection has grown from the subterminal cell to fuse with the clamp, where the septum in the main hypha has been formed behind the tip of the backwards bent clamp (M. rotula). (Phase contrast)

Fig. 3. Hypha on which two clamps have appeared simultaneously within 50 of each other (arrows) (M. androsaceus). (Phase contrast).


1


2
5μ


3
5μ

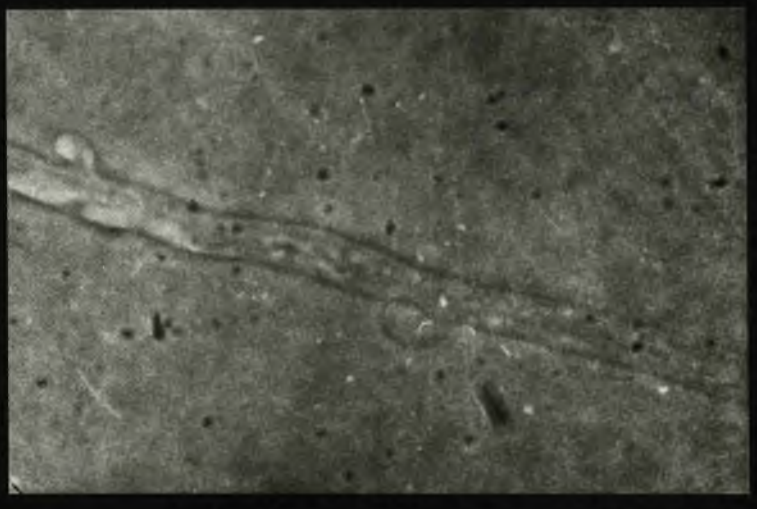
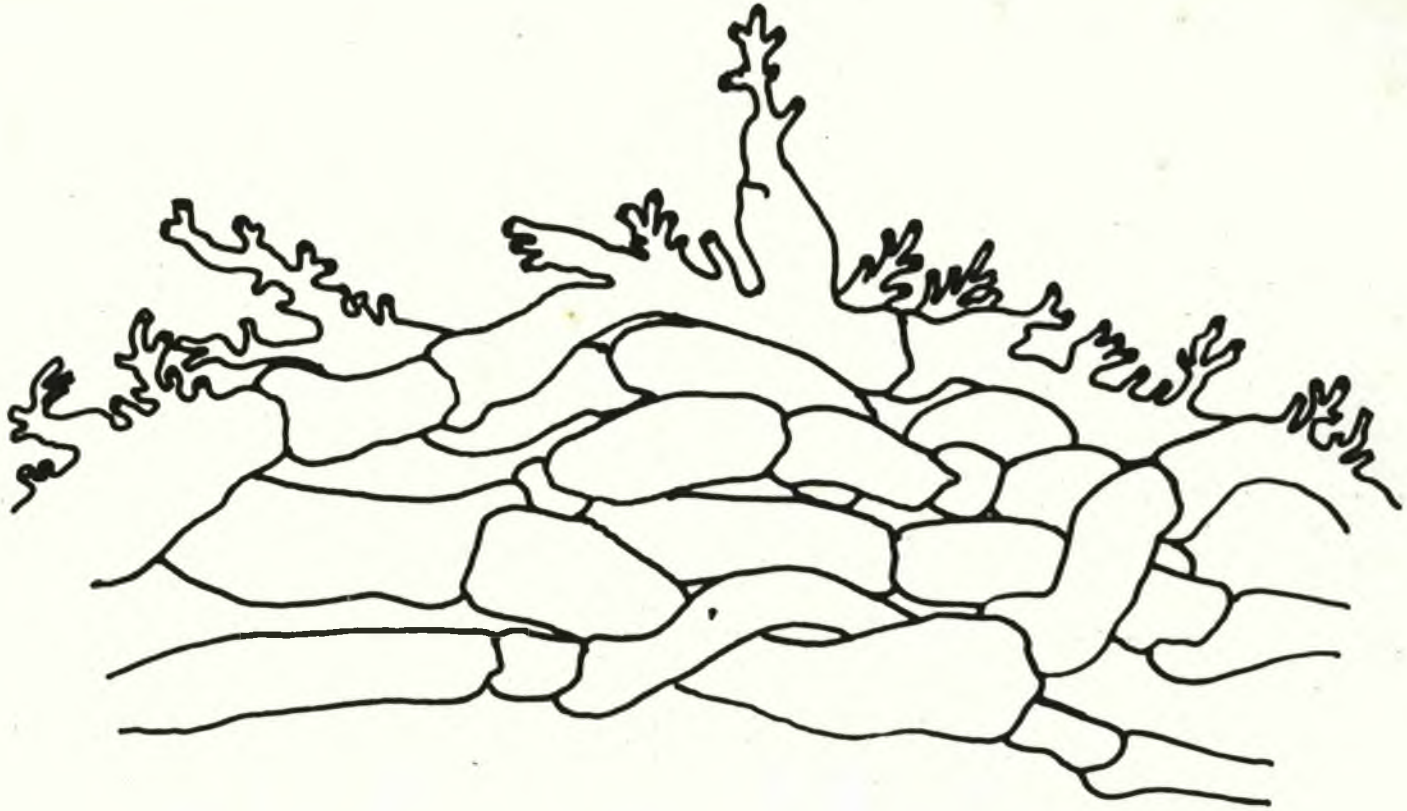


PLATE XIV.

Camera lucida drawing showing the numerous, long, irregular hyphae from which branched finger-like outgrowths project, which run transversely across the cap of M. androsaceus.



10μ

PLATE XV.

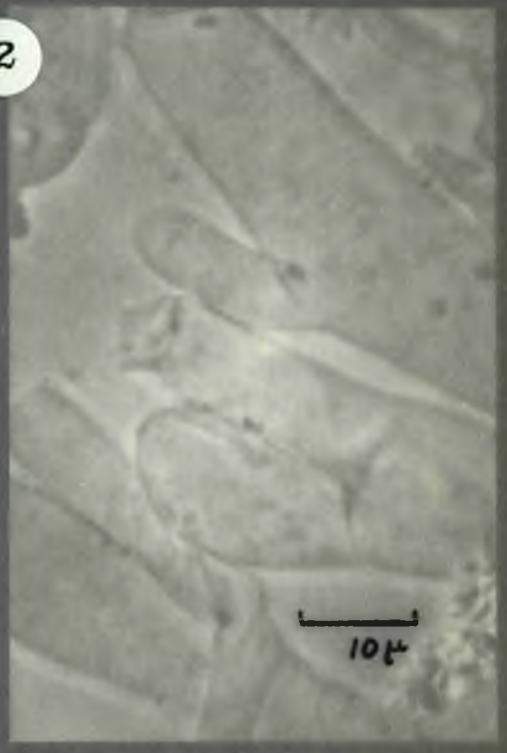
Fig. 1. 'Herisses en brosse' cells which cover the surface of the cap of M. androsaceus. (Phase contrast).

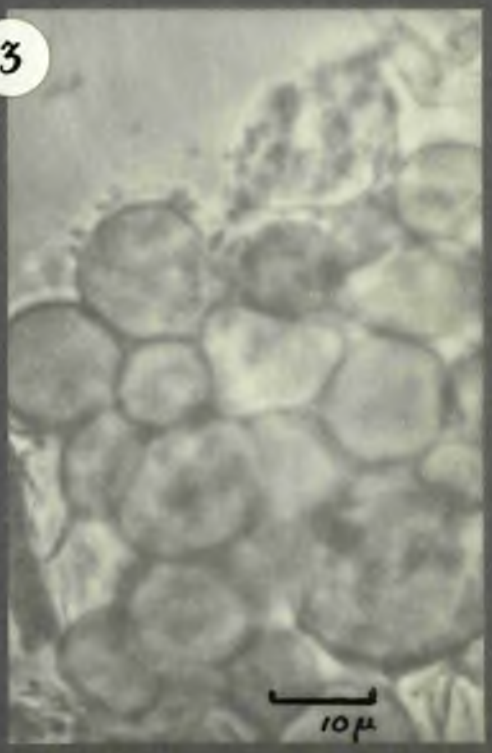
Fig. 2. Large thin-walled hyphae found in the pileus flesh of M. androsaceus. (Phase contrast).

Fig. 3. Surface view of the club-shaped cells which form the epicutis of the cap of M. rotula. (Phase contrast).

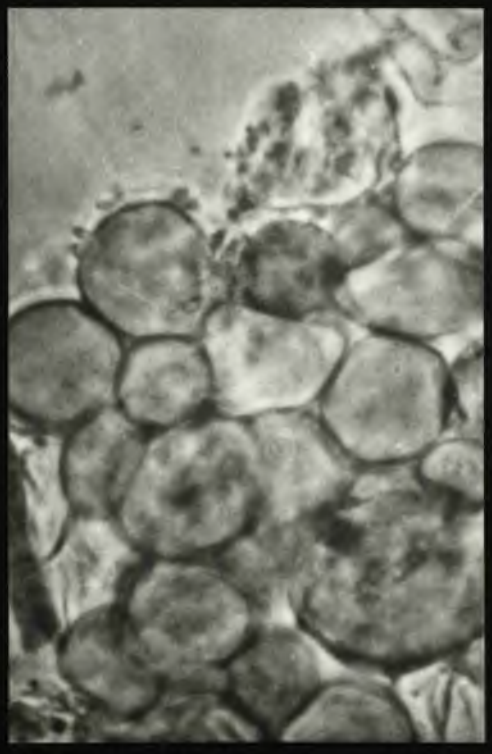
Fig. 4. Single club-shaped cell from the epicutis of the cap of M. rotula. (Phase contrast).


1
10μ


2
10μ


3
10μ


4
10μ



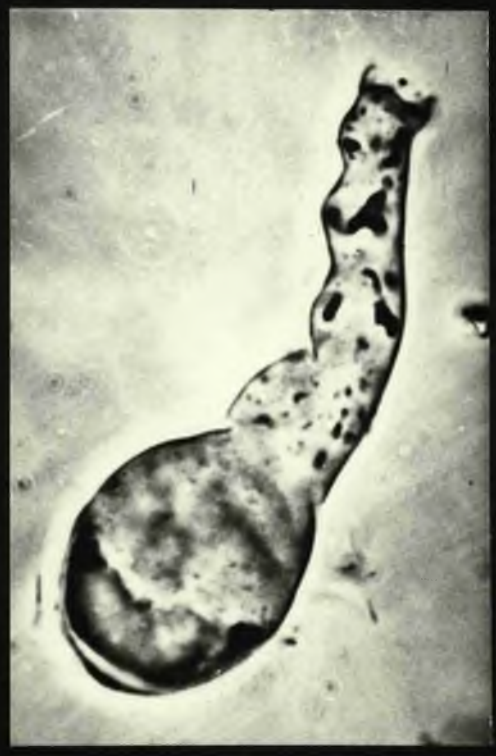
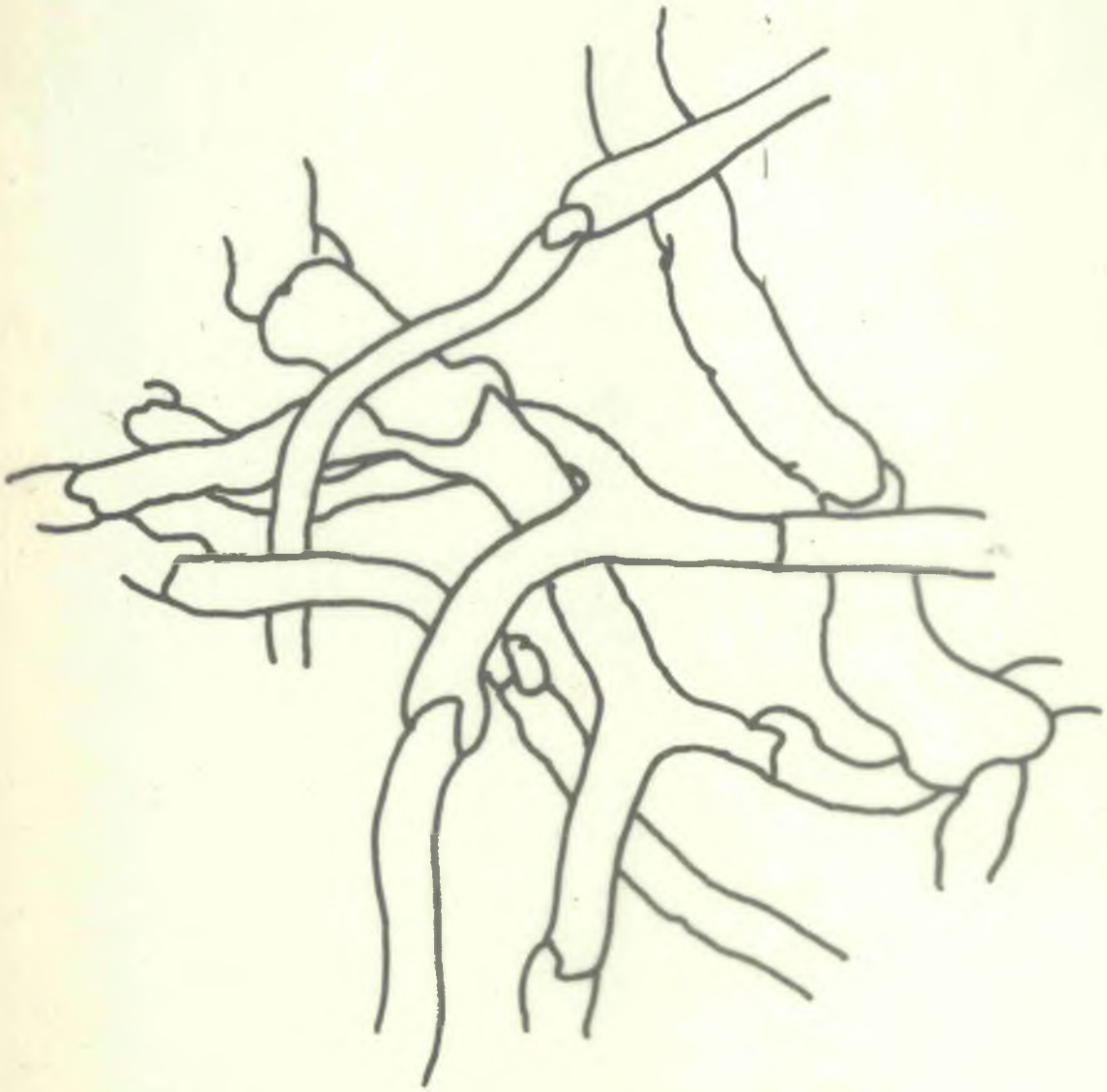


PLATE XVI.

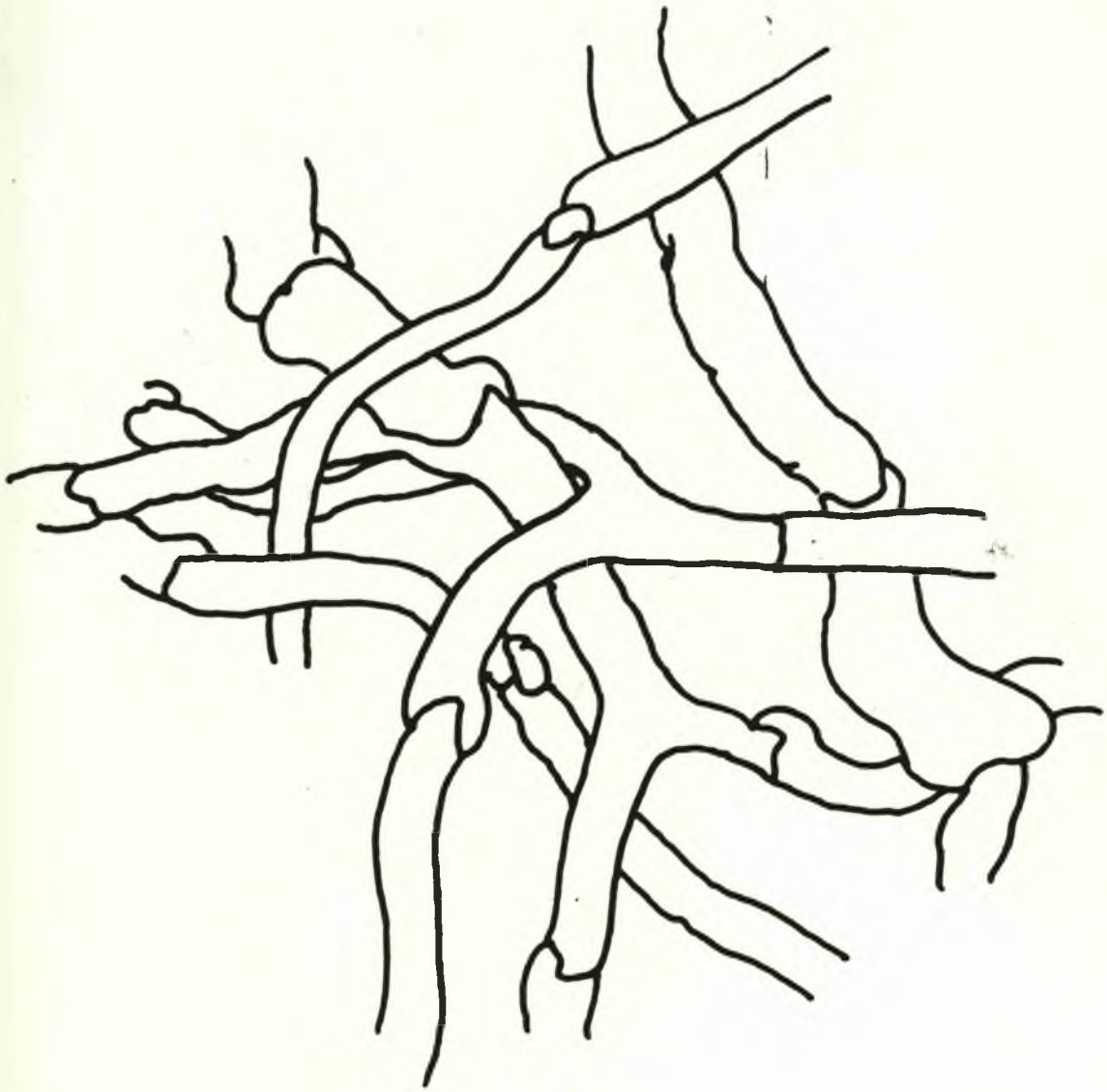
Camera lucida drawing of the loosely interwoven, thin-walled hyphae which form the flesh of the pileus and trama of the gills of M. androsaceus.



10 μ

PLATE XVI.

Camera lucida drawing of the loosely interwoven, thin-walled hyphae which form the flesh of the pileus and trama of the gills of M. androsaceus.



10 μ

PLATE XVII.

Camera lucida drawing of a portion of a vertical section of a gill of M. androsaceus showing the arrangement of the cells in trama, sub-hymenium and hymenium.

10µ
trama
sub hymenium
hymenium

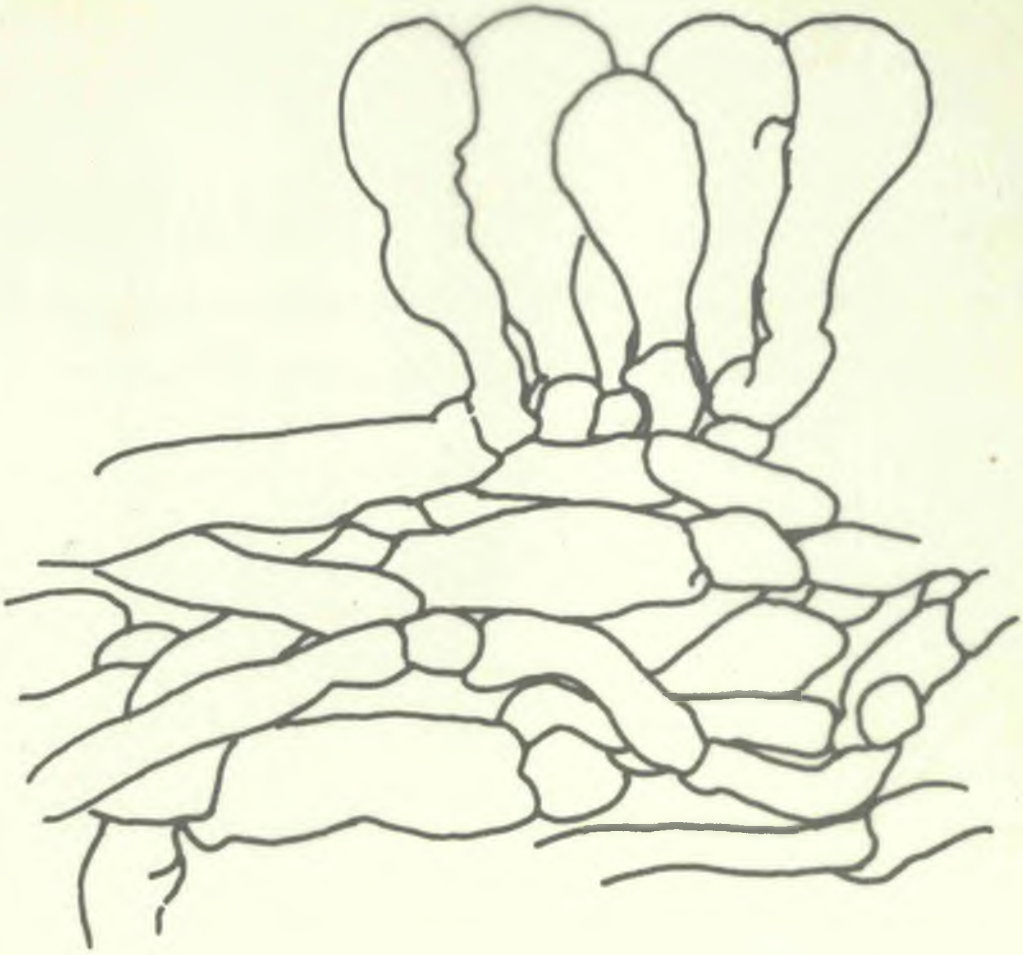
## PLATE XVII.

Camera lucida drawing of a portion of a vertical section of a gill of M. androsaceus showing the arrangement of the cells in trama, sub-hymenium and hymenium.

10μ
trama
sub hymenium
hymenium

# PLATE XVIII.

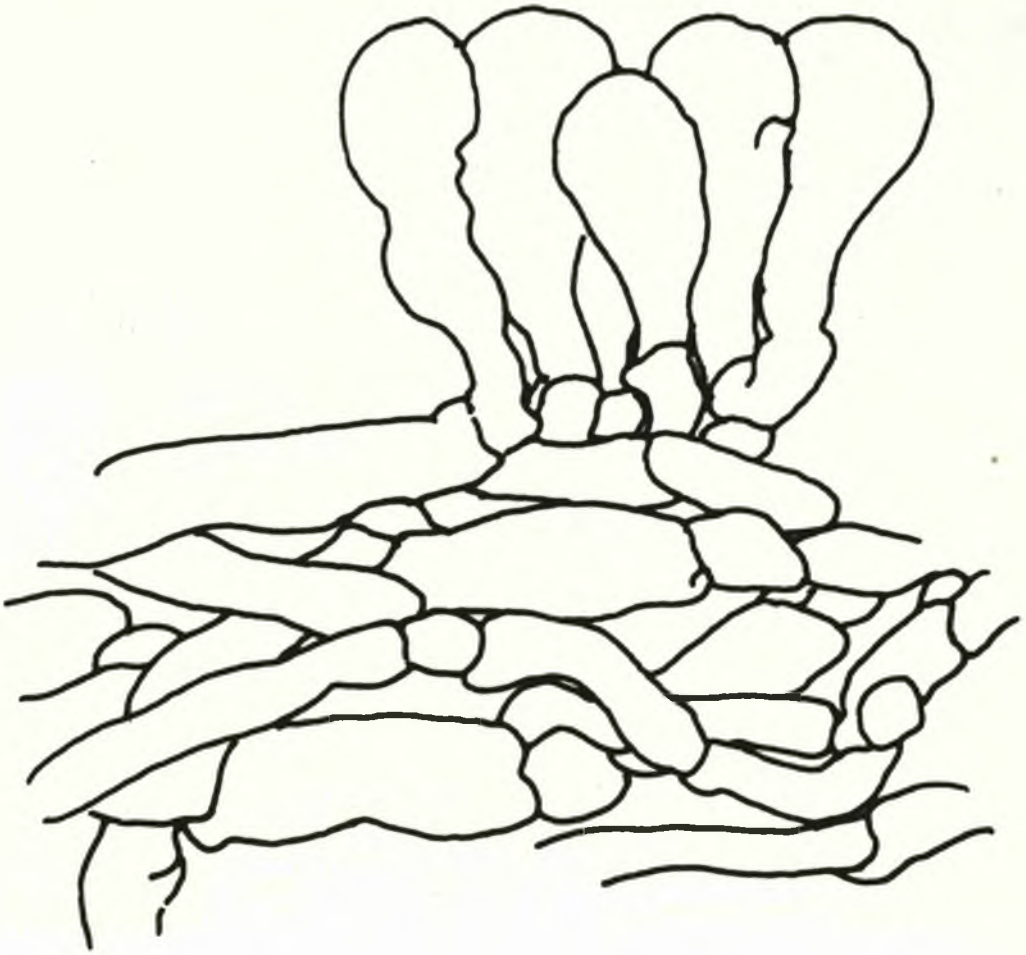
Camera lucida drawing of a portion of a section through the cap of M. rotula showing the outermost hyphae of the cap and the club-shaped cells which form the epicutis.



۱۰ μ

# PLATE XVIII.

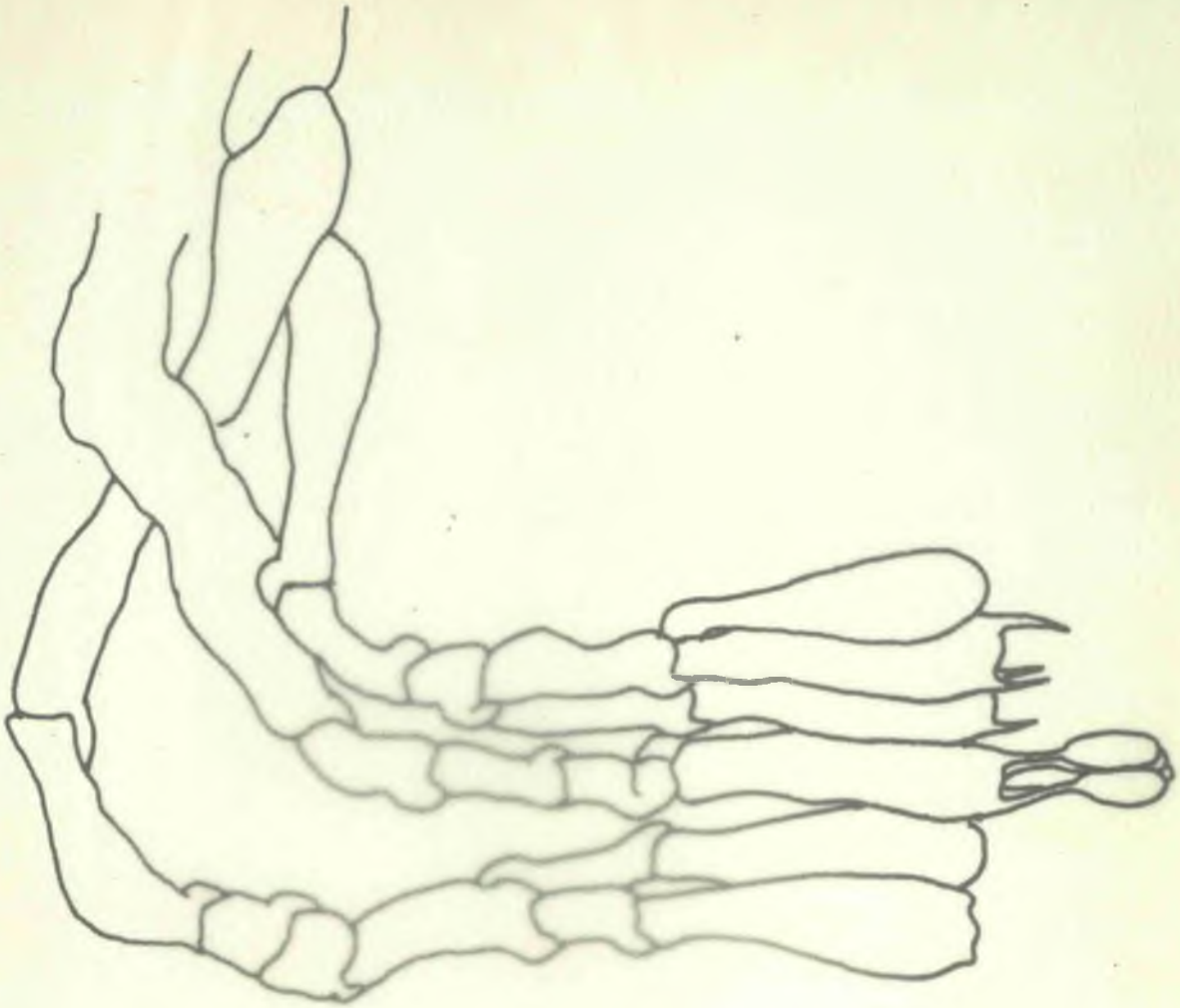
Camera lucida drawing of a portion of a section through the cap of *M. rotula* showing the outermost hyphae of the cap and the club-shaped cells which form the epicutis.

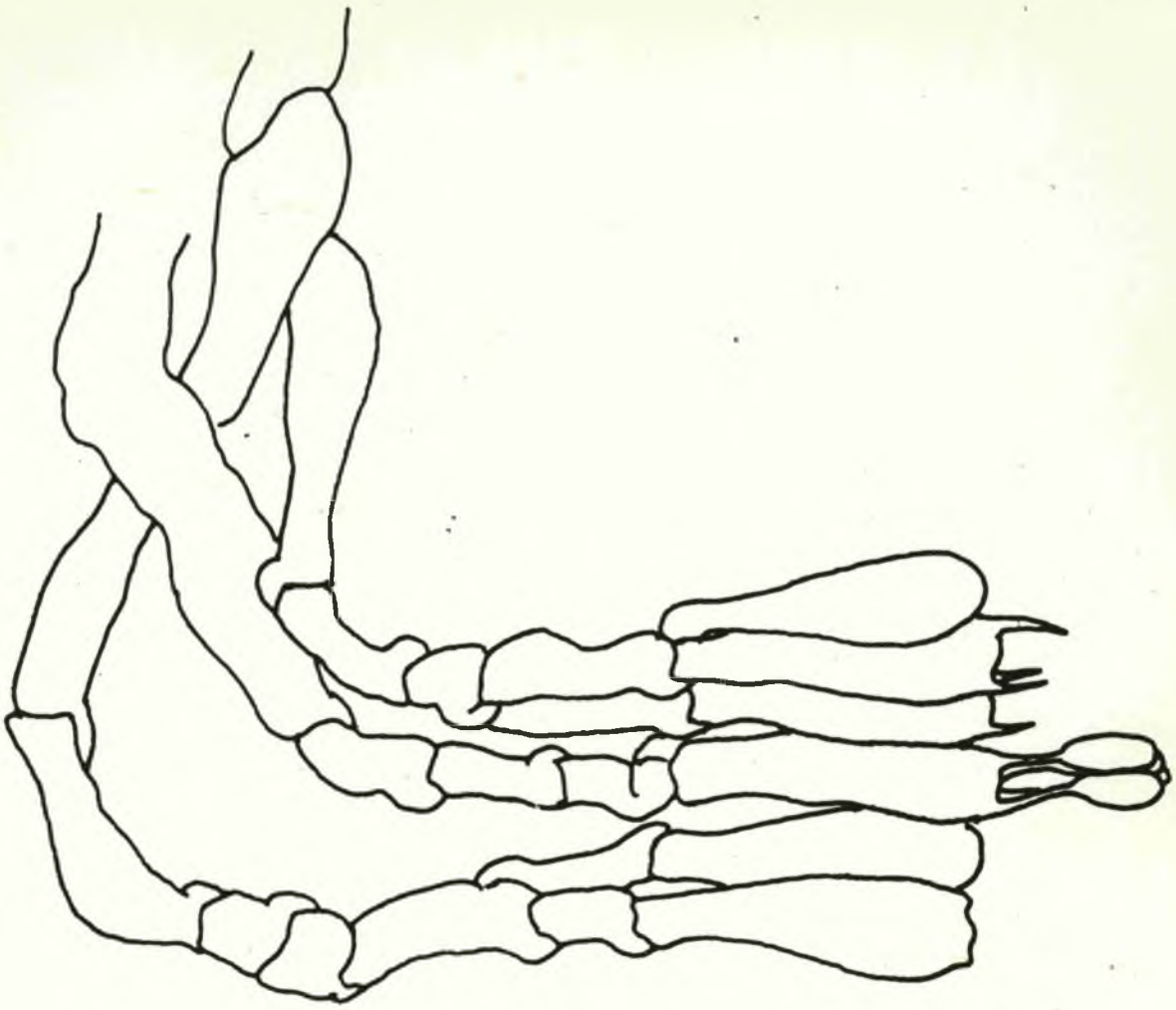


10 μ

PLATE XIX.

Camera lucida drawing of a portion of a section of a gill of *M. rotula* showing the arrangement of cells in trama, sub-hymenium and hymenium.





10 μ

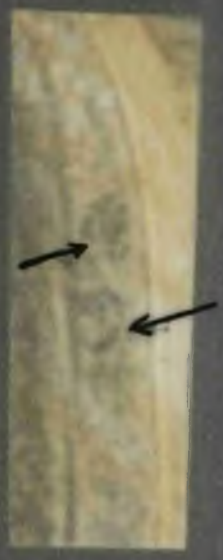
PLATE XX.

Fig. 1. Hyphae from trama of gills of M. androsaceus showing non-migratory nuclei, each consisting of a loosely coiled chromatin strand to which is attached an eccentrically placed nucleolus (arrows). Aceto-orcein stained; bright light.

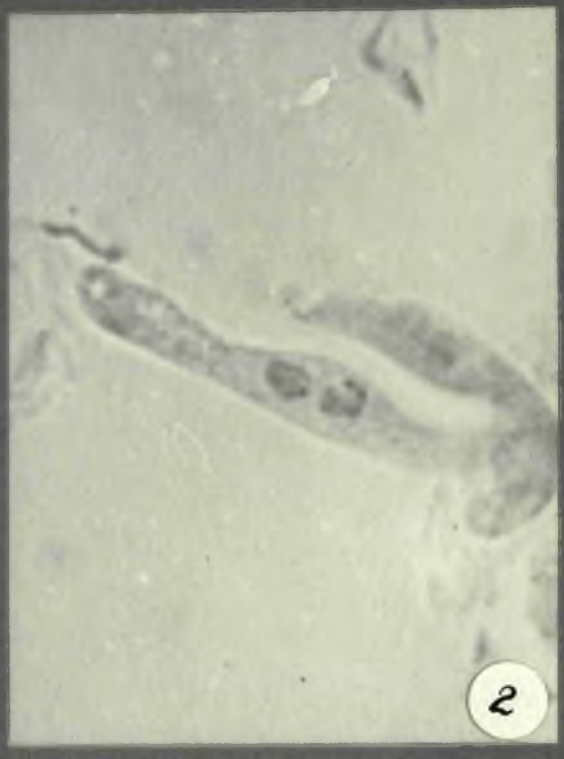
Fig. 2. Hyphal tip showing migratory nuclei, each consisting of a central nucleolus around which the chromatin strand is closely appressed. Aceto-orcein stained; bright light.

Fig. 3. Nuclei in early stages of division in ultimate clamp. Chromatin seen as two strands attached to nucleolus (arrow) in nucleus (a) and as one strand appeared to be doubled on itself in nucleus (b). Aceto-orcein stained; bright light.

Fig. 4. Ultimate clamp and nuclei in which the chromatin is deeply stained. The nucleolus is indistinct. Aceto-orcein stained; bright light.



1



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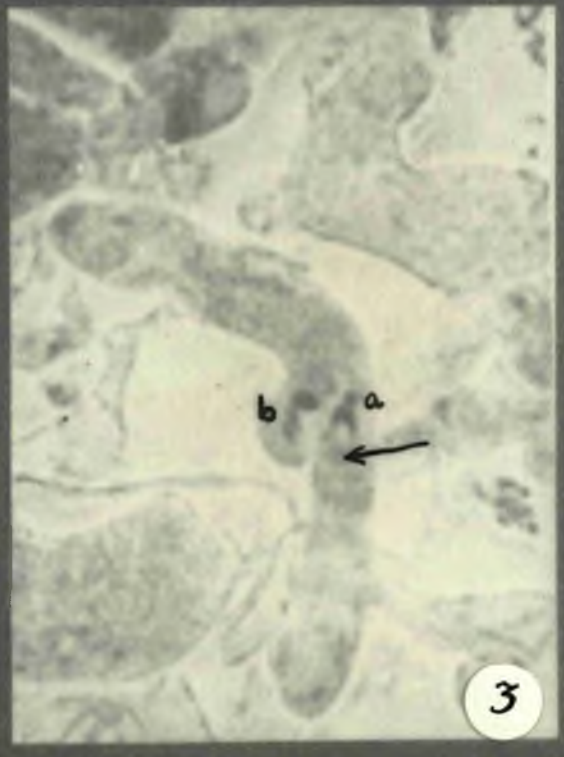
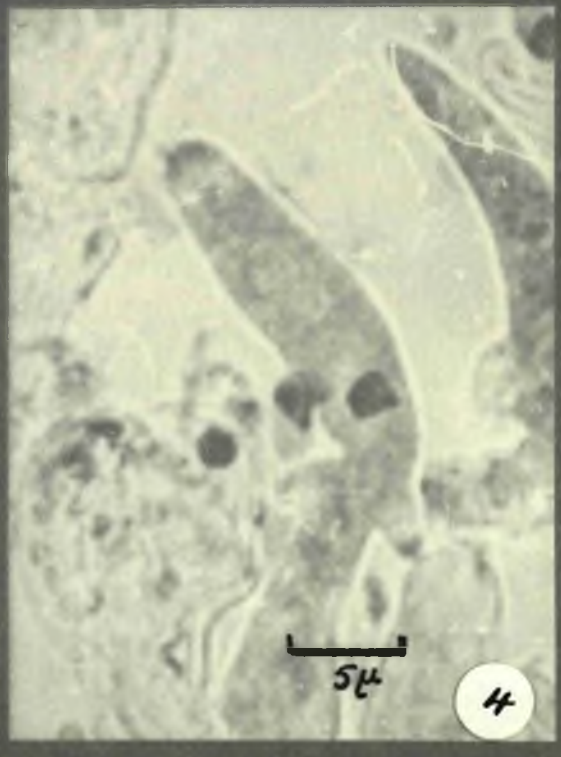
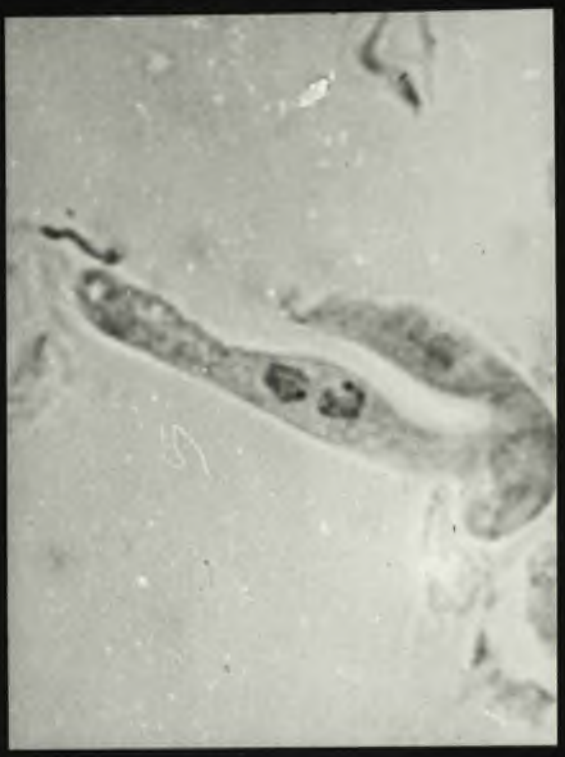
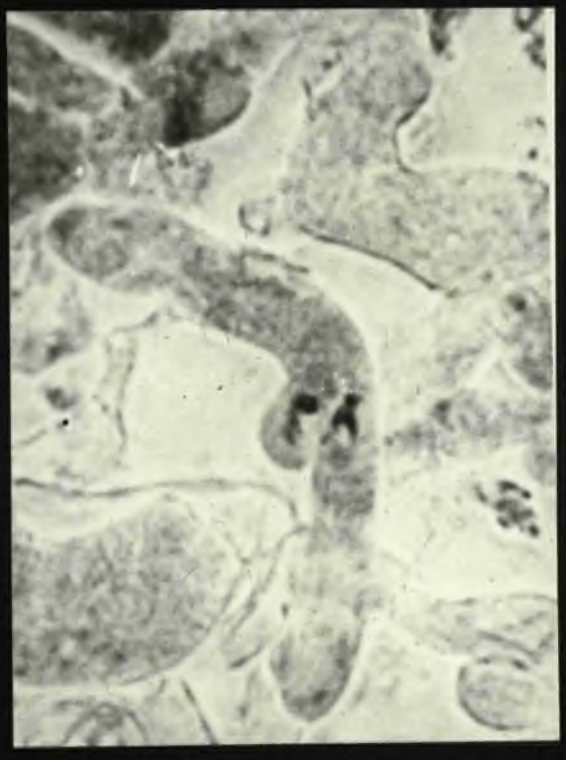


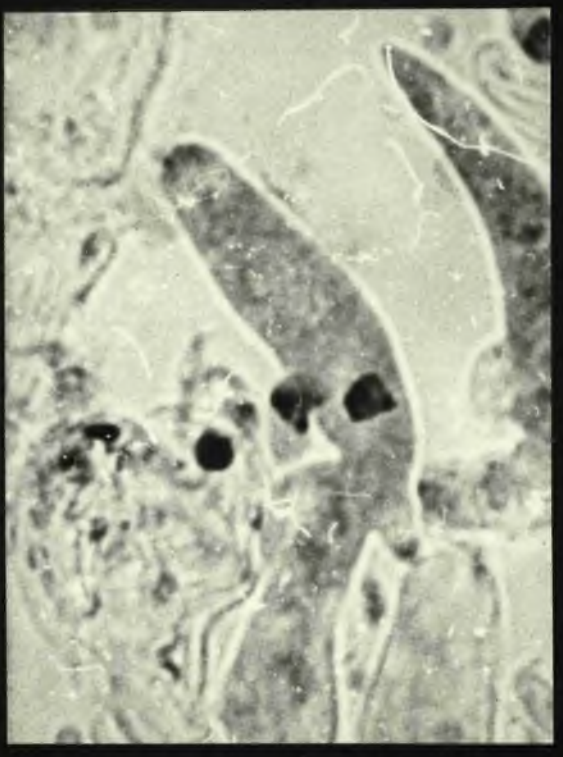

3




4







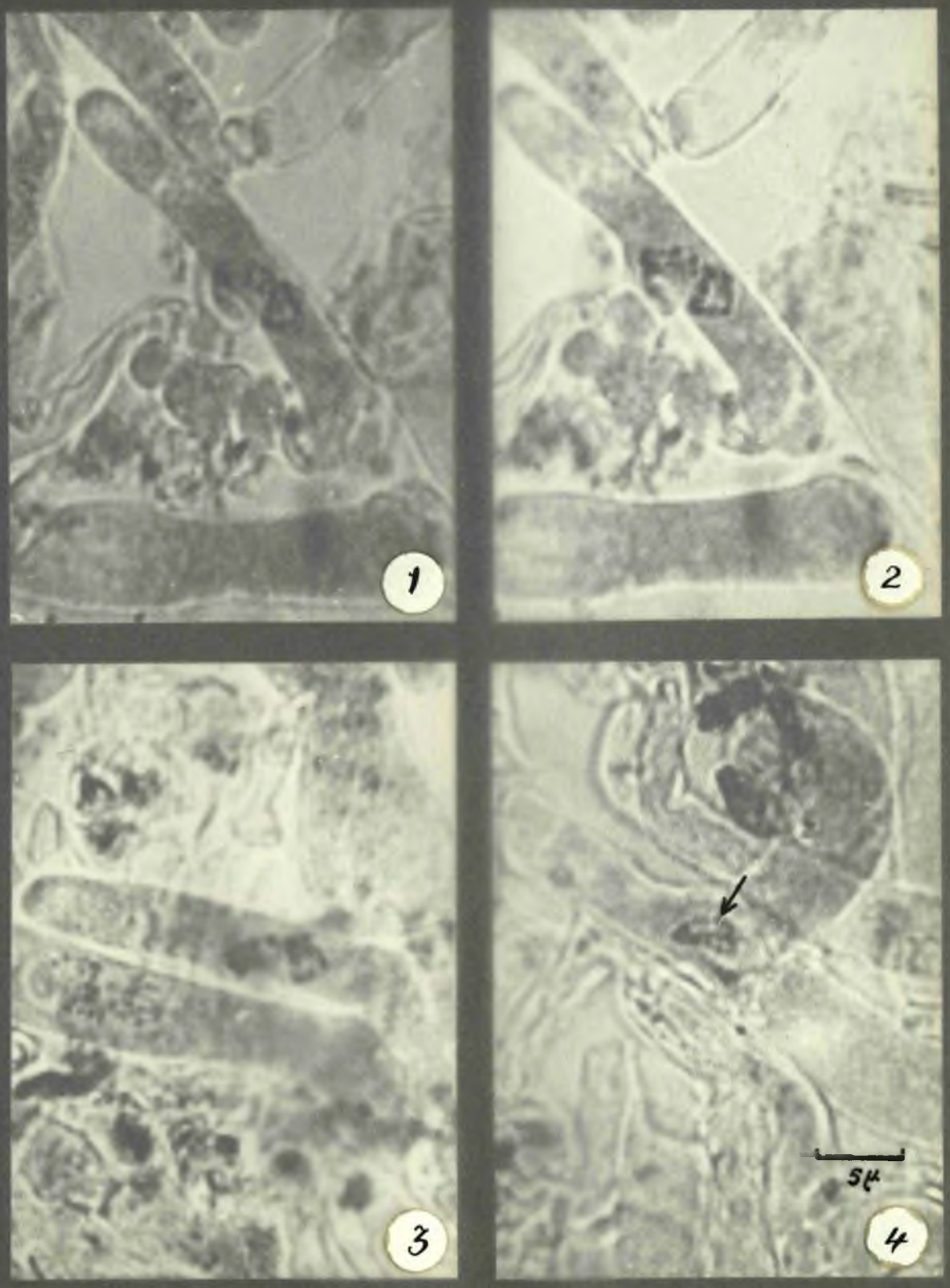
# PLATE XXI.

Nuclear division in the ultimate clamp of *M. androsaceus*; early stages of 'prophase'.

Figs. 1 & 2. Different focuses of nuclei of ultimate clamp, showing double strand of beaded chromatin. One strand is in focus in each figure. Aceto-orcein stained; bright light.

Fig. 3. Ultimate clamp showing nuclei consisting of beaded strands of chromatin. Aceto-orcein stained; bright light.

Fig. 4. Ultimate clamp; one nucleus only is seen (arrow) consisting of double loops of beaded chromatin strand. Aceto-orcein stained; bright light.


1
2
3
5μ
4

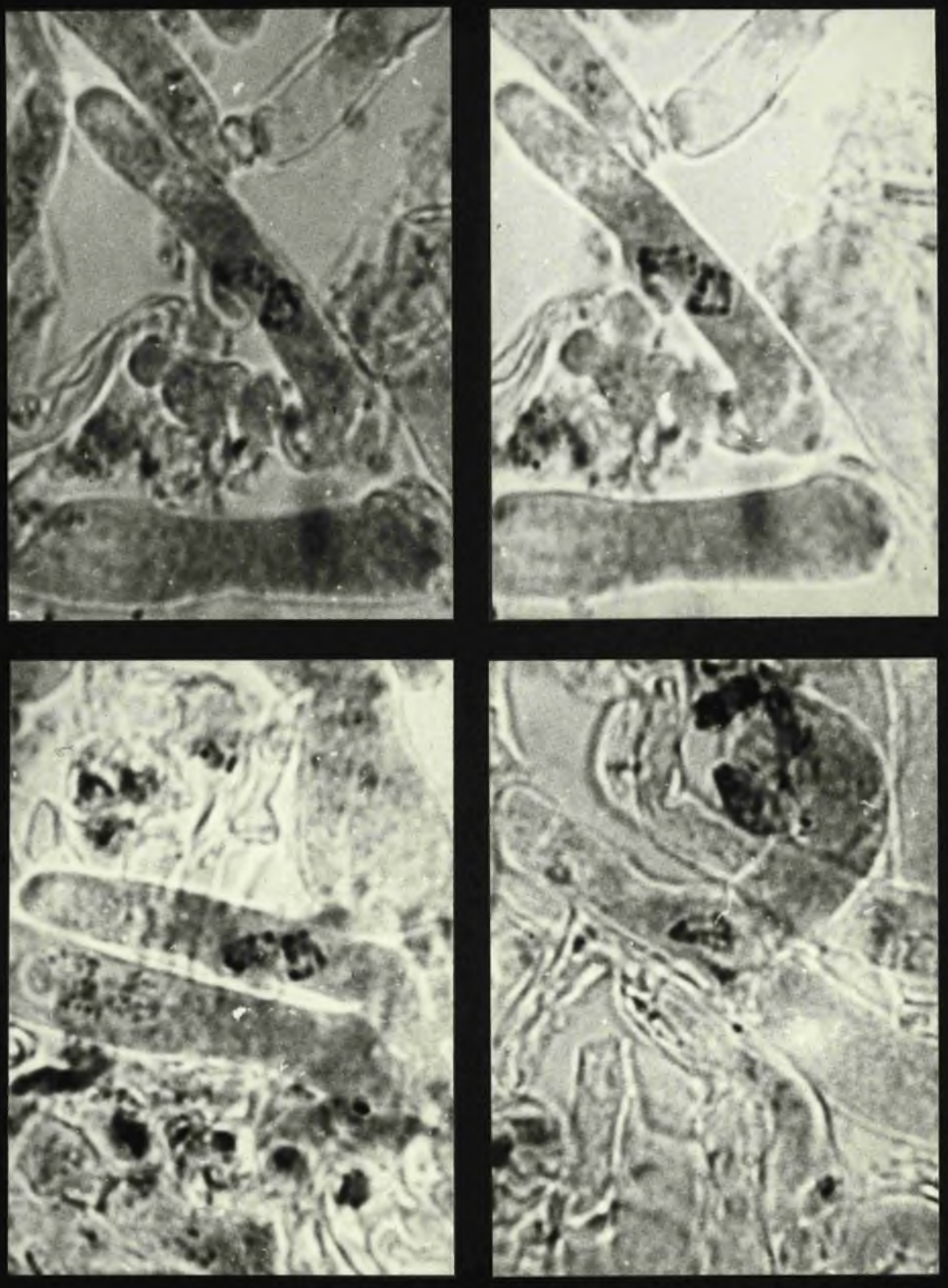


PLATE XXII.

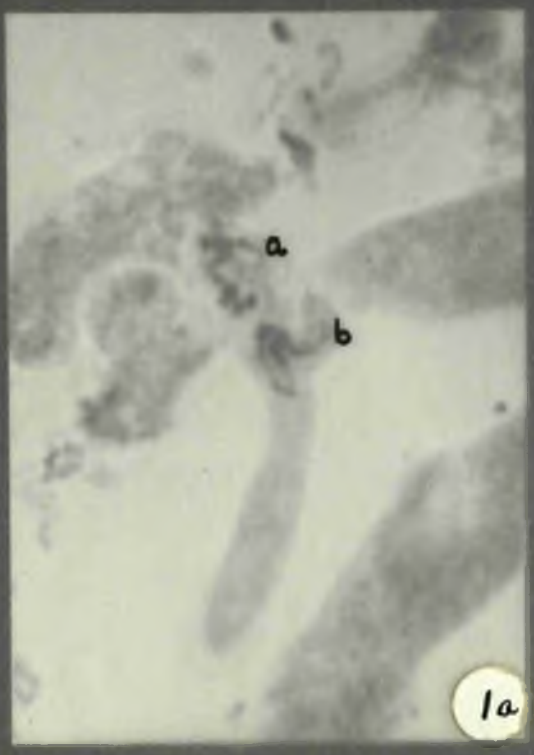
Nuclear division in the ultimate clamp of M. androsaceus; late stages of 'prophase'.

Fig. 1a. Nuclei undergoing twist leading to formation of figure of eight. Nucleus (a) opened out in large ring; nucleus (b) showing twist. Aceto-orcein stained; bright light.

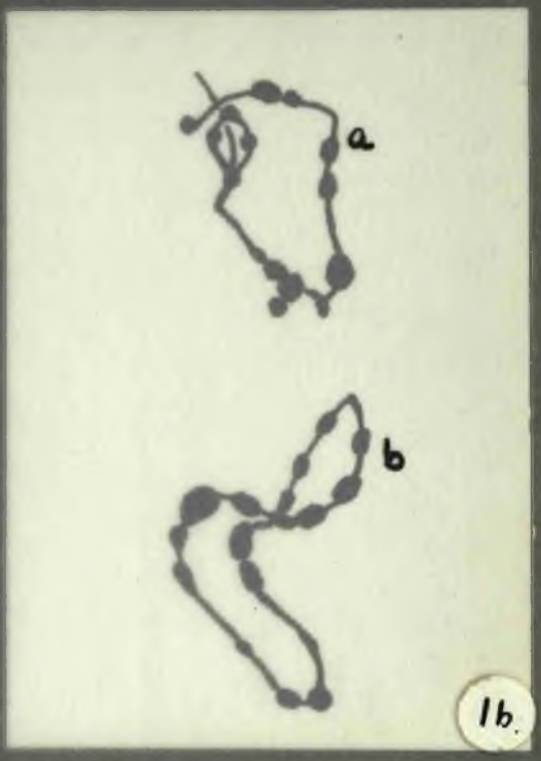
Fig. 1b. Drawing of nuclei above.

Fig. 2a. Nuclei undergoing twist leading to formation of figure of eight. Nucleus (a) already twisted and doubled over on itself. Aceto-orcein stained; bright light.

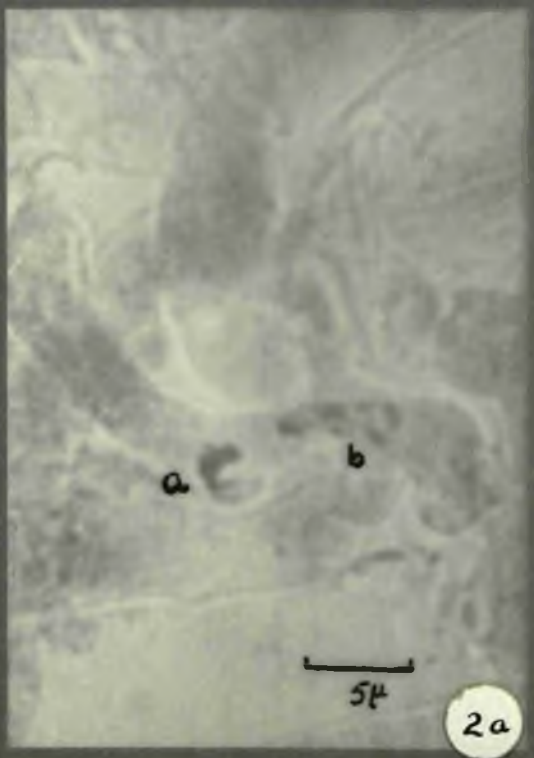
Fig. 2b. Drawing of nuclei above.




1a

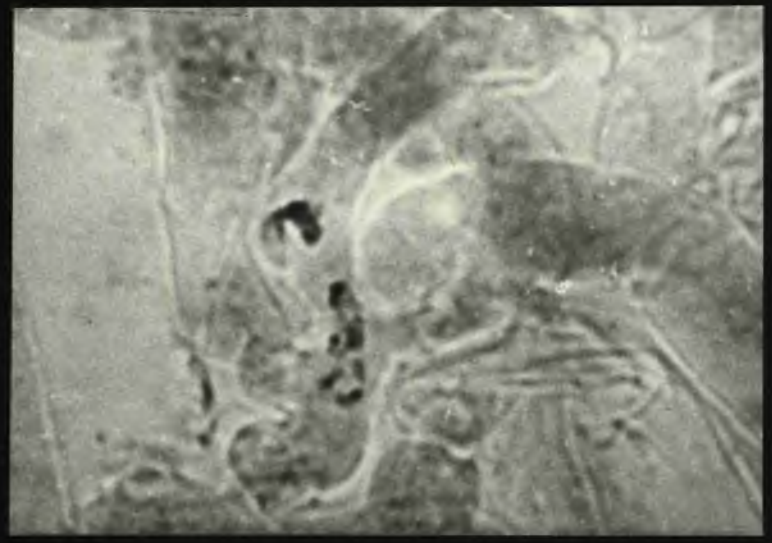


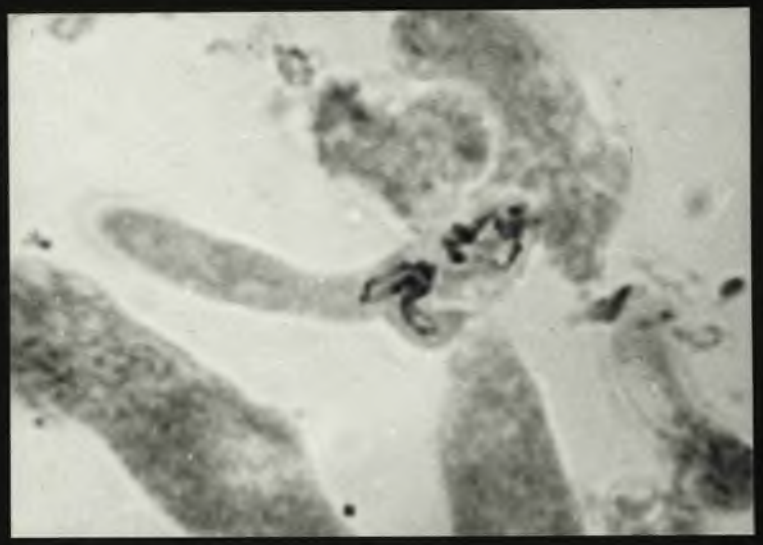

1b.

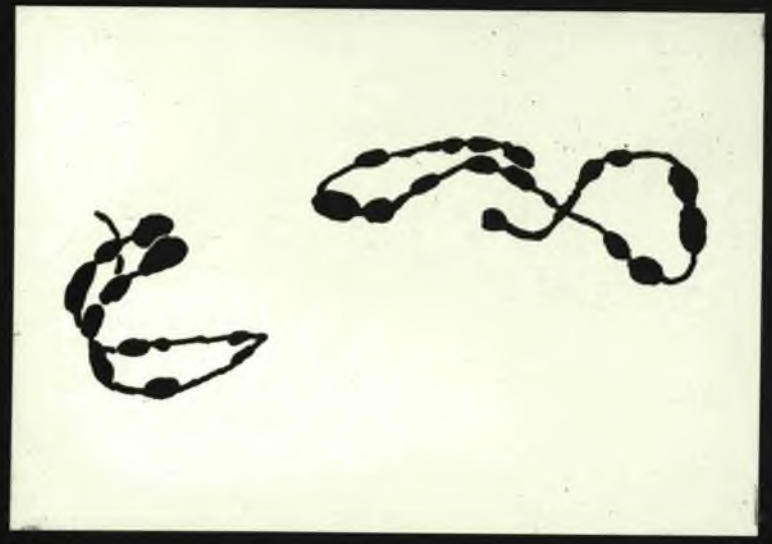



2a


2b







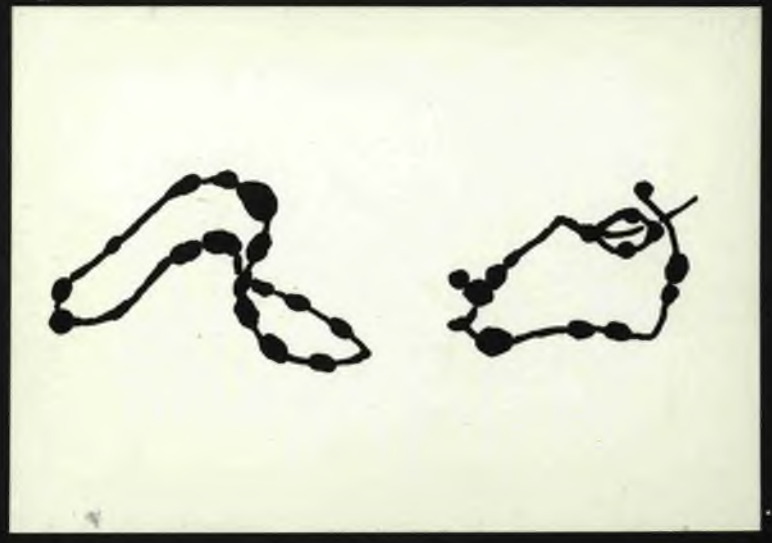


PLATE XXIII.

'Pre-metaphase'-'metaphase' in M. androsaceus.

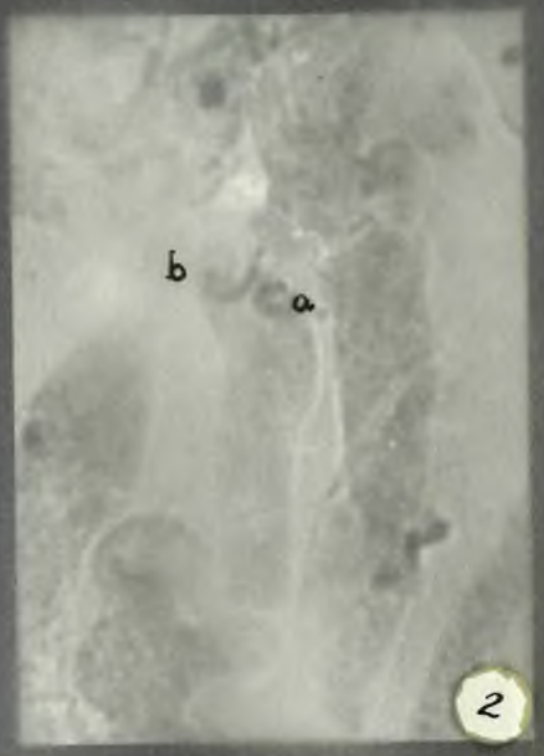
Fig. 1. Ultimate clamp showing nuclei consisting of double rings of beaded chromatin formed as a result of the doubling over of the figure of eight. Aceto-orcein stained; bright light.

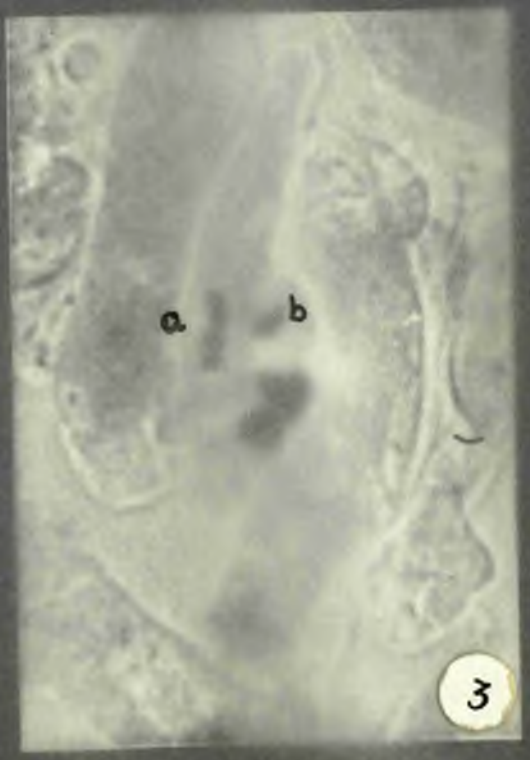
Fig. 2. Ultimate clamp showing two nuclei:(a) double ring of chromatin; (b) double ring of chromatin in which a break has occurred. Aceto-orcein stained; bright light.

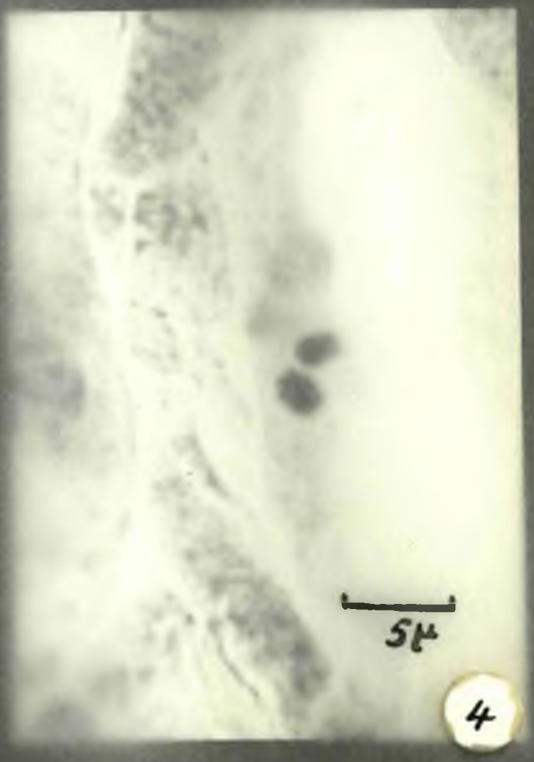
Fig. 3. Ultimate clamp showing(a) nucleus in which the arms of the double ring of chromatin have extended linearly after the break; (b) shows another ring of beaded chromatin stage. Aceto-orcein stained; bright light.

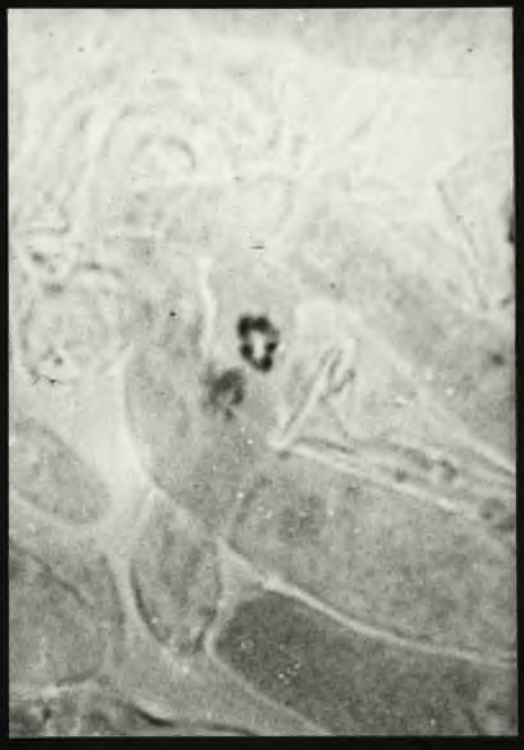
Fig. 4. Ultimate clamp showing nuclei in double ring of beaded chromatin stage. Aceto-orcein stained; bright light.

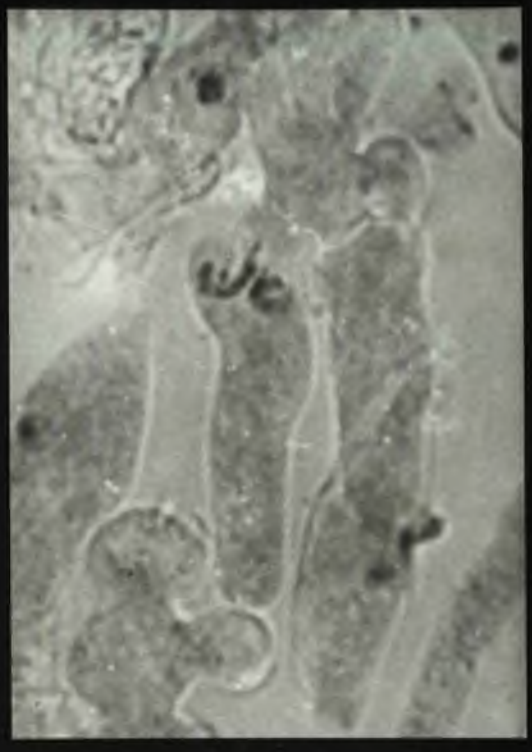

1

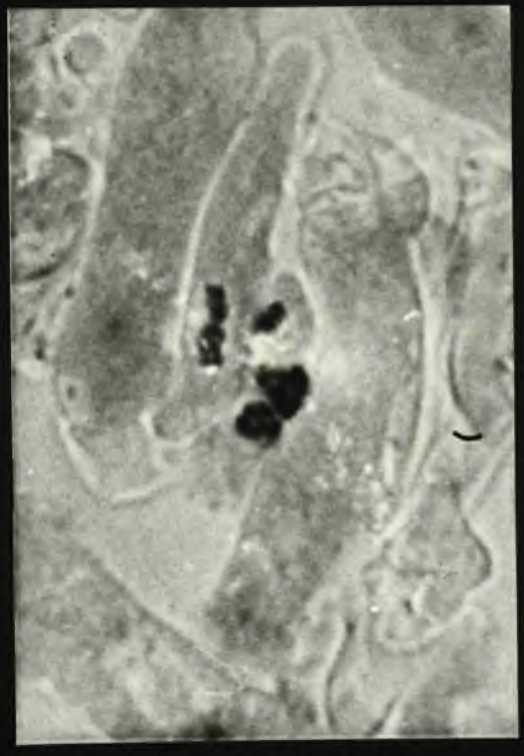

b
a
2


a
b
3


5μ
4







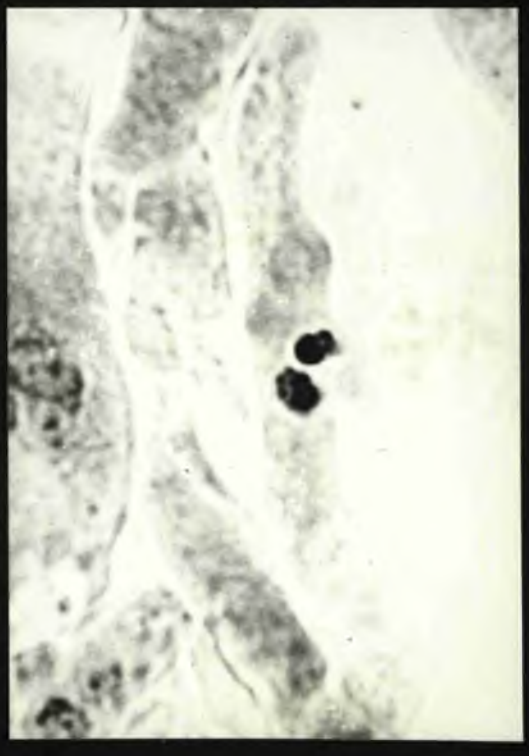


PLATE XXIV.

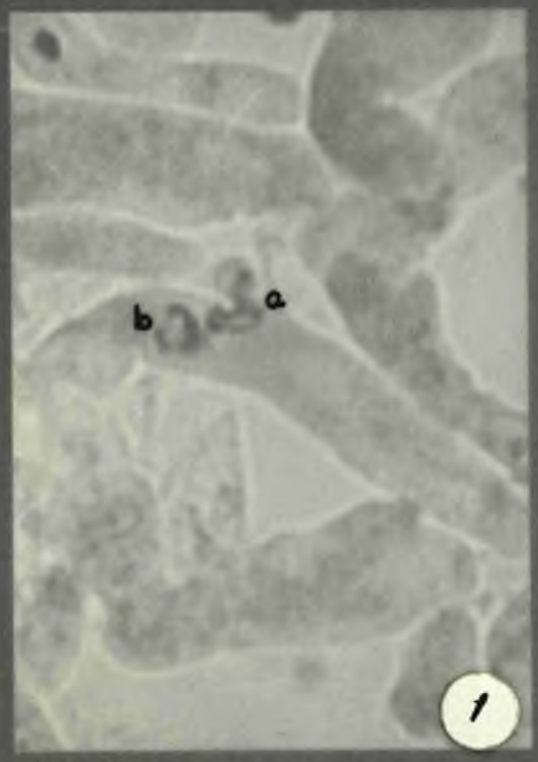
Nuclear division in the 'ultimate clamp' of M. androsaceus. 'Pre-metaphase' - 'metaphase'.

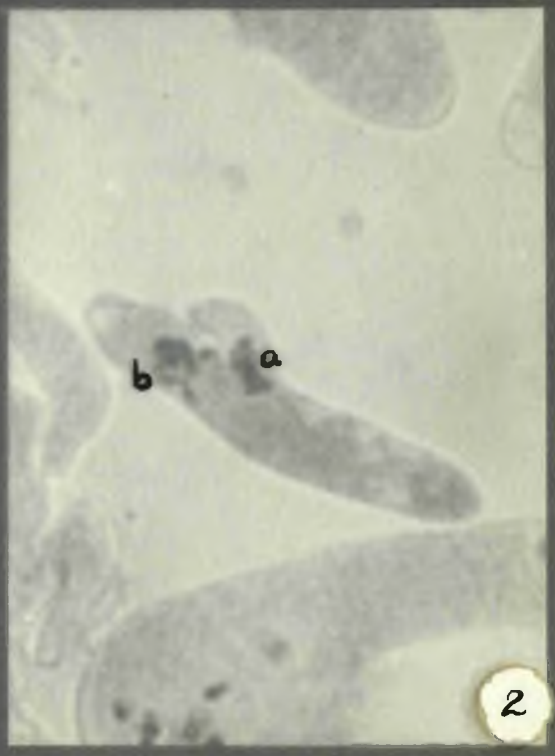
Fig. 1. Nucleus (a) undergoing twist leading to the formation of figure of eight; nucleus (b) a stage in advance of (a) showing double ring of chromatin. Aceto-orcein stained; bright light.

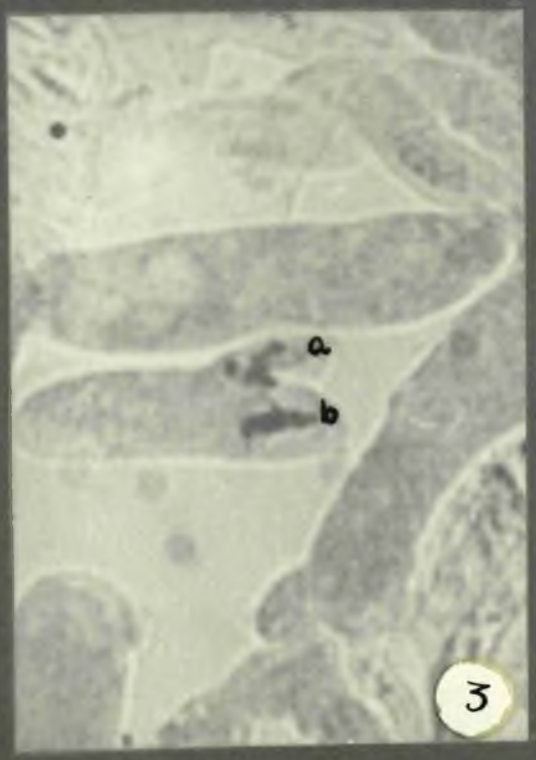
Fig. 2. Nucleus (a) showing chromatin 'metaphase' rods crossed on each other; nucleus (b) double ring of chromatin. Aceto-orcein stained; bright light.

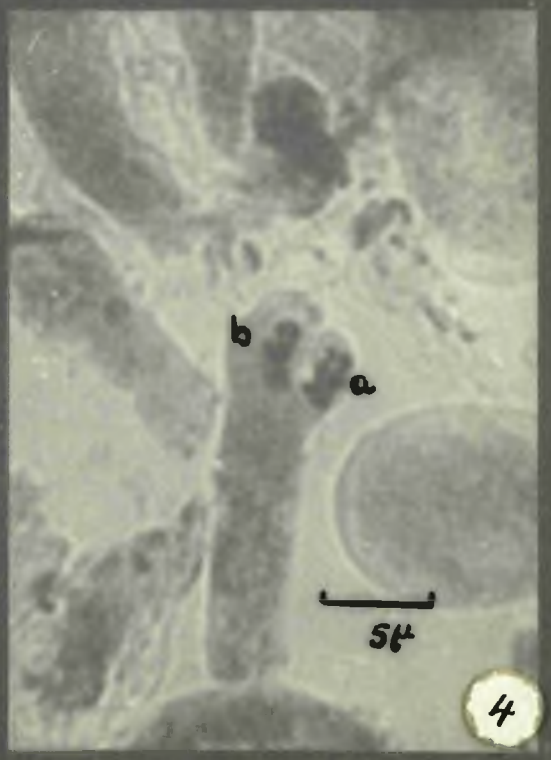
Fig. 3. Nuclei (a) and (b) in different stages of twist leading to formation of double ring of chromatin. Aceto-orcein stained; bright light.

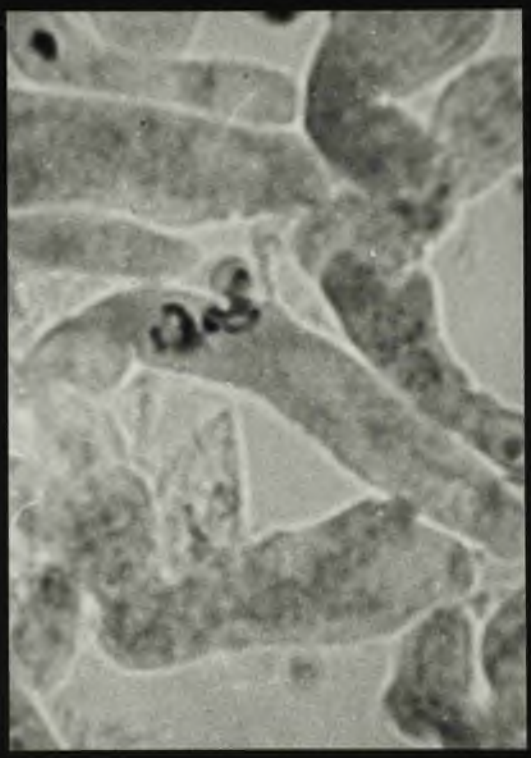
Fig. 4. Nucleus (a) showing two chromatin rods; nucleus (b) arms of chromatin rings opening out to give 'metaphase' rods. Aceto-orcein stained; bright light.

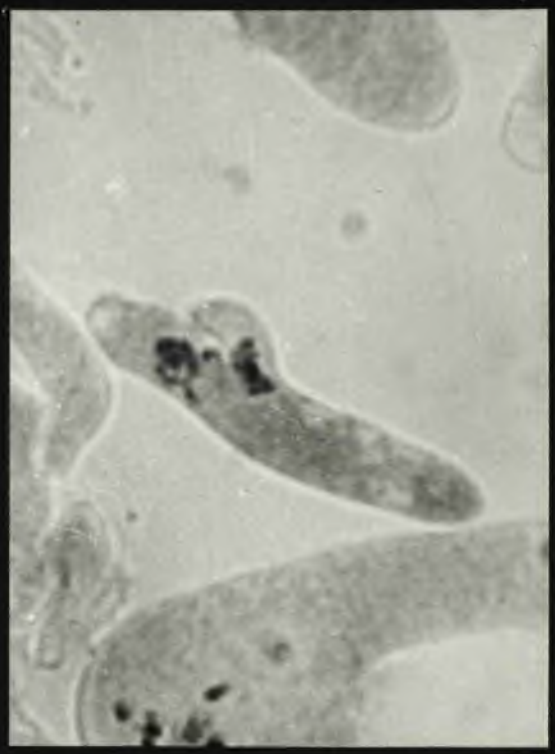

b
a
1

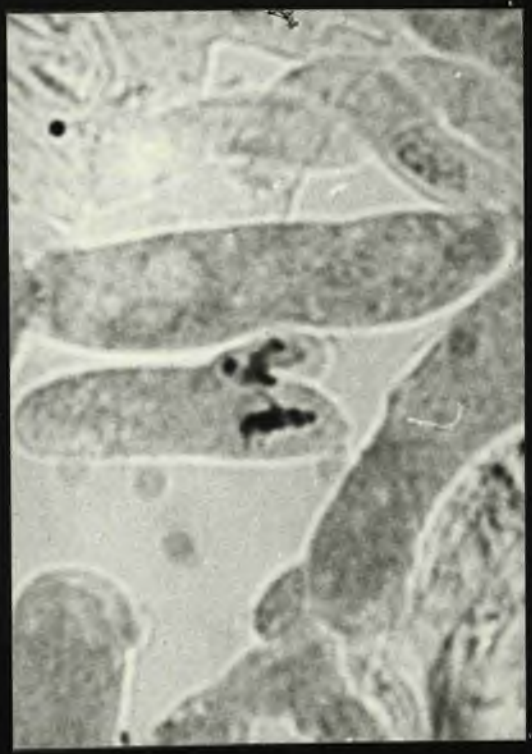

b
a
2


a
b
3


b
a
5μ
4







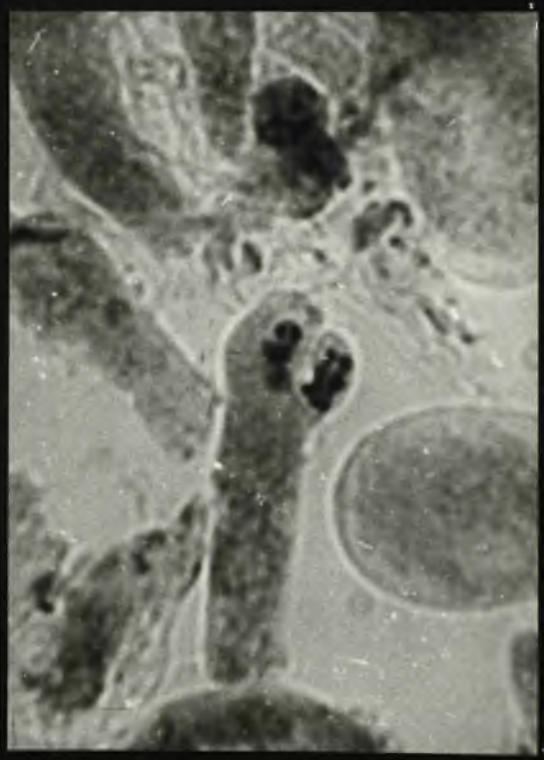


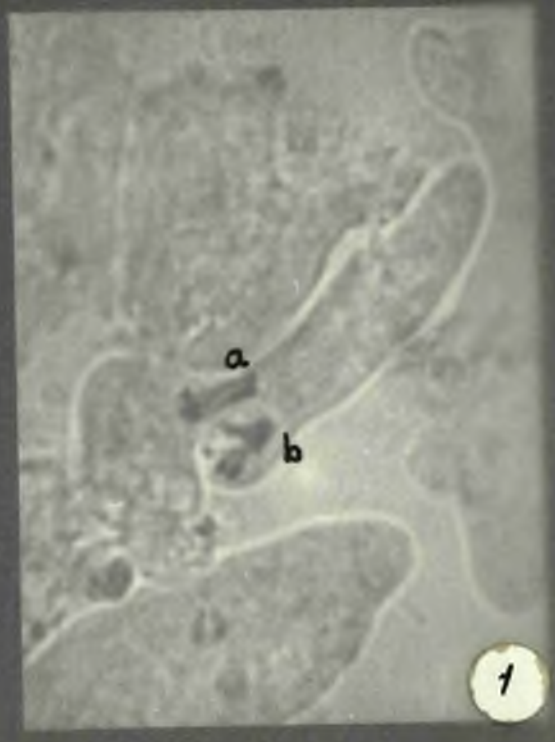
PLATE XXV.

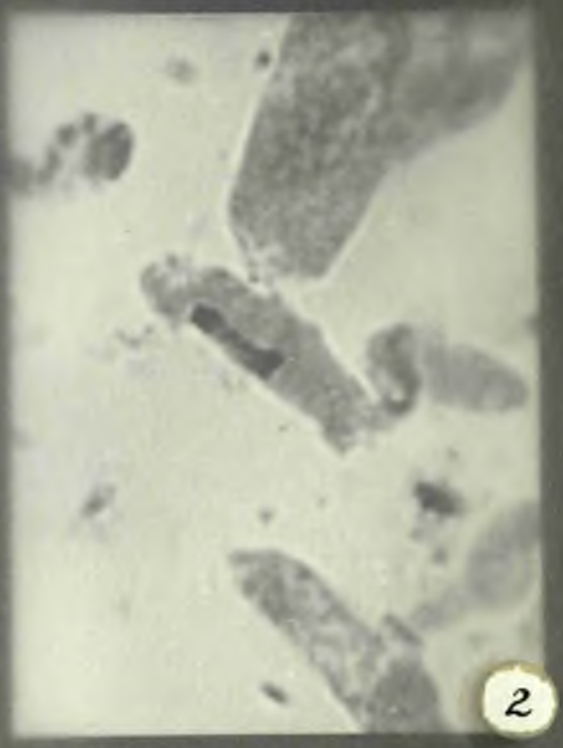
Nuclear division in the ultimate clamp of M. androsaceus. 'Metaphase' - 'anaphase'.

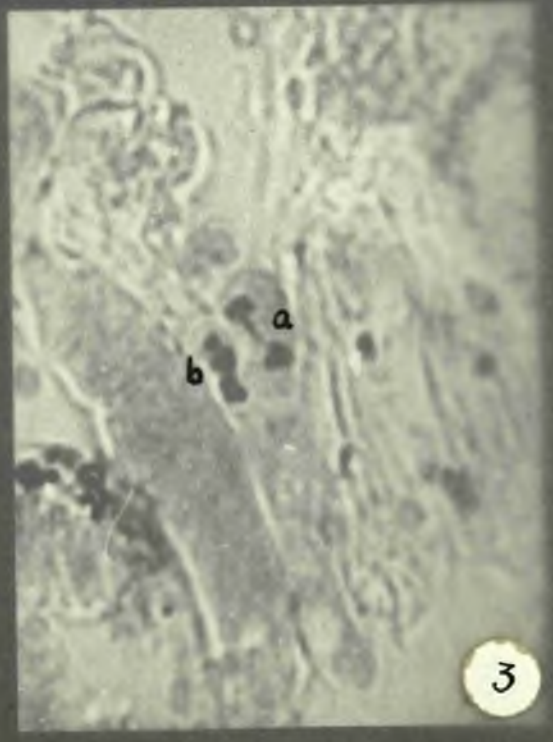
Fig. 1. (a) 'Metaphase' rods fully extended; (b) arms in process of extending to form metaphase rods. Aceto-orcein stained; bright light.

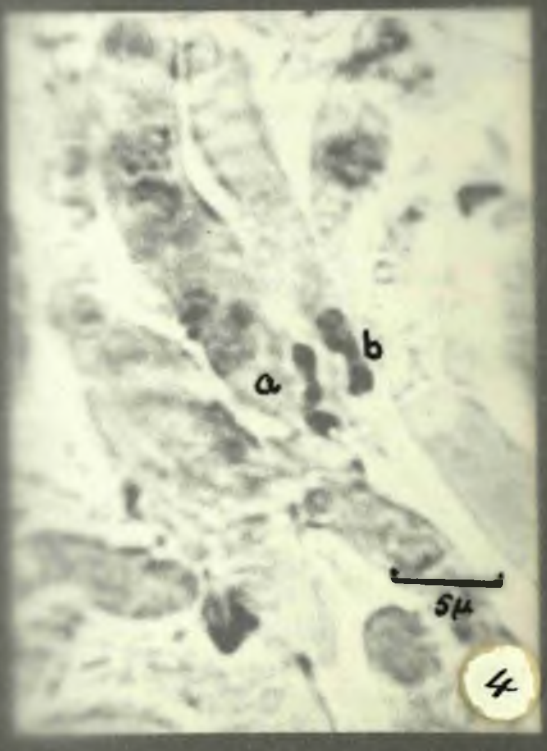
Fig. 2. 'Anaphase' separation of chromosome groups in nucleus of main hypha. Clamp has been torn away in preparation. Movement of chromosome groups parallel to direction in which metaphase rods lay. Aceto-orcein stained; bright light.

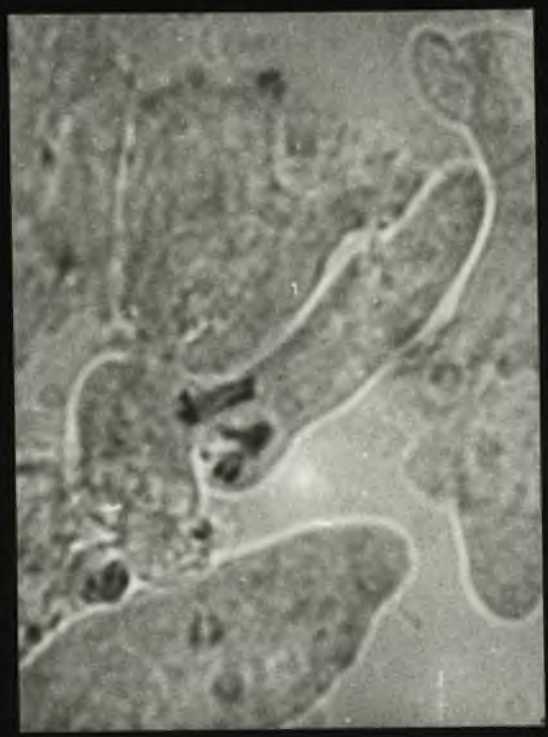
Figs. 3 & 4. 'Anaphase' separation of chromosome groups; (a) in both figures showing bridges. Beaded appearance of rods evident in (b) in both cases. Aceto-orcein stained; bright light.

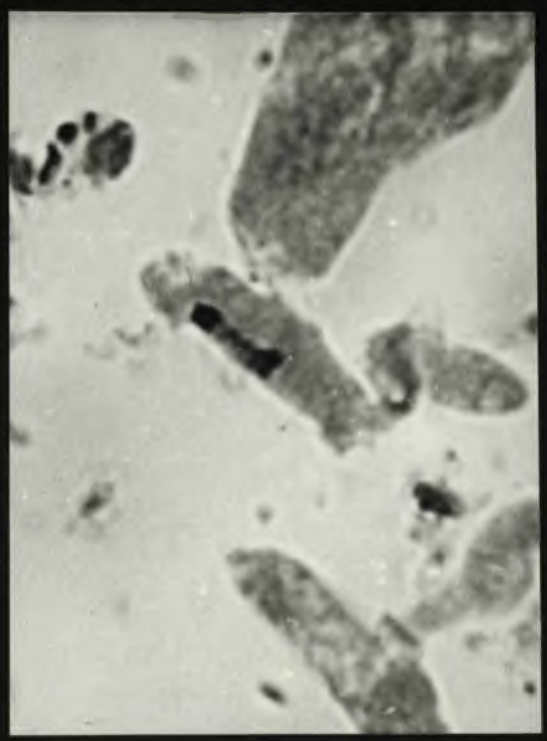

a
b
1

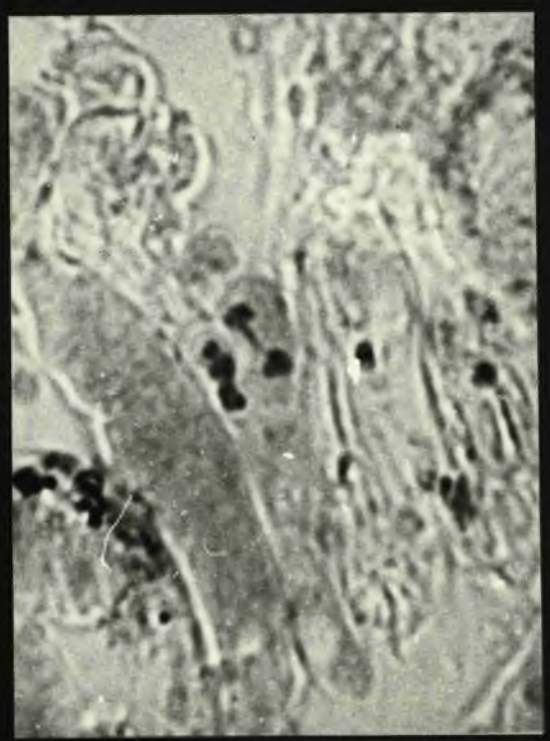

2

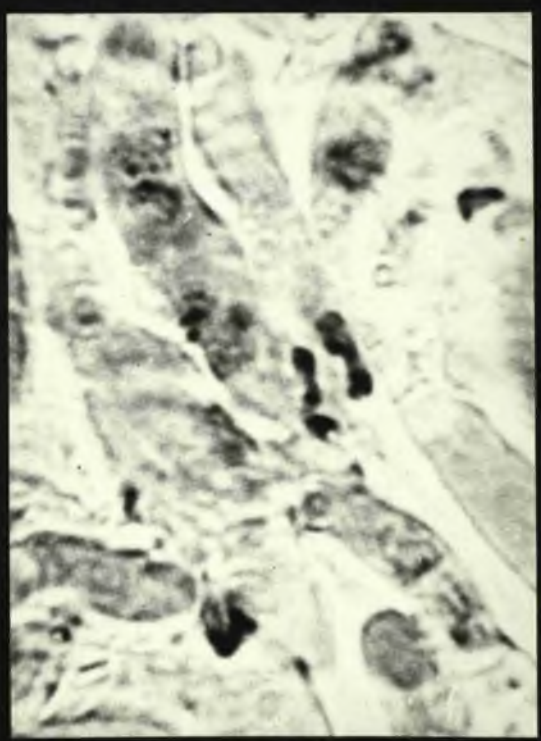

a
b
3


a
b
5μ
4









## PLATE XXVI.

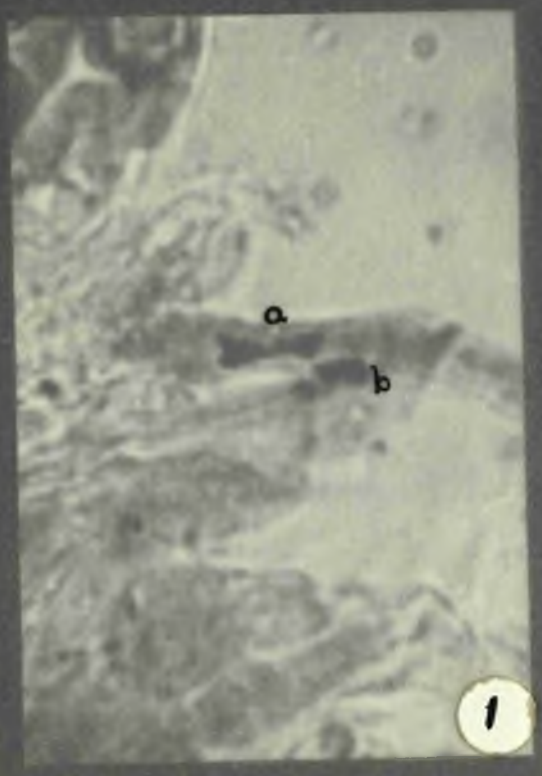
Nuclear division in ultimate clamp of M. androsaceus. 'Anaphase'.

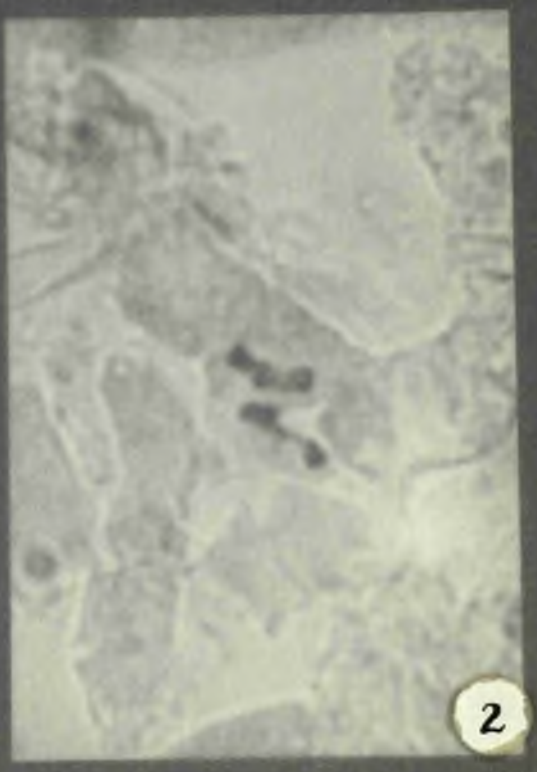
Fig. 1. Separation in (a) in advance of separation in (b). Aceto-orcein stained; bright light.

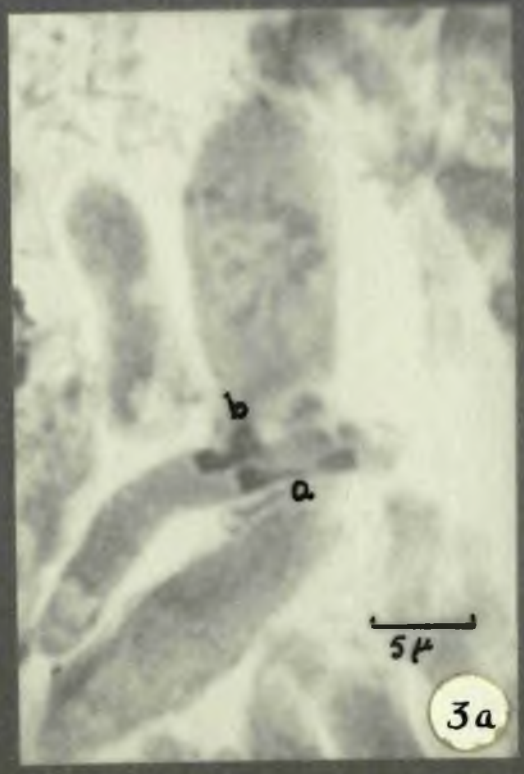
Fig. 2. Separation in both nuclei approximately at the same stage. Bridges are evident in both nuclei. Aceto-orcein stained; bright light.

Fig. 3a. (a) in an advanced state of separation over (b); 'chromosomes' seen in groups moving to the left. Aceto-orcein stained; bright light.

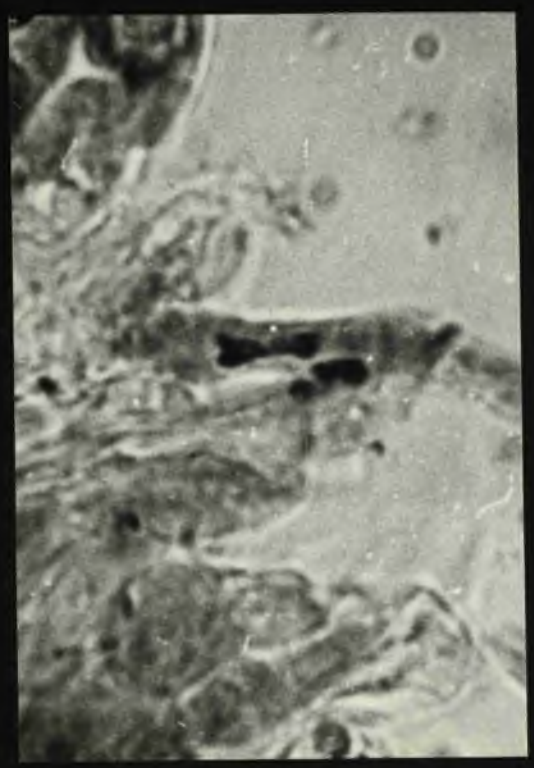
Fig. 3b. Drawing of nuclei seen in Fig. 3a.

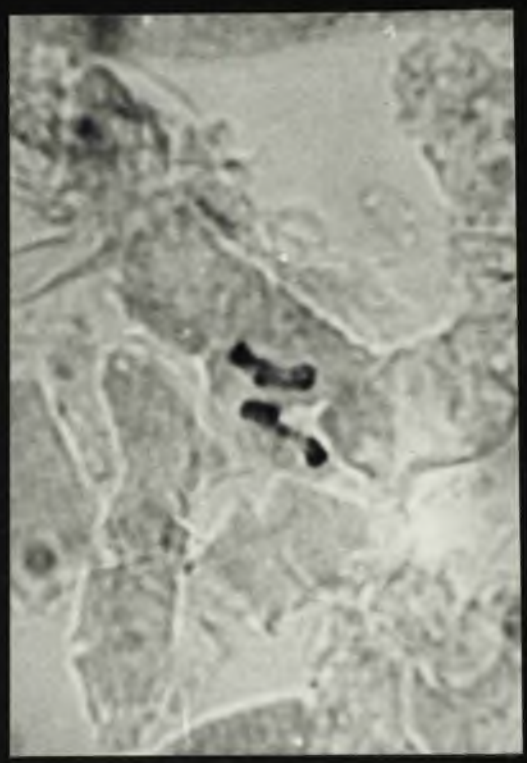

a
b
1

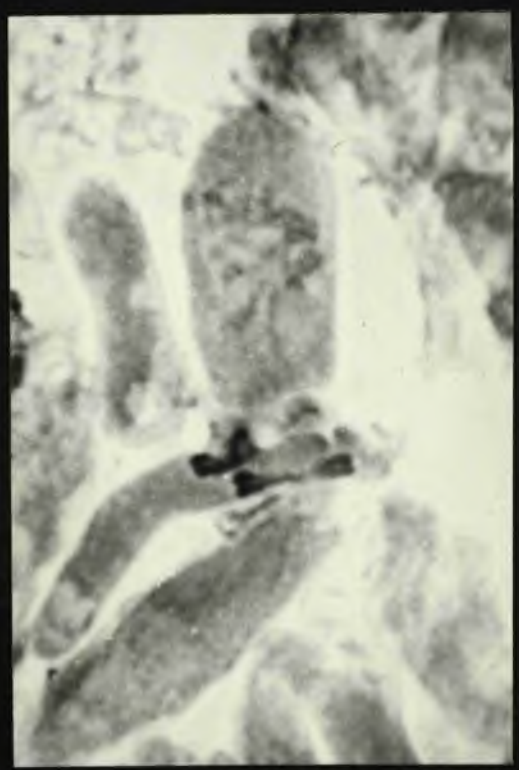

2


b
a
5μ
3a


a
b
3b







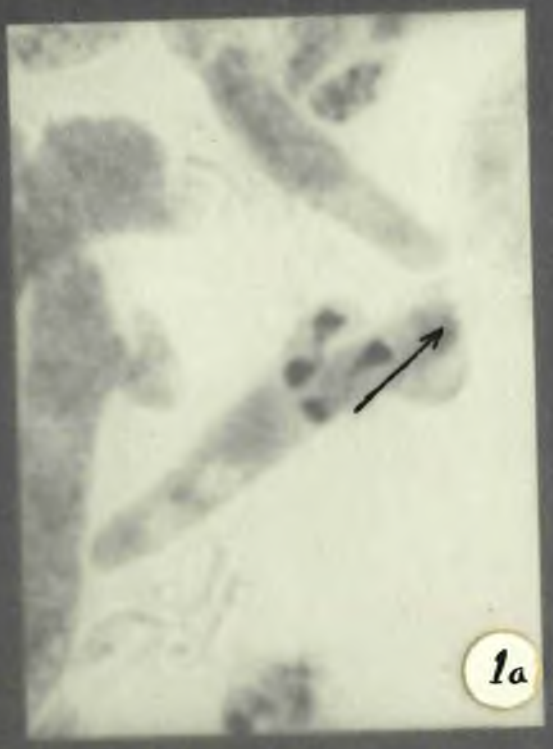
# PLATE XXVII.

Nuclear division in ultimate clamp of M. androsaceus. 'Late anaphase'.

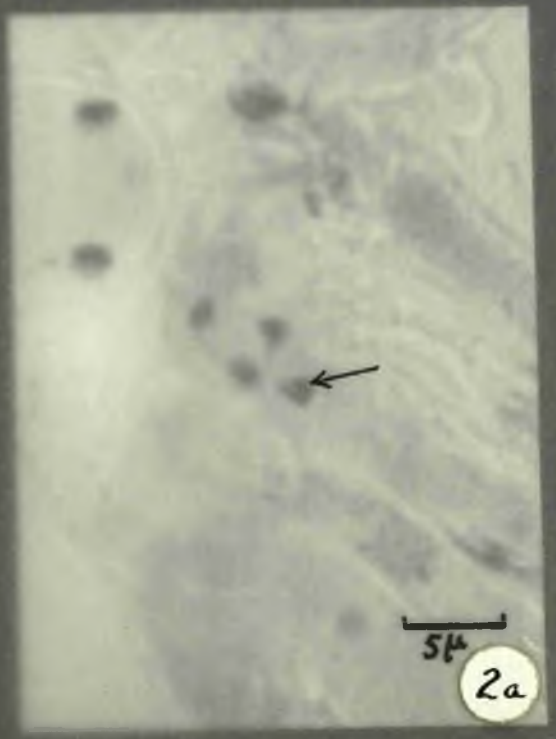
Fig. 1a. Bridges are seen connecting chromosome groups Aceto-orcein stained.

Fig. 1b. Drawing of nuclei above.

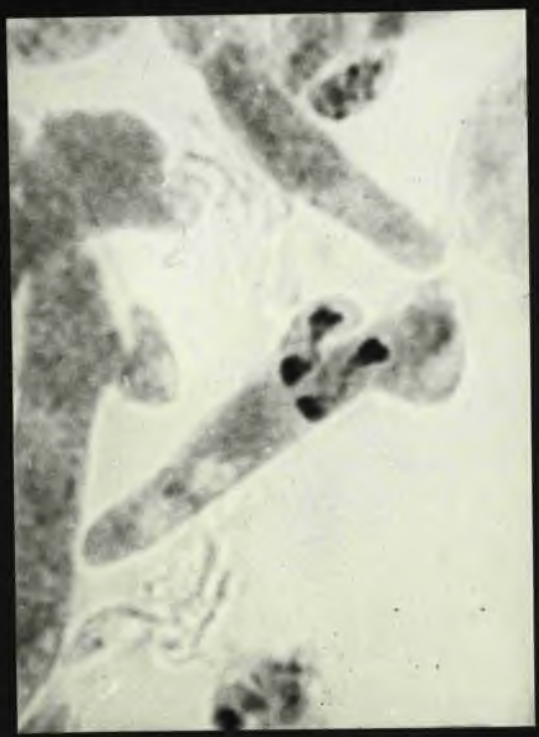
Figs. 2a and 2b. Nuclei seen with bridges between chromosome groups. Arrow indicates daughter nucleus in which 3 of the 4 'chromosomes' can be seen. Aceto-orcein stained; bright light.

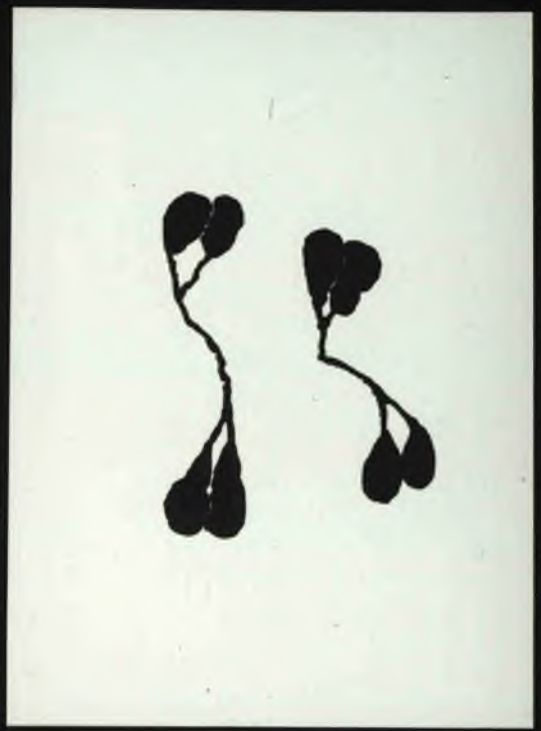

1a

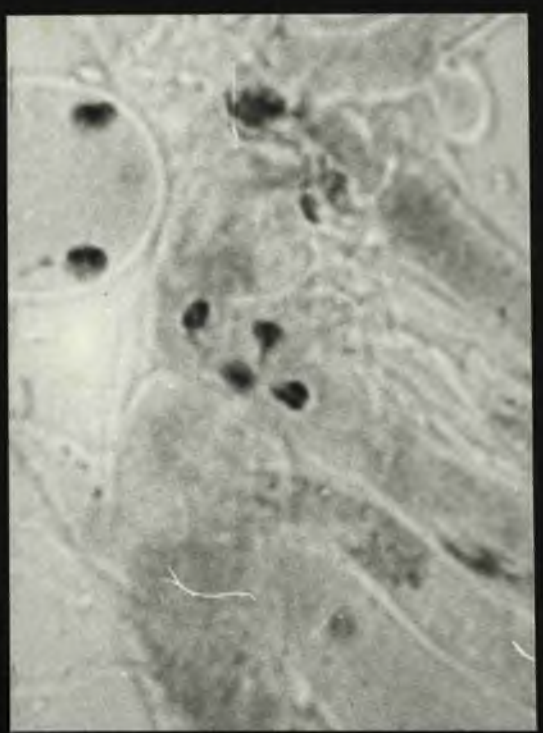

1b


5μ
2a


2b



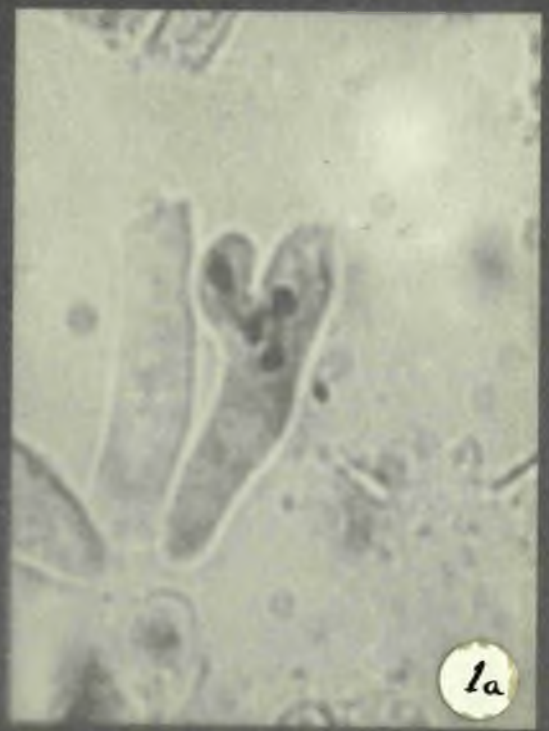




# PLATE XXVIII.

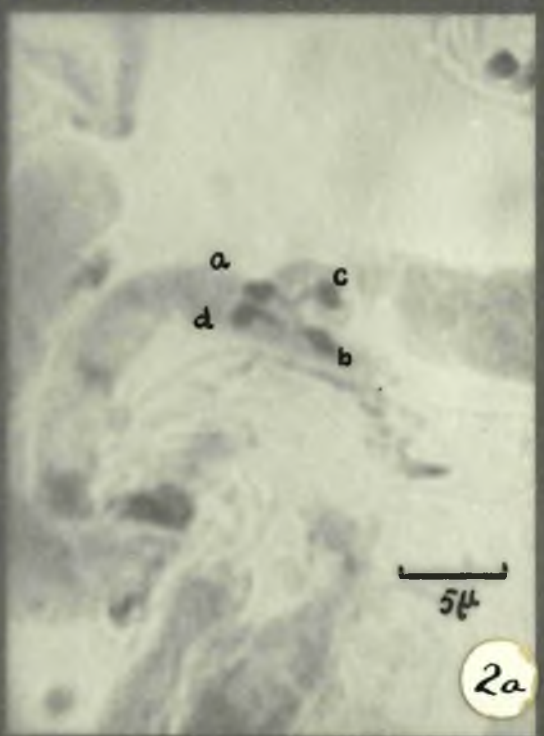
Nuclear division in ultimate clamp of *M. androsaceus*.
Late 'anaphase' - early 'telophase'.

Figs. 1a and 1b. Daughter nuclei showing chromatin rounded off into four 'chromosomes'.
Figs. 2a and 2b. Bridges still persist. Aceto-orcein stained; bright light.

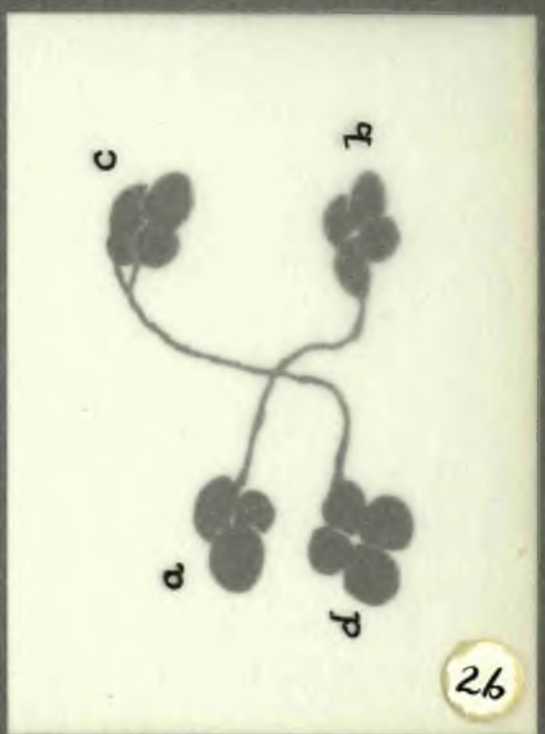


1a

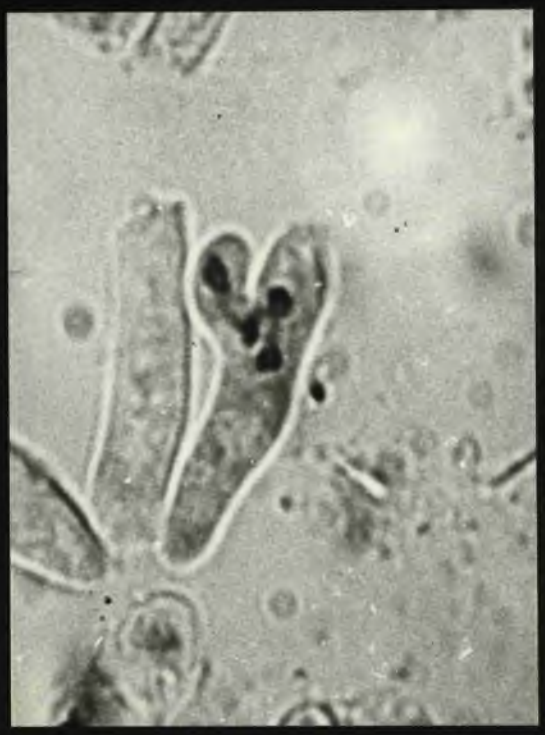
1b

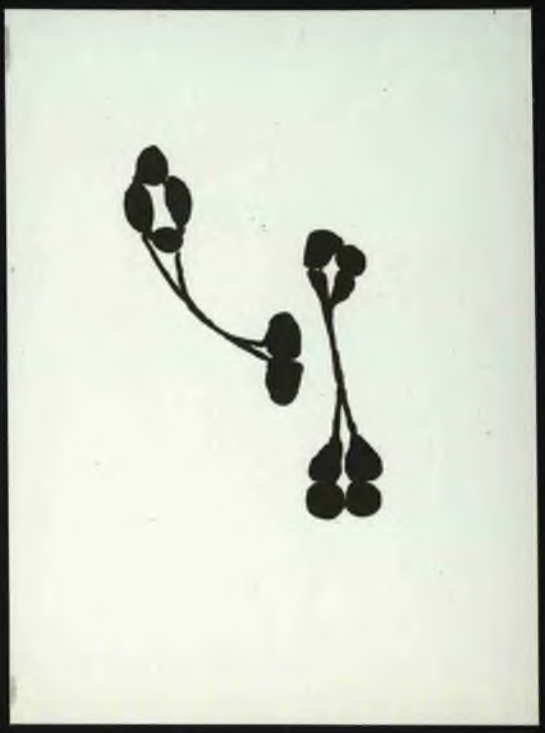


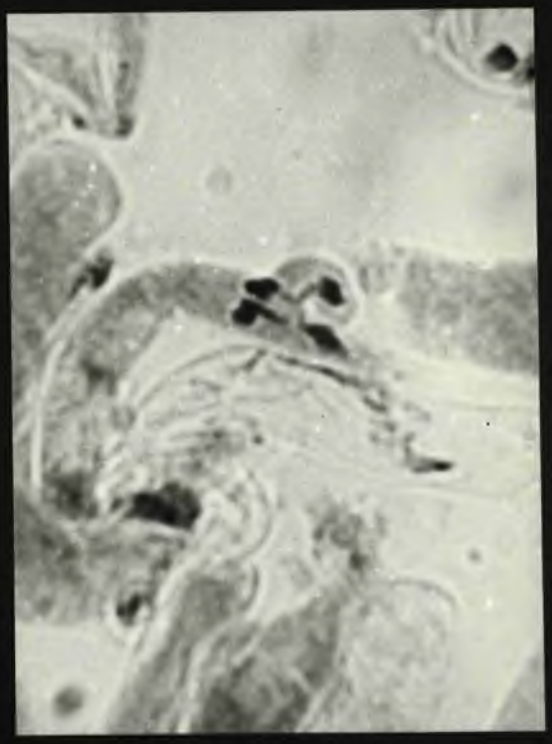

2a

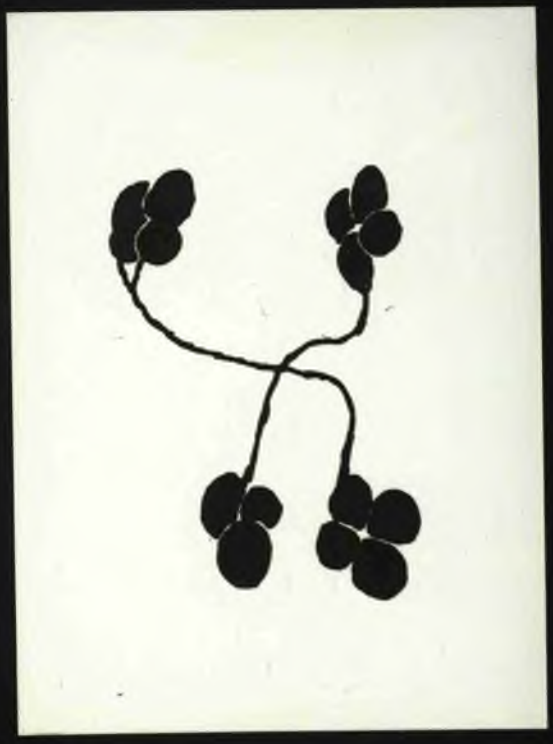


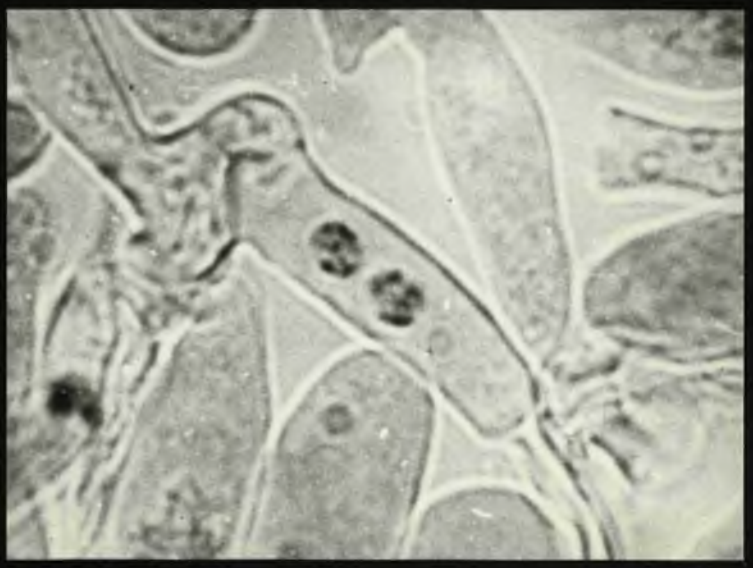

2b











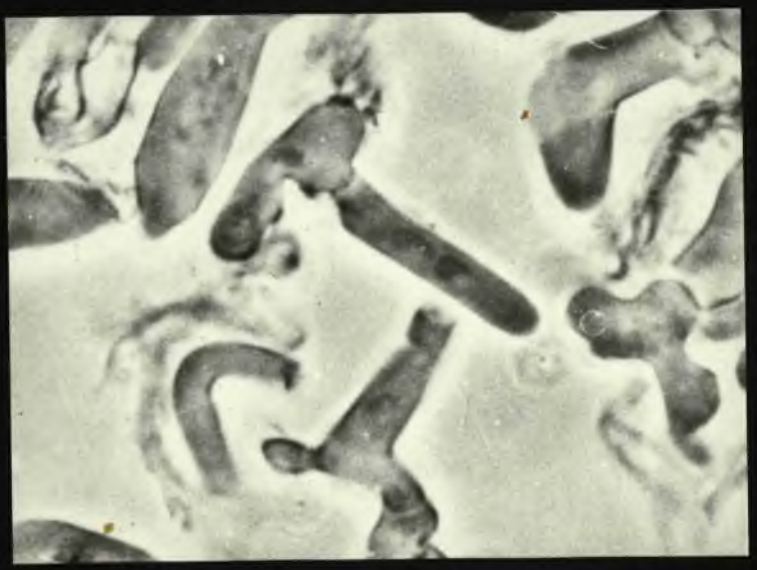
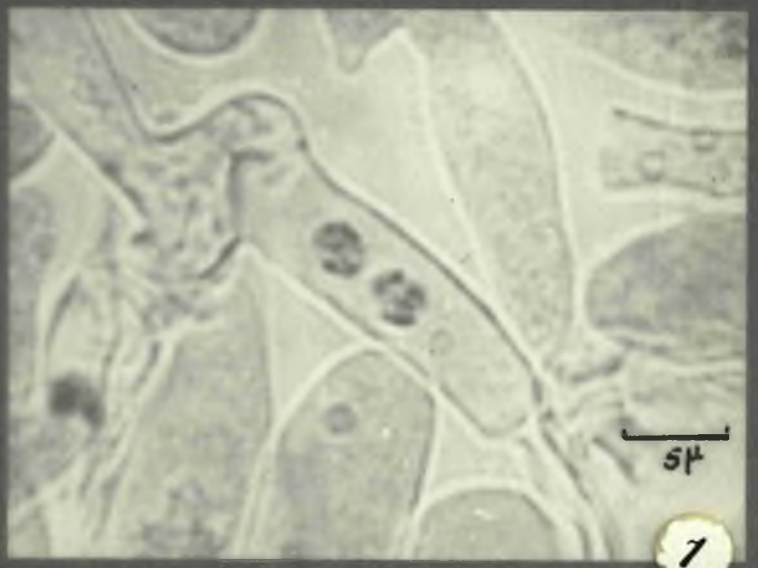


PLATE XXIX.

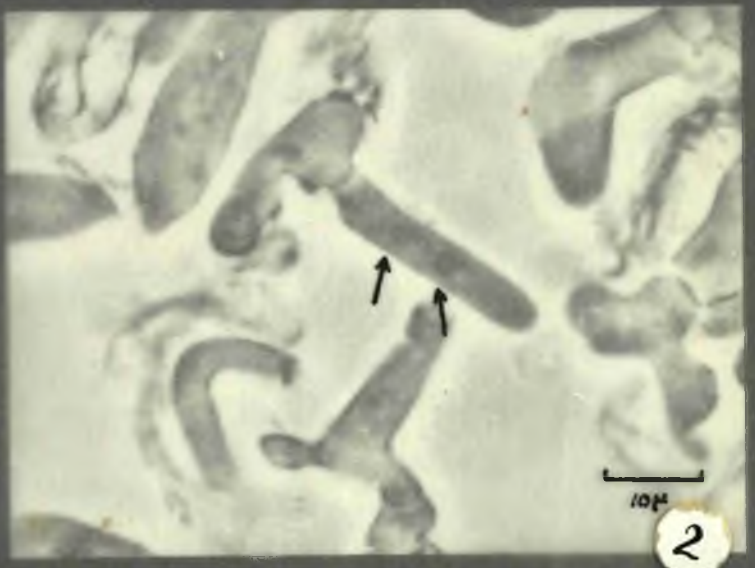
Young basidia of *M. androsaceus*.

Fig. 1. Migratory nuclei within young basidium. Granular chromatin surrounding central nucleus. Aceto-orcein stained; bright light.

Fig. 2. A later stage than Fig. 1. Nuclei move to centre of basidium prior to fusion. Nucleoli (arrows) lead the way; chromatin massed at rear of nucleolus. Feulgen stained; phase contrast.




1




2

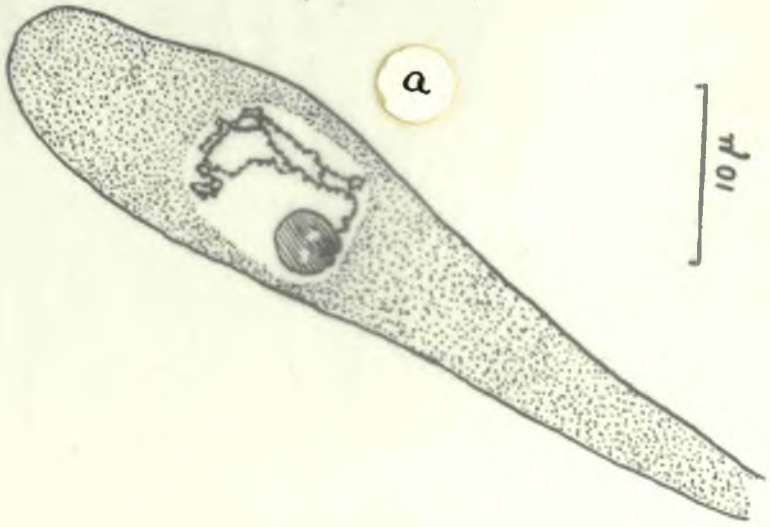
PLATE XXX.

Drawings of basidia of *M. androsaceus*.

Fig. (a) Large fusion nucleus showing prominent nucleolus with attached long, beaded chromatin strand. (see Plate XXXI, fig. 2).

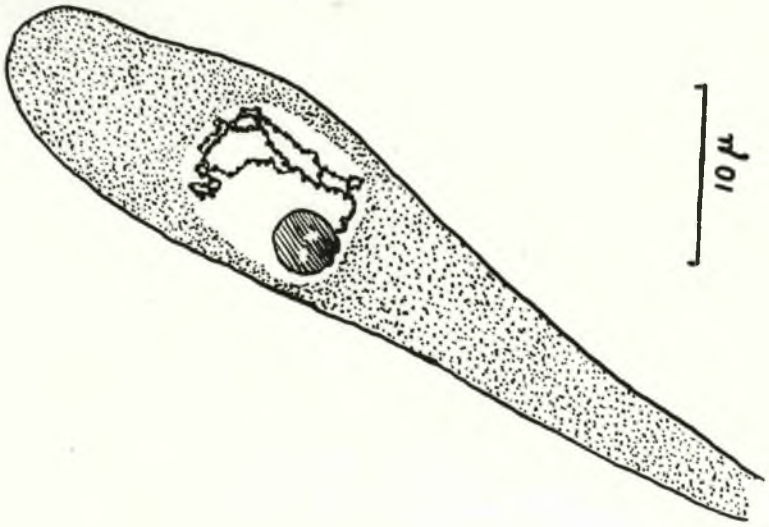
Fig. (b) Nuclei approach each other prior to fusion; thin cytoplasmic barrier exists between them. Nucleoli prominent with attached chromatin strands.

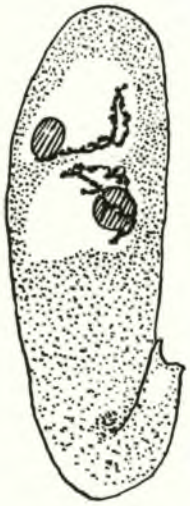
Fig. (c) Cytoplasmic barrier between nuclei has broken down; nucleoli fuse.


a
10 μ


b
10 μ


c
10 μ


10 μ


10 μ

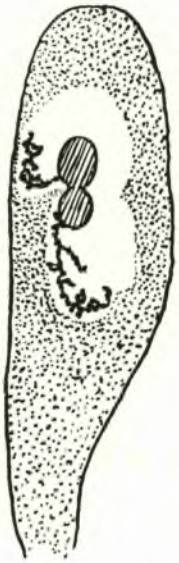

10 μ

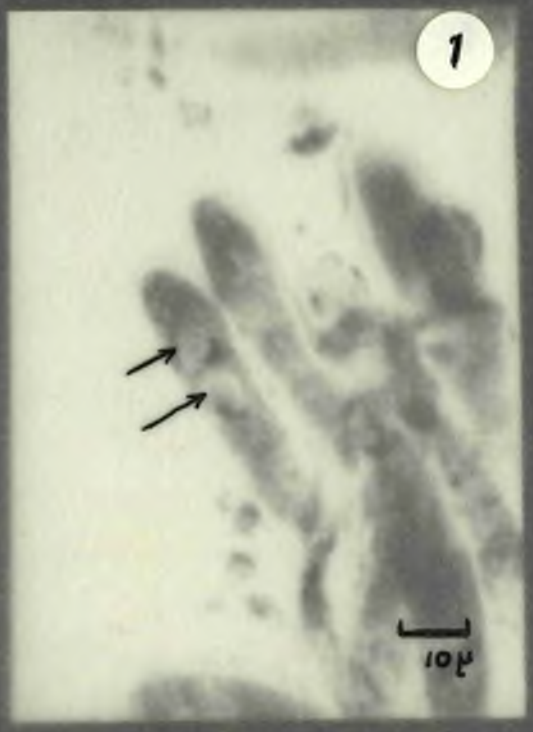
PLATE XXXI.

Young basidia of M. androsaceus.

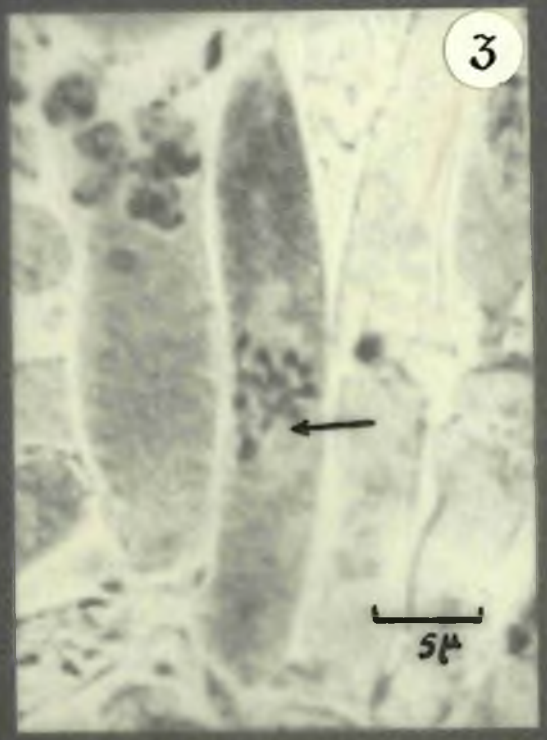
Fig. 1. Nuclei approaching each other prior to fusion; thin cytoplasmic barrier exists between them. Nucleoli (arrows) prominent. Aceto-orcein stained; bright light.

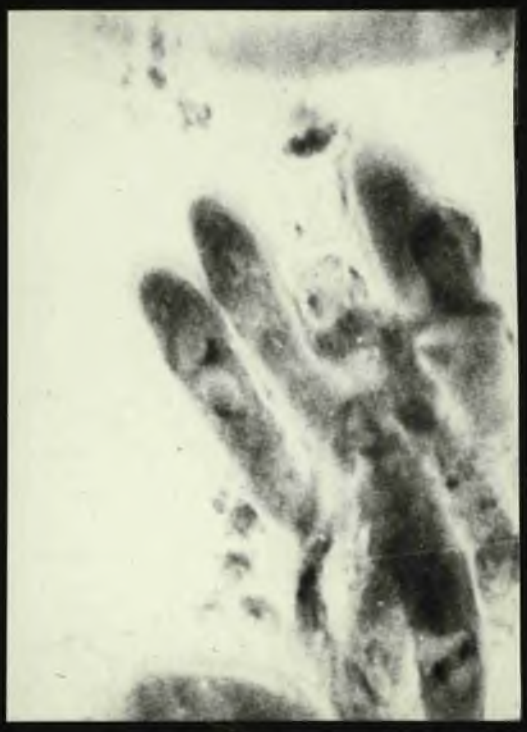
Fig. 2. Fusion nucleus, showing prominent nucleolus with attached chromatin strand. Aceto-orcein stained; bright light.

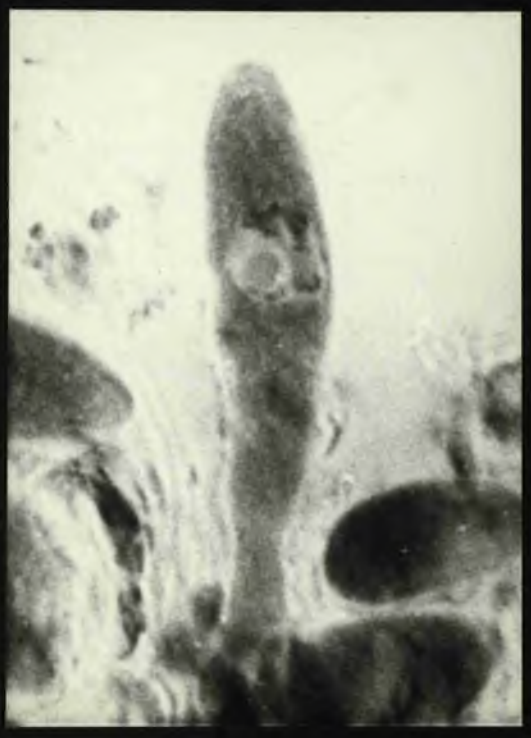
Fig. 3. Fusiform cell of hymenium of M. androsaceus showing large central fusion nucleus with nucleolus (arrow) and prophase-like chromosomes. Aceto-orcein stained; bright light.


1
10μ


2
10μ


3
5μ





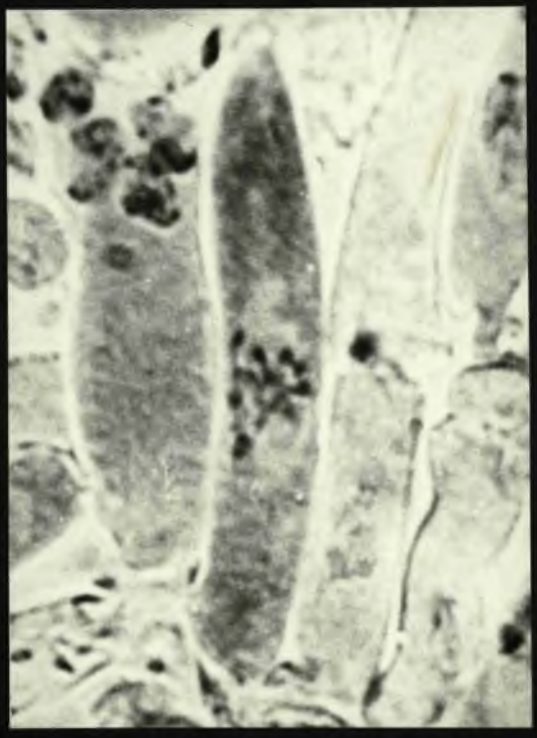


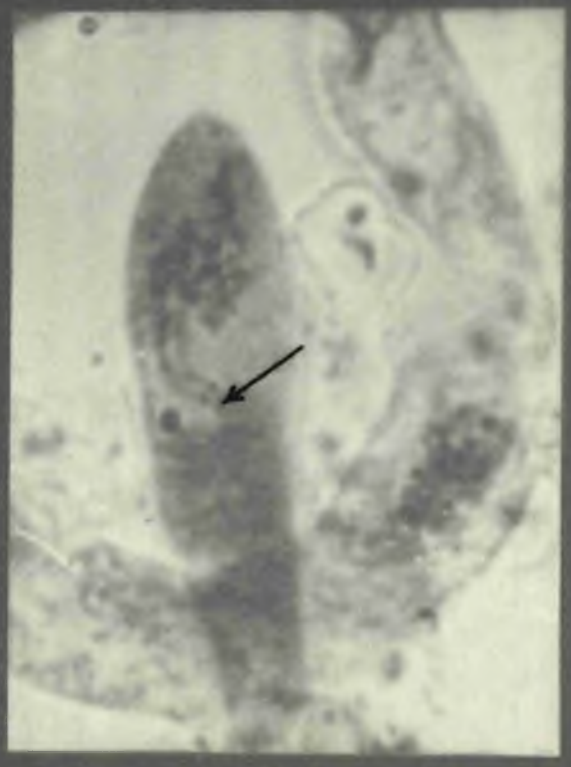
PLATE XXXII.

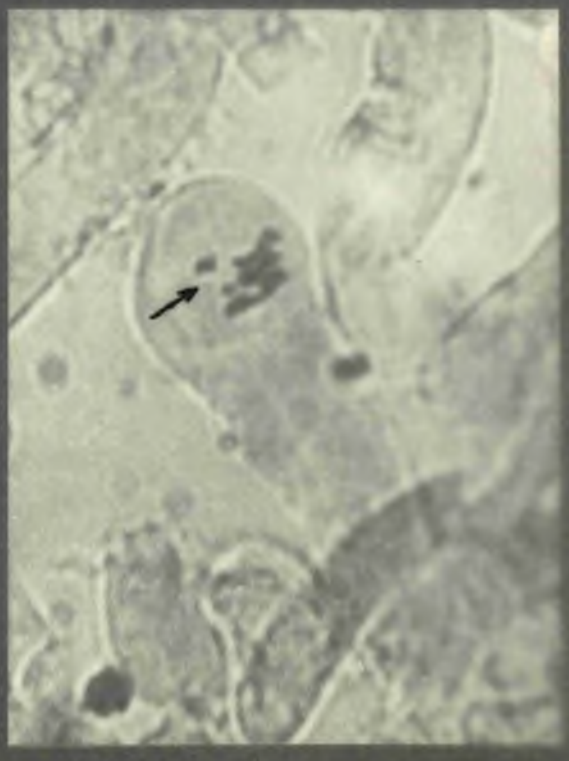
Meiosis in basidium of M. androsaceus.

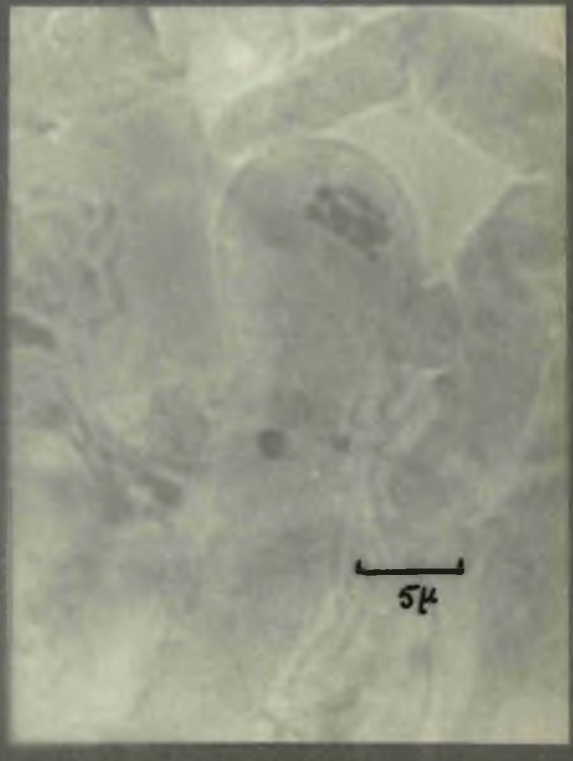
Figs. 1a & b. Zygonema. Four pairs of greatly elongated chromosomes are seen, one of which is attached to the nucleolus (arrow); pairing of 'beads' on homologous chromosomes seen. Aceto-orcein stained; bright light.

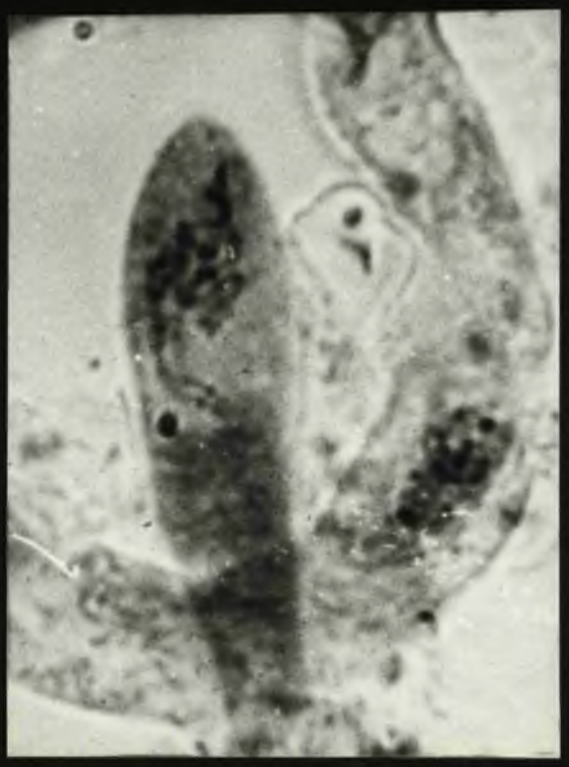
Fig. 2. Pachytene. Bivalents seen lying free in cytoplasm, showing orientation parallel to each other and at right angles to long axis of basidium. Beaded appearance still evident. Satillited chromosome II (arrow) seen. Aceto-orcein stained; phase contrast.

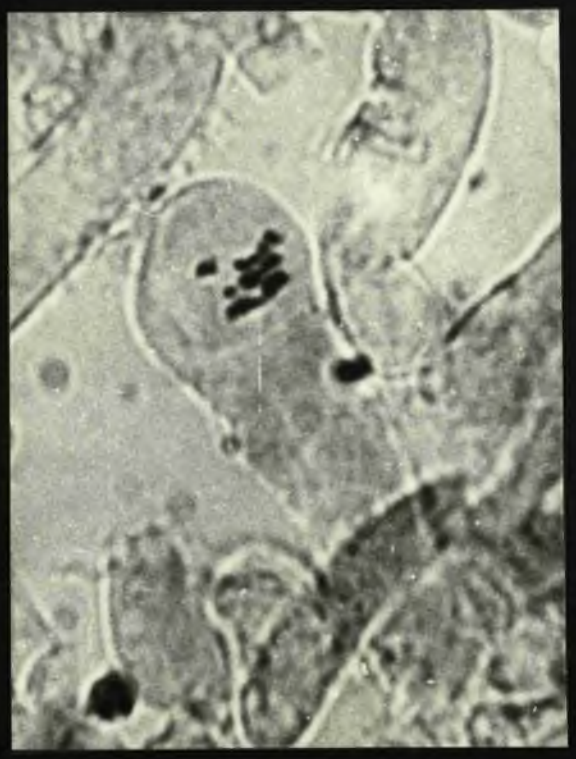
Fig. 3. Pachytene. Bivalents free in cytoplasm of basidium; nucleolus has disappeared; chromosomes all parallel to each other and at right angles to short axis of the basidium. Aceto-orcein stained; bright light.






5μ





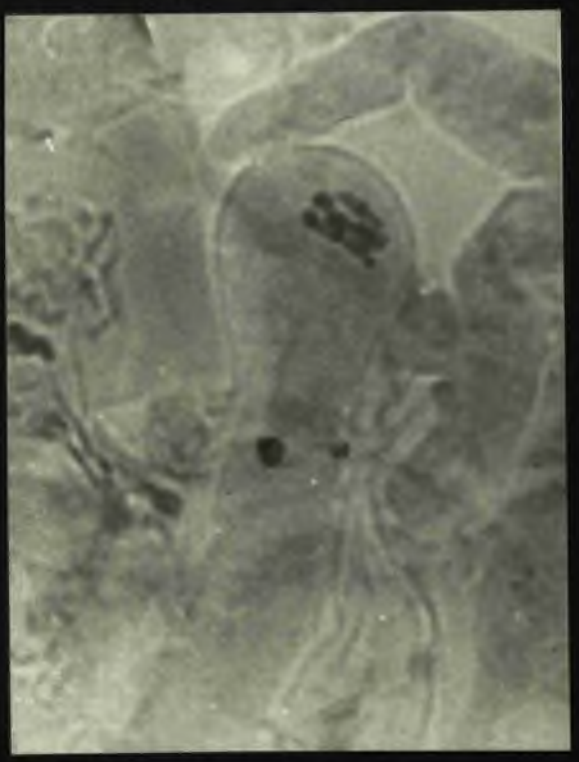


PLATE XXXIII.

Meiosis in the basidium of M. androsaceus.

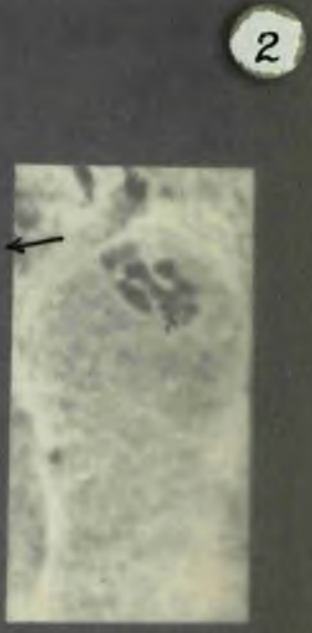
Fig. 1. Pachytene. Bivalents parallel to each other; nucleolus has disappeared. Aceto-orcein stained; phase contrast.

Fig. 2. Pachytene. Four bivalents seen; beaded appearance still evident. Chiasma (arrow) seen in longest chromosome. Aceto-orcein stained; bright light.

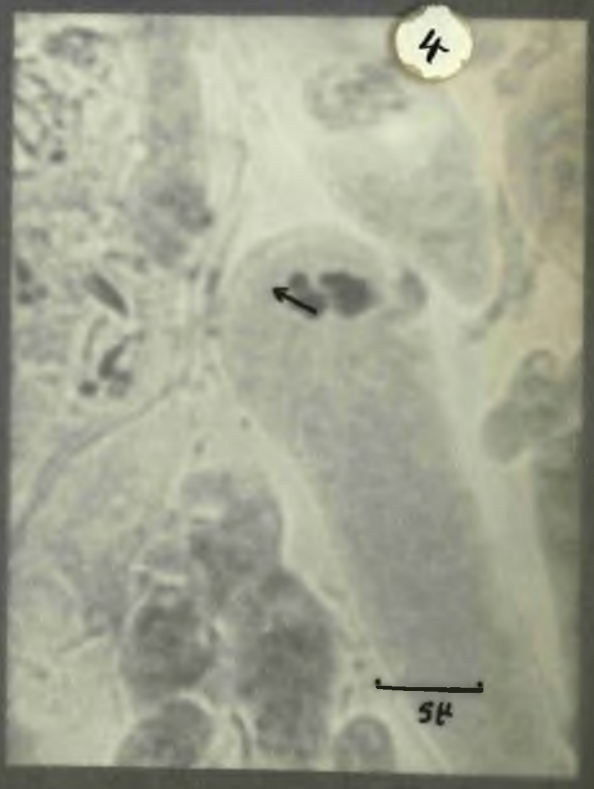
Fig. 3. Diakinesis. Bivalents have lost their beaded appearance, but are still parallel to each other and are now connected one to the other by a faintly stained thread. Aceto-orcein stained; phase contrast.

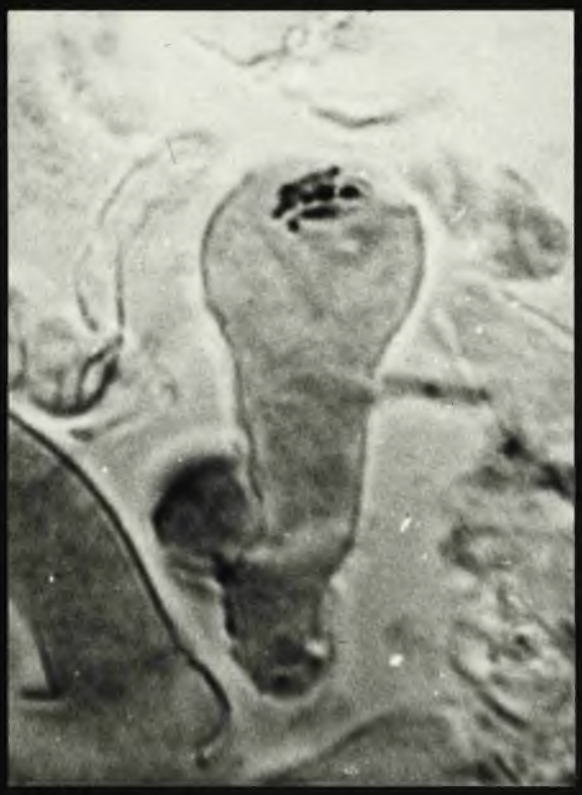
Fig. 4. Pre-metaphase. Bivalents connected by a thread one to the other, arranged in ring. Duality of arrowed bivalent is seen. Aceto-orcein stained; bright light.

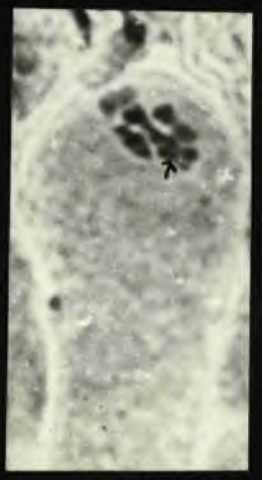

1

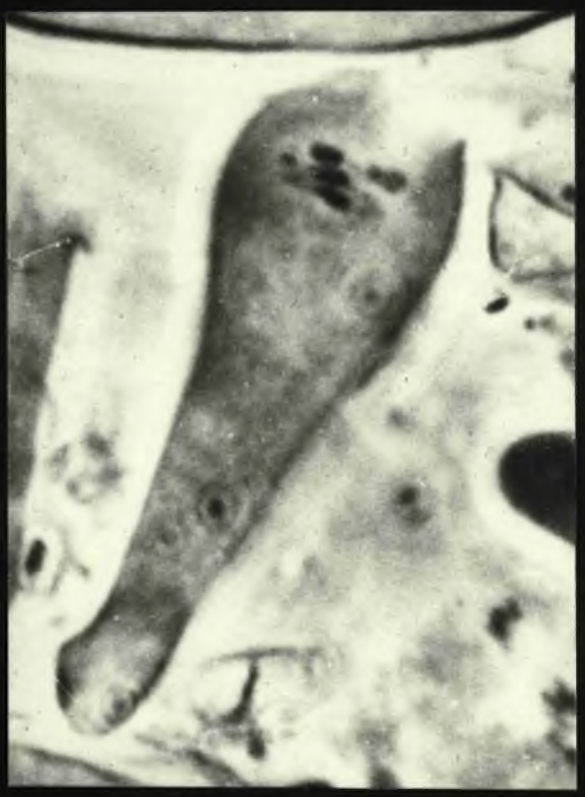

2


3


4
5μ







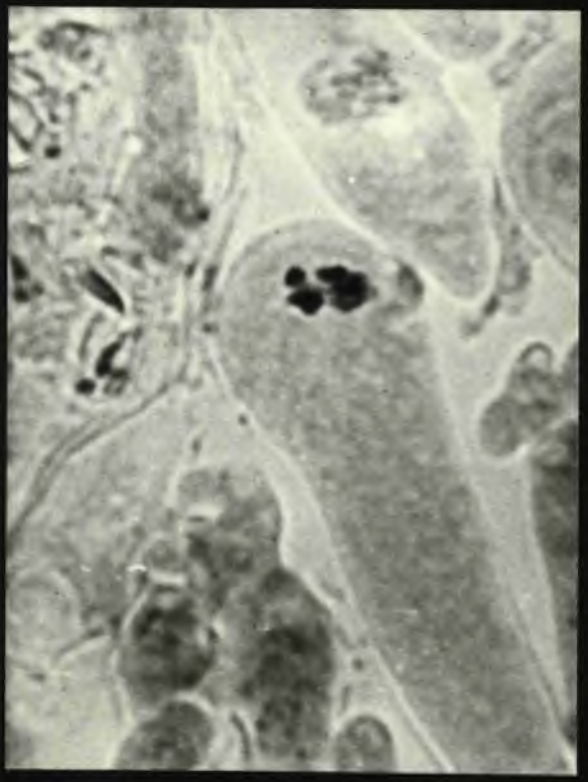
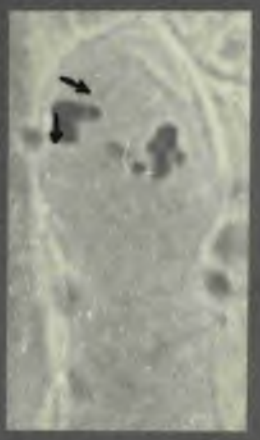


PLATE XXXIV.

Meiosis in the basidium of M. androsaceus.

Figs. 1 a & b. Anaphase. Four chromosomes seen in each group; satillited chromosome (arrowed). Faint thread still connects the chromosomes to each other and one group to the other, forming a 'bridge'. Aceto-orcein stained; bright light.

Figs. 2 a & b. Anaphase. Four chromosomes are seen in each group; smallest chromosomes lagging behind. Connecting thread still evident and forming a 'bridge' between the groups. Aceto-orcein stained; bright light.



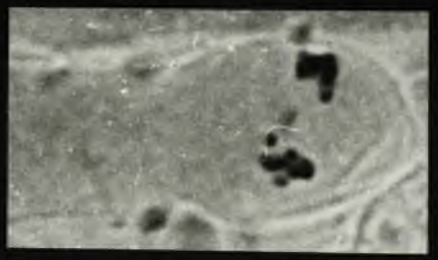
1a

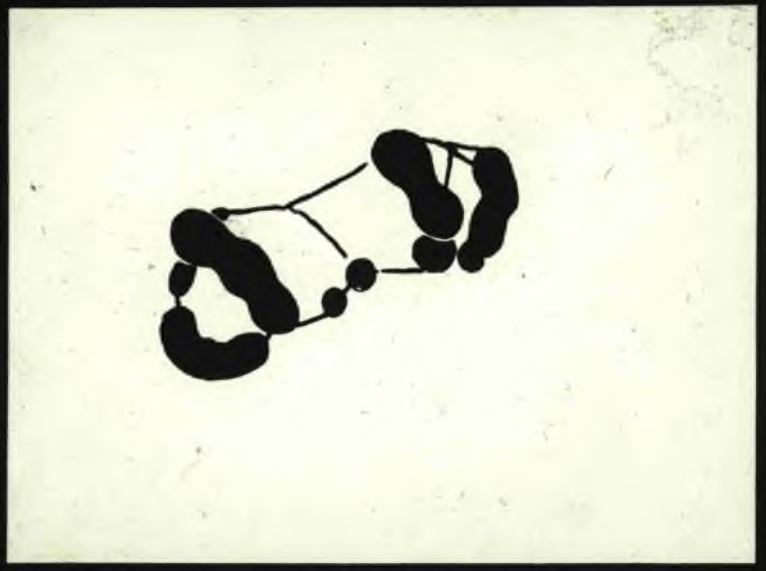
1b


5μ

2a


2b





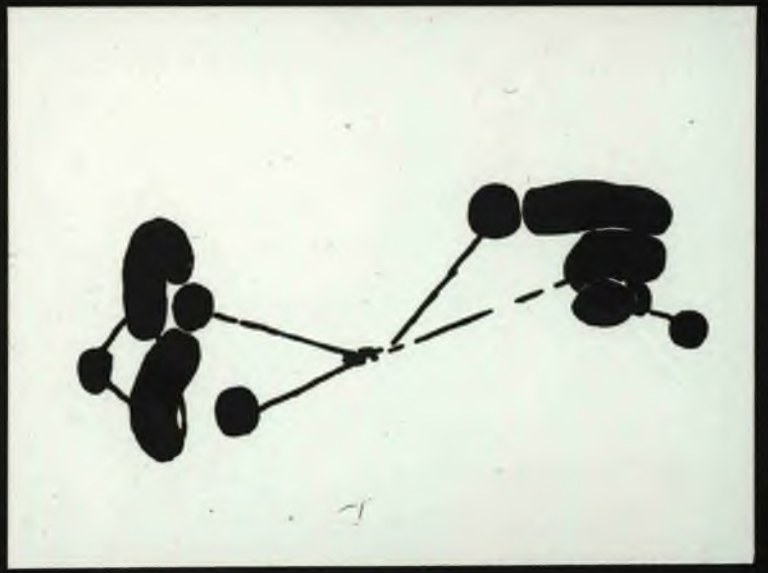


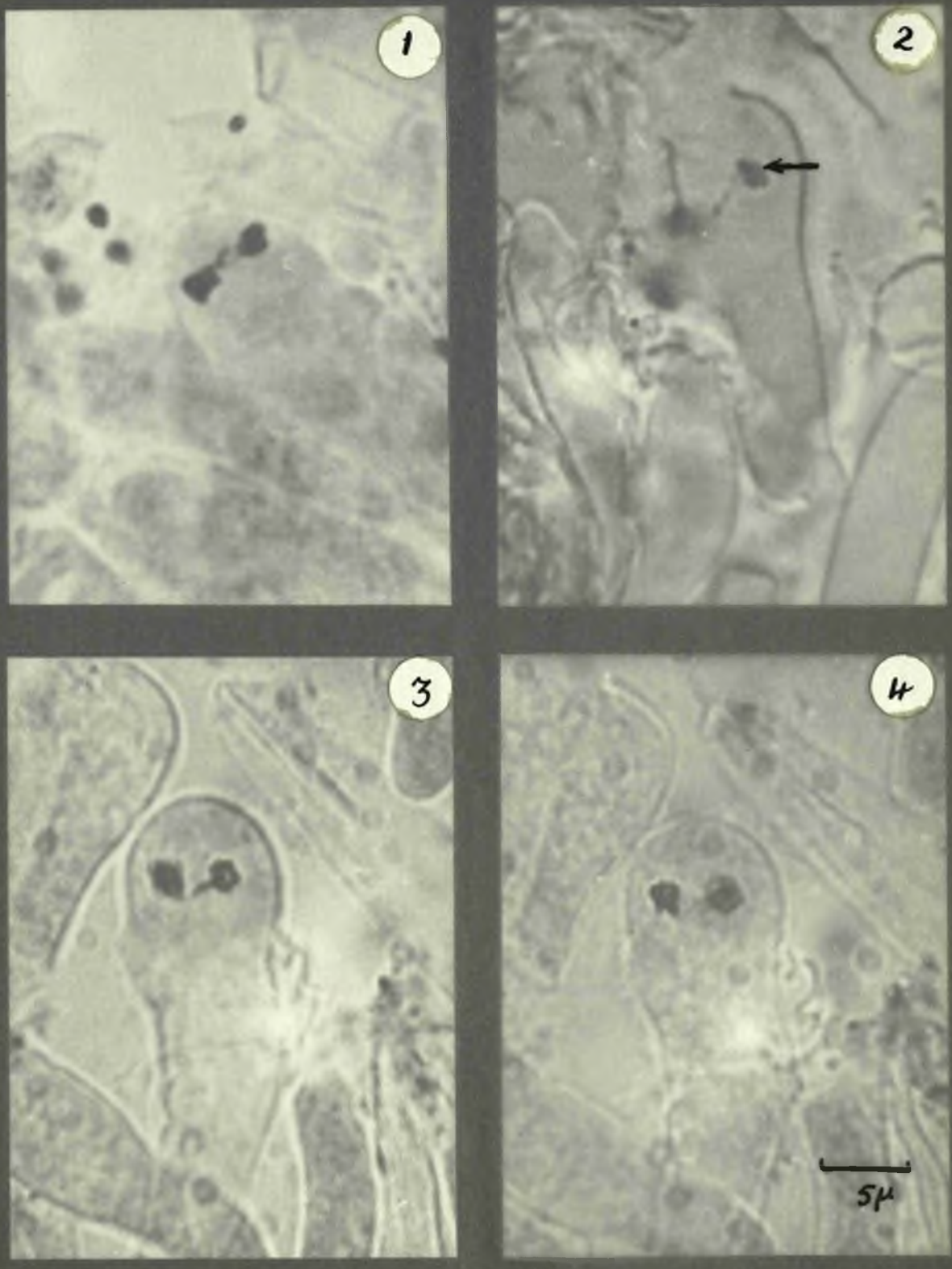
PLATE XXXV.

Meiosis in the basidium of M. androsaceus.

Fig. 1. Anaphase separation, a bridge exists between the two groups of chromosomes. Aceto-orcein stained; bright light.

Fig. 2. Late anaphase - early telophase. Four chromosomes evident in arrowed nucleus. Bridge persists. Feulgen stained; phase contrast.

Figs. 3 & 4. Different focuses of early telophase. Small rounded chromosomes seen; bridge between daughter nuclei broken. Aceto-orcein stained; bright light.


1
2
3
4
5μ

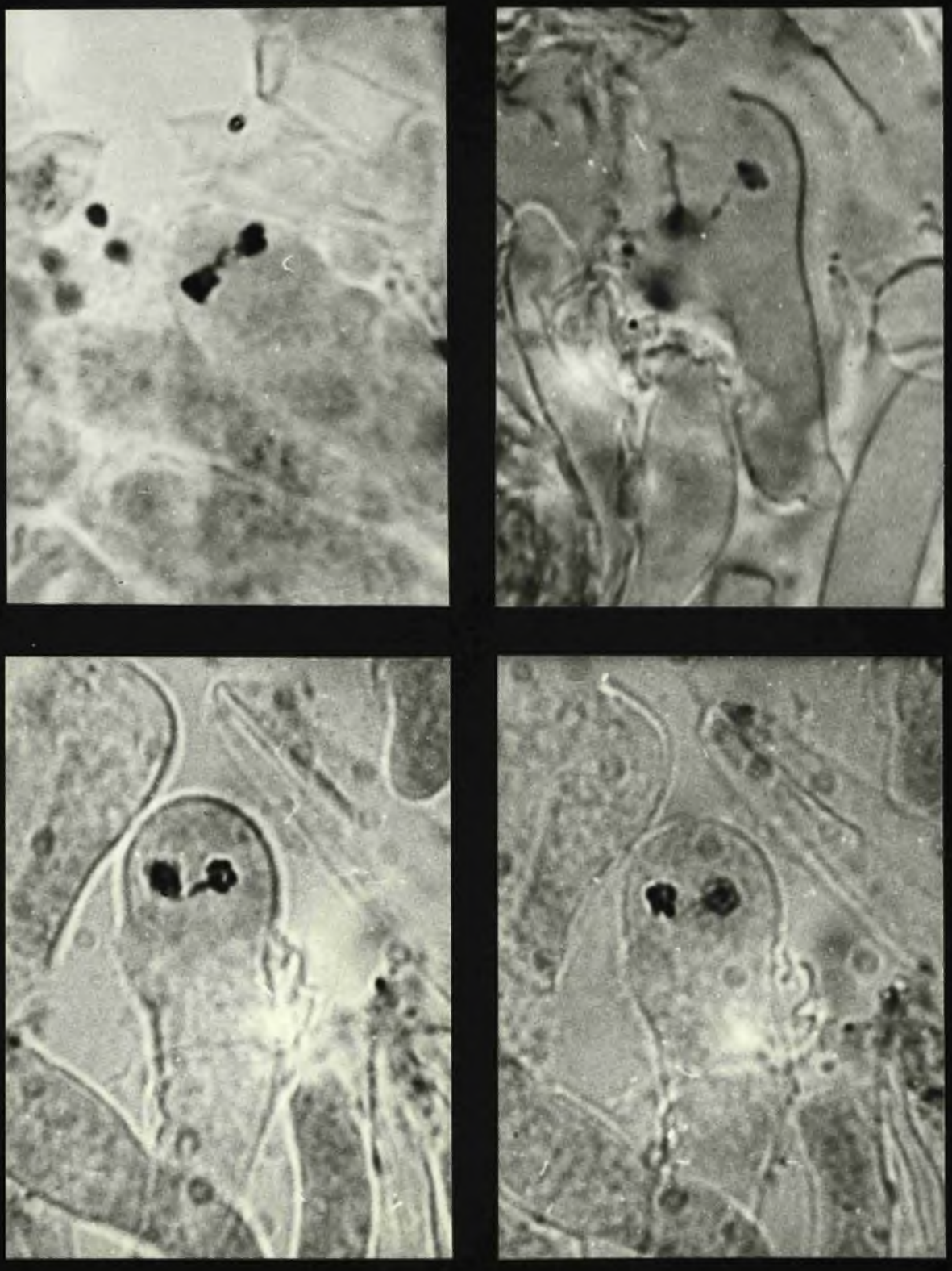
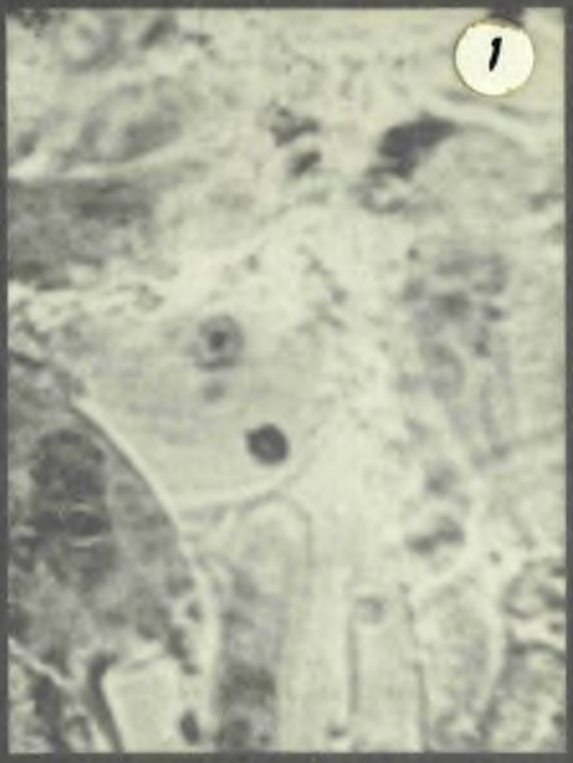


PLATE XXXVI.

Meiosis in basidium of *M. androsaceus*.

Figs. 1 & 2. Different focuses of telophase. Four rounded chromosomes in ring.

Fig. 3. Reconstituted nuclei. Nucleoli (arrows) to which looped strands of beaded chromatin are attached are seen. Feulgen-Fast Green stained; bright light.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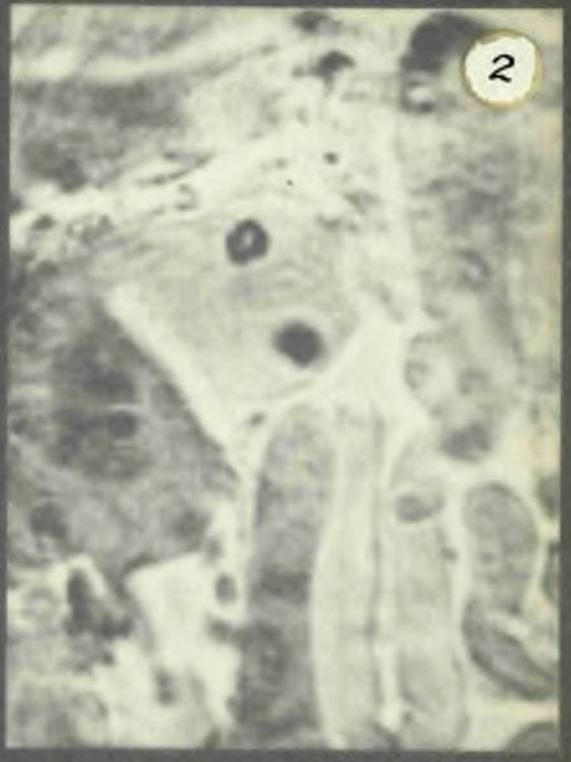
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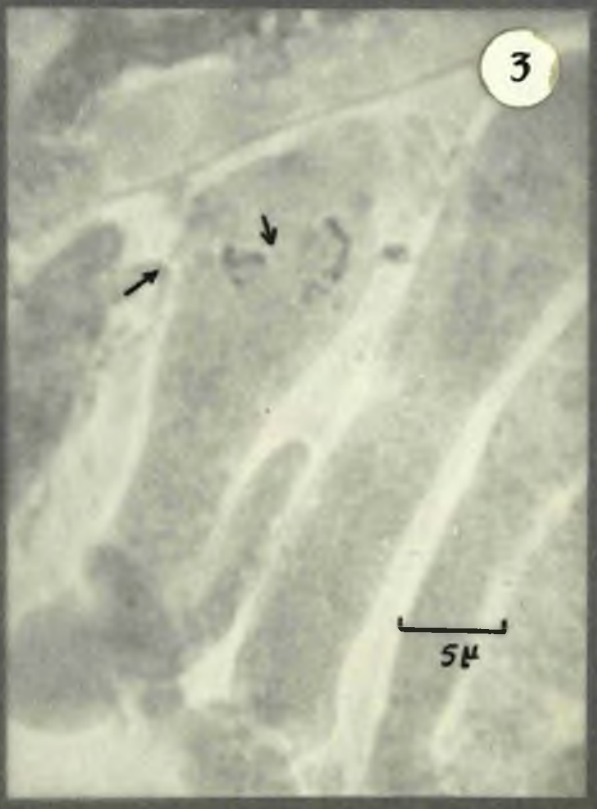
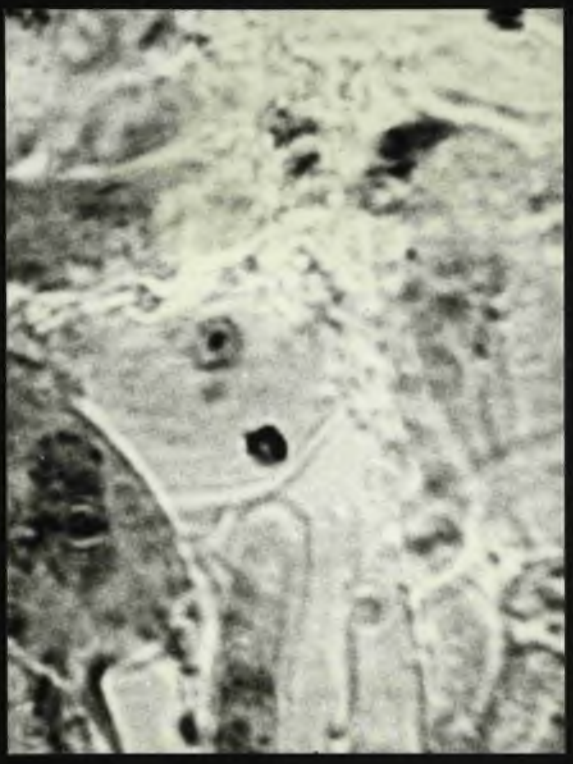
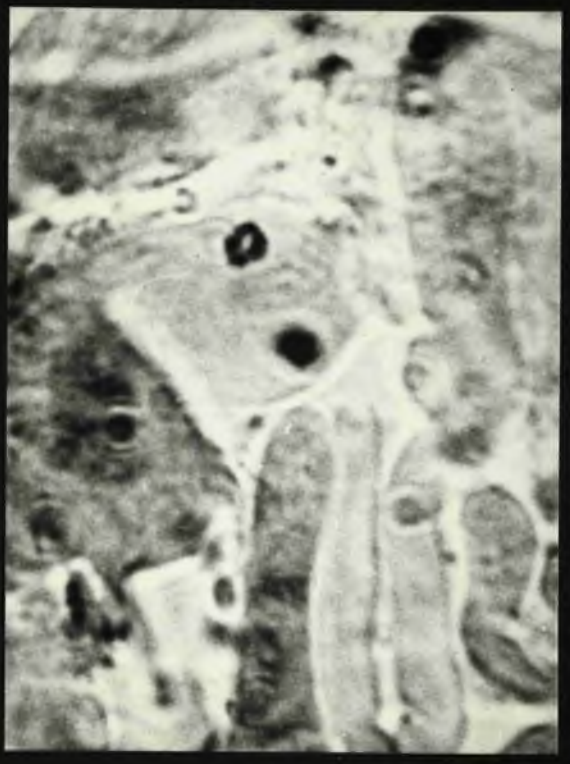
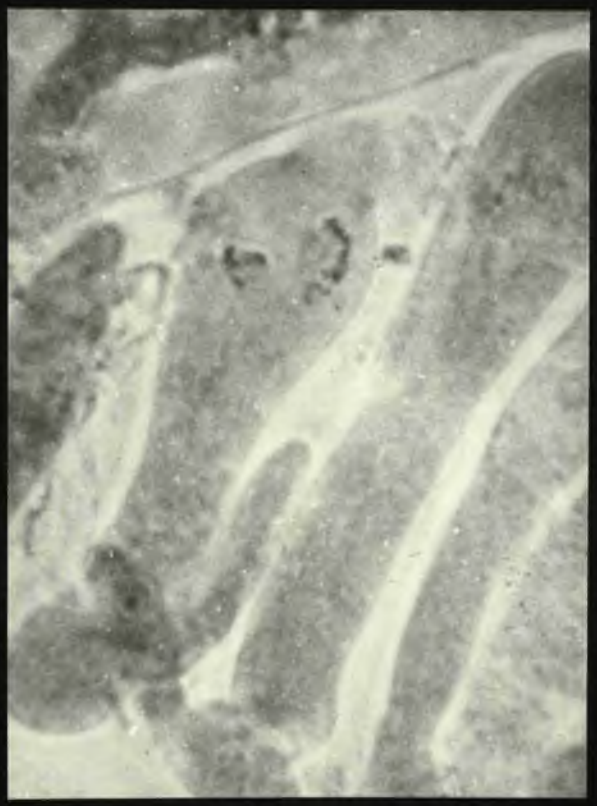

3
5μ



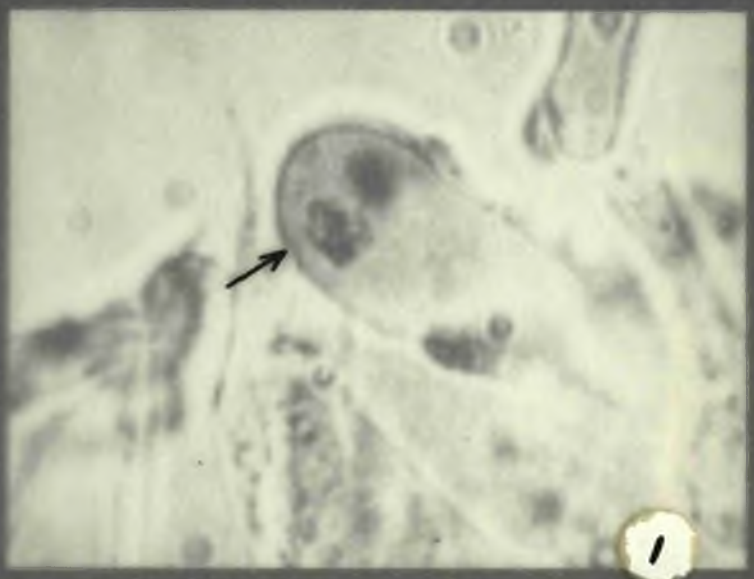


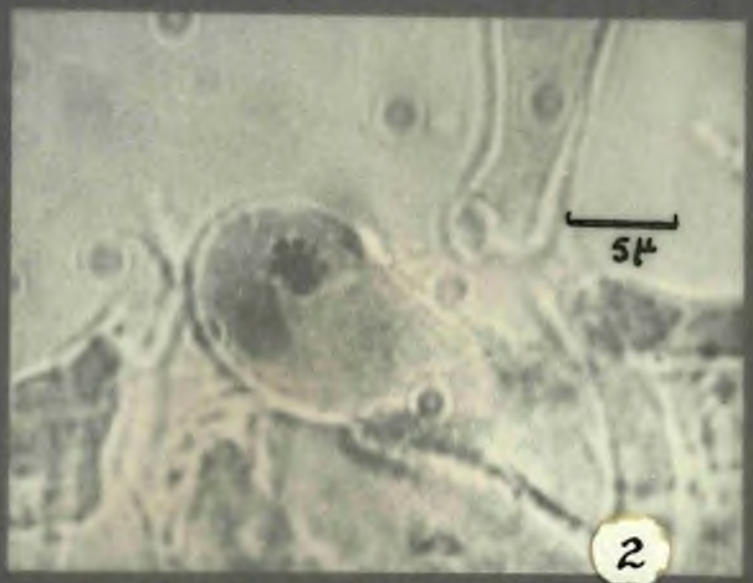


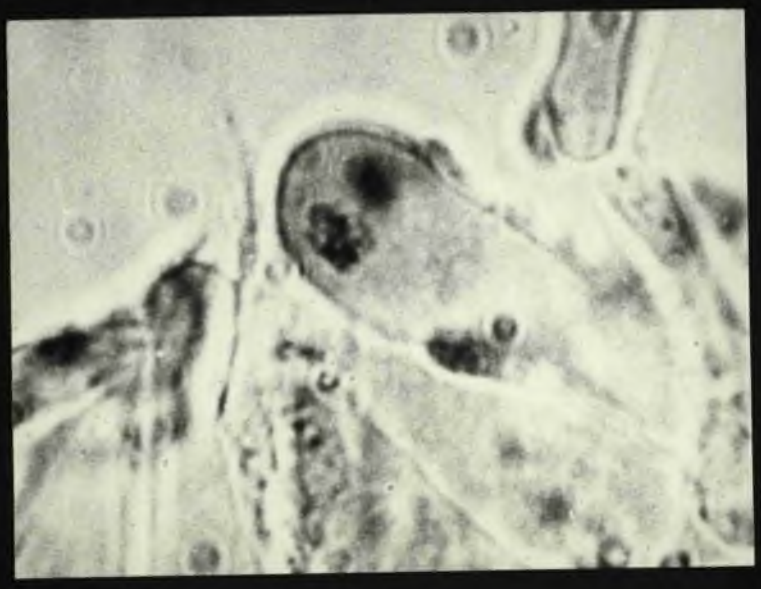
# PLATE XXXVII.

Meiosis II in basidium of *M. androsaceus*.

Figs. 1 and 2. Different focuses of early prophase nuclei. Arrowed nucleus shows greatly coiled double strand of beaded chromatin. Aceto-orcein stained; bright light.


1


5μ
2



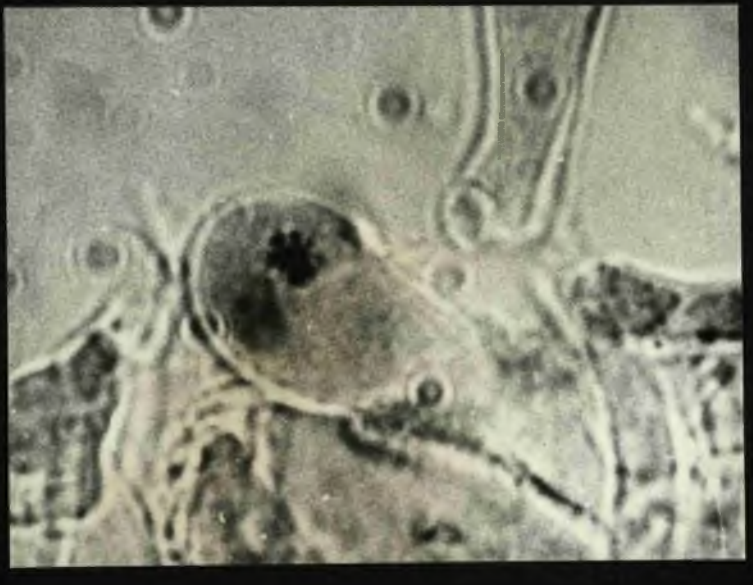


PLATE XXXVIII.

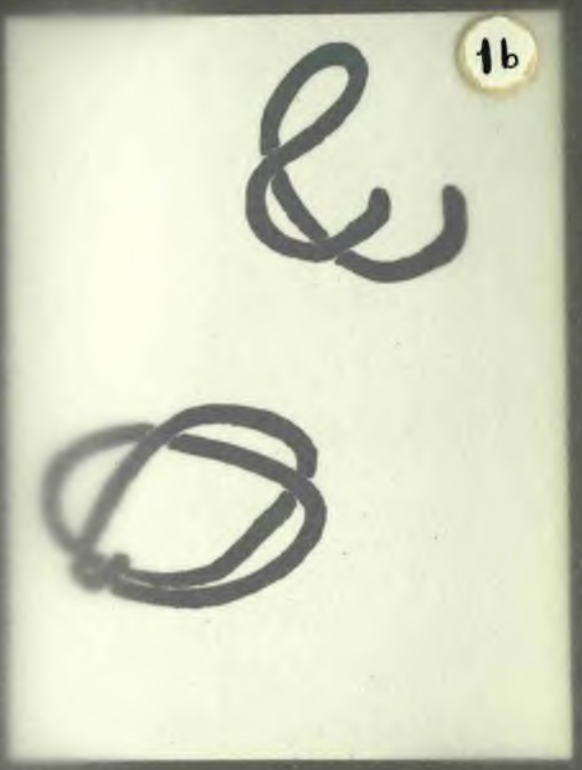
Meiosis II in the basidium of M. androsaceus.

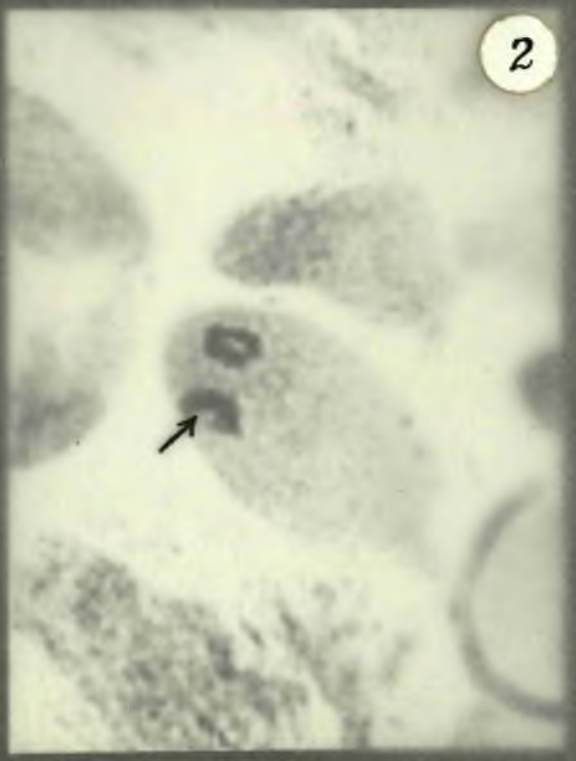
Figs. 1 a & b. Double rings of beaded chromatin formed as a result of the folding over of the loop of the figure of eight. 'Metaphase'. Aceto-orcein stained; bright light.

Fig. 2. Double rings of chromatin. Arrowed nucleus beginning to open out linearly. Aceto-orcein stained; bright light.

Fig. 3. As above; Feulgen stained; phase contrast.


1a


1b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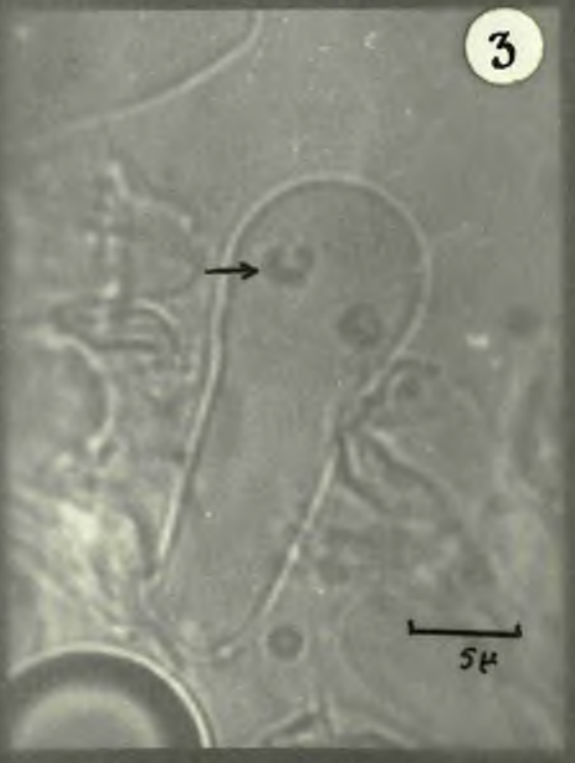
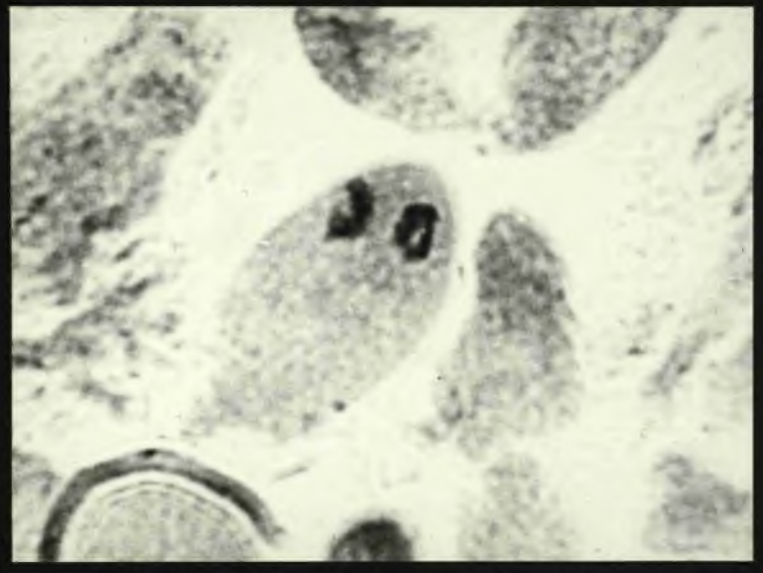
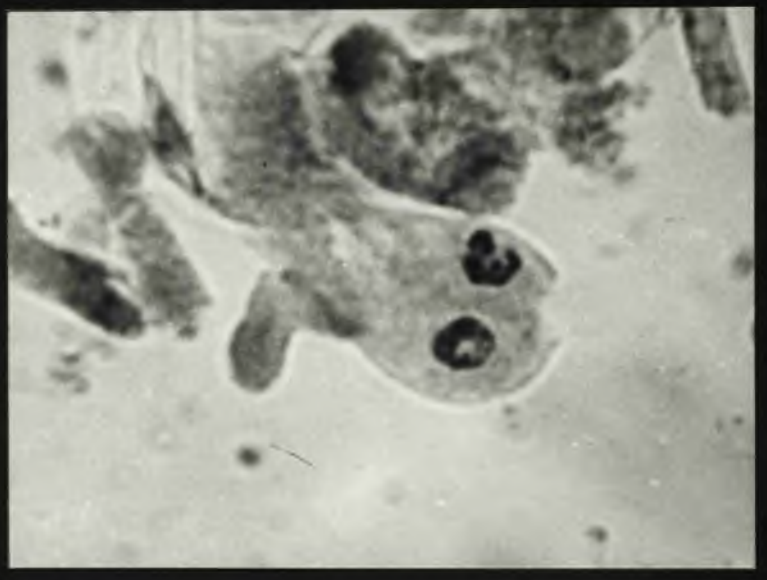
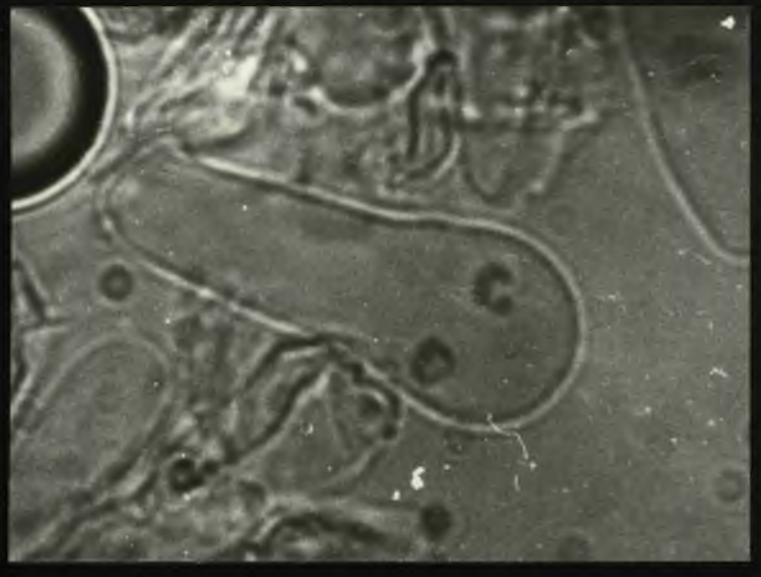
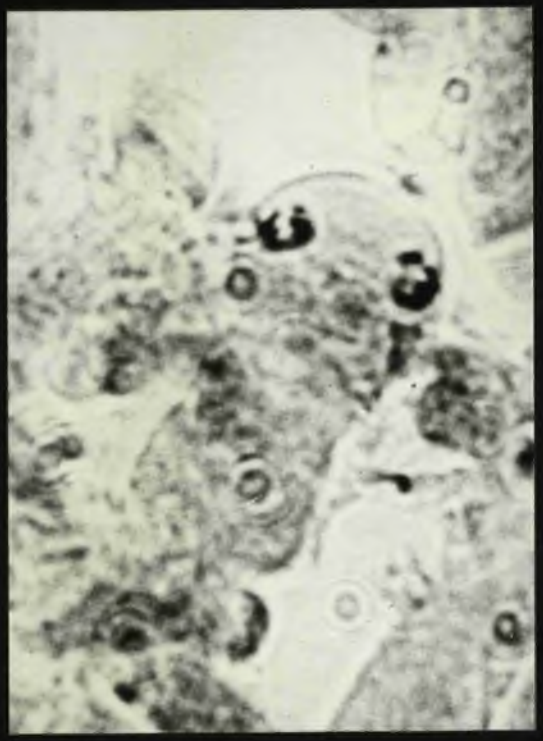
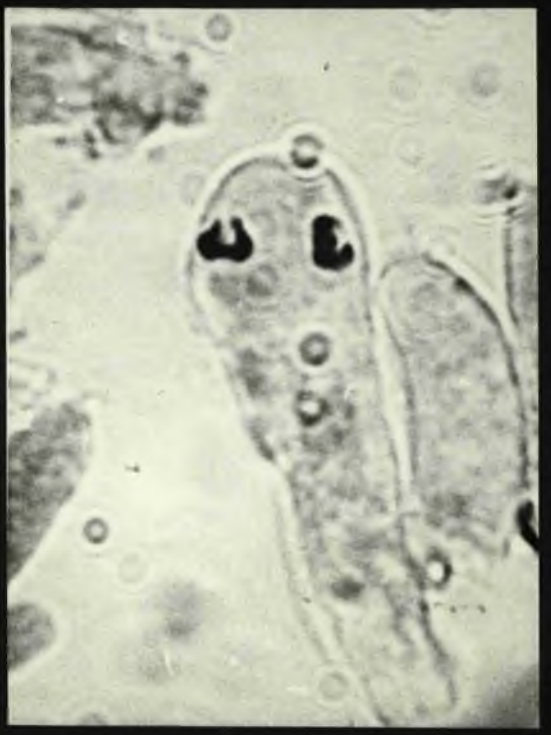

3
5μ

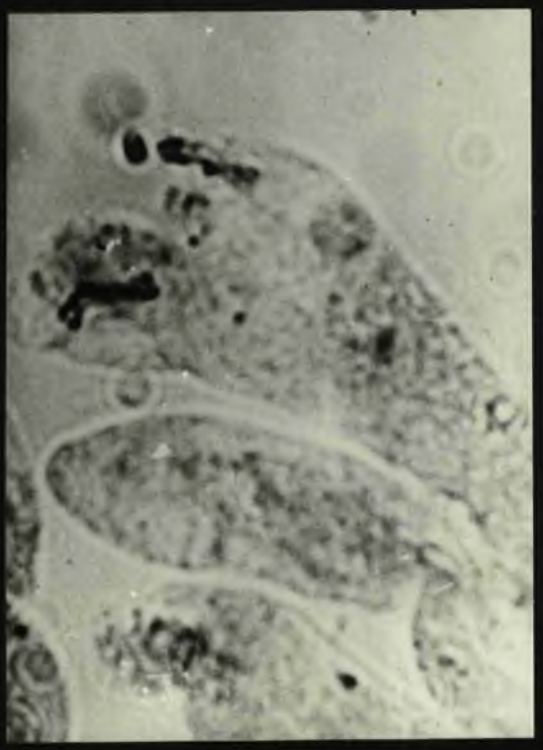


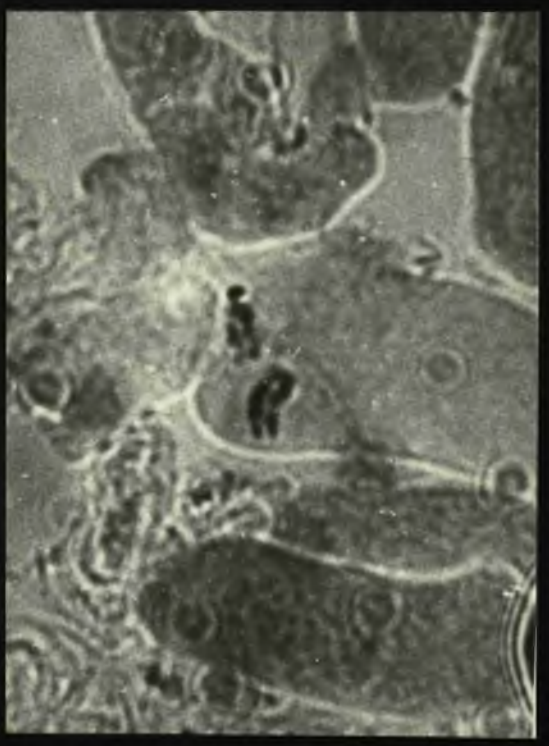












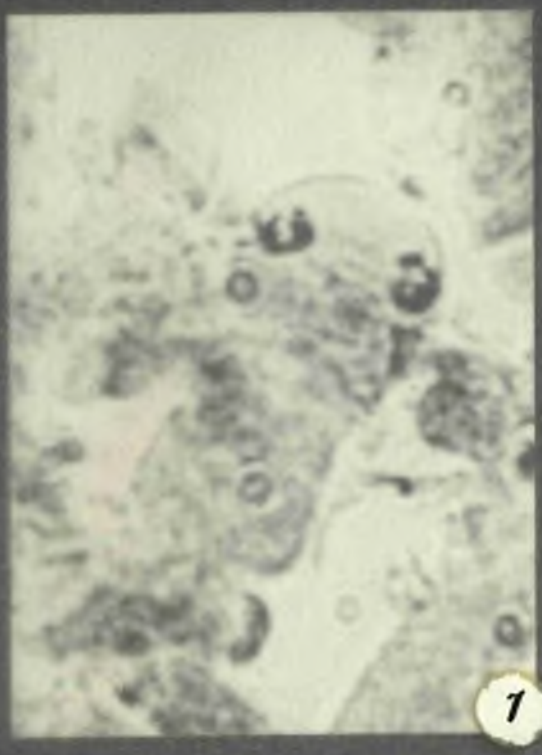
# PLATE XXXIX.

Meiosis II in basidium of *M. androsaceus*.

Figs. 1 & 2. Double rings of chromatin in which break has taken place, opening out linearly. Beaded nature of arrowed nucleus in fig. 2 is seen. Aceto-orcein stained; bright light.

Fig. 3. 'Metaphase' rods of chromatin greatly extended, formed by the opening out of the arms of the metaphase rings. (a) rods crossed on one another. Aceto-orcein stained; bright light.

Fig. 4. 'Metaphase' rods of chromatin, showing beaded appearance. Aceto-orcein stained; bright light.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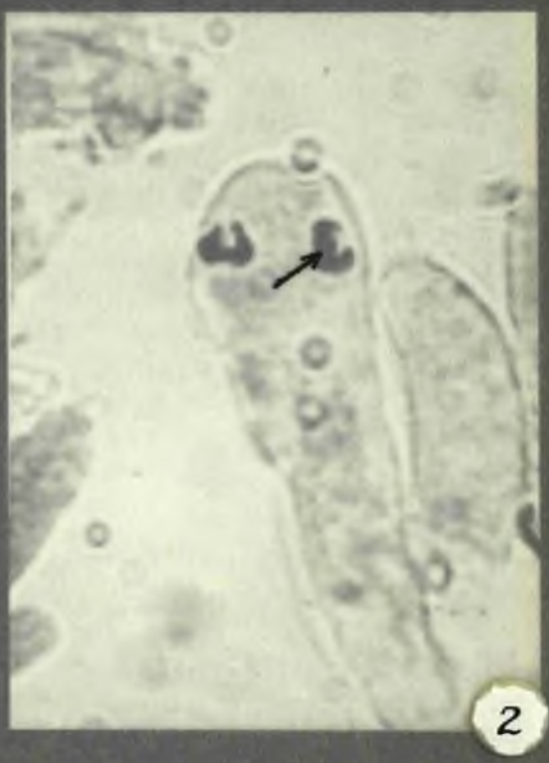
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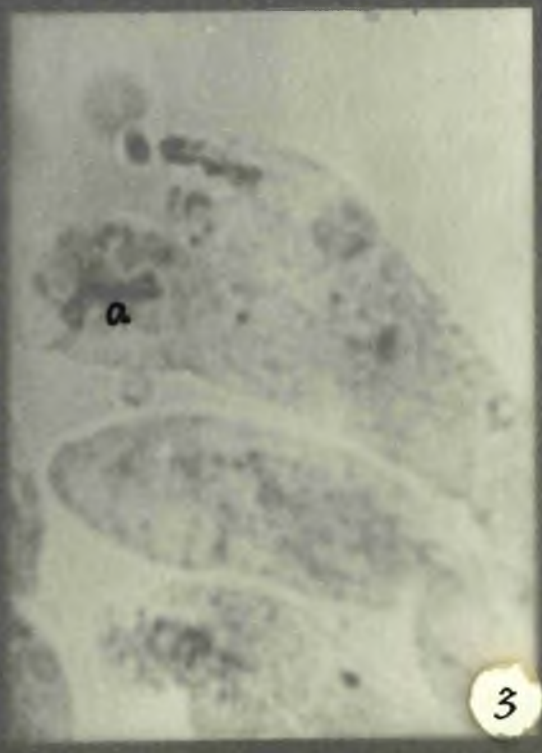

a
3

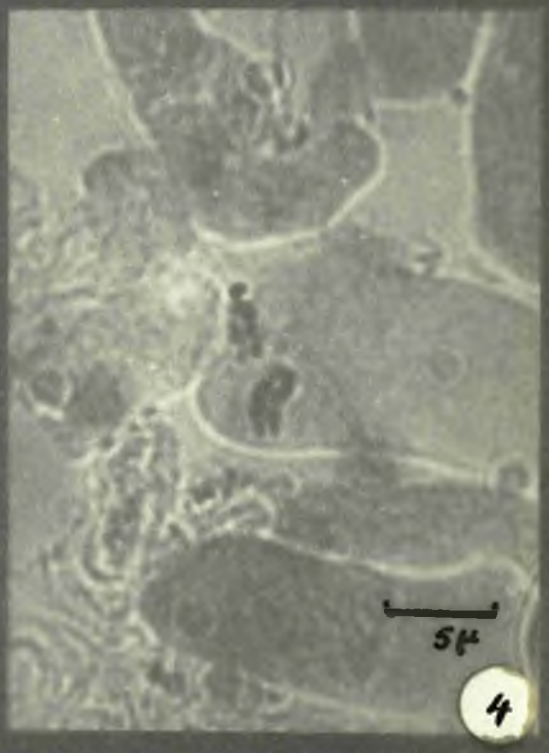
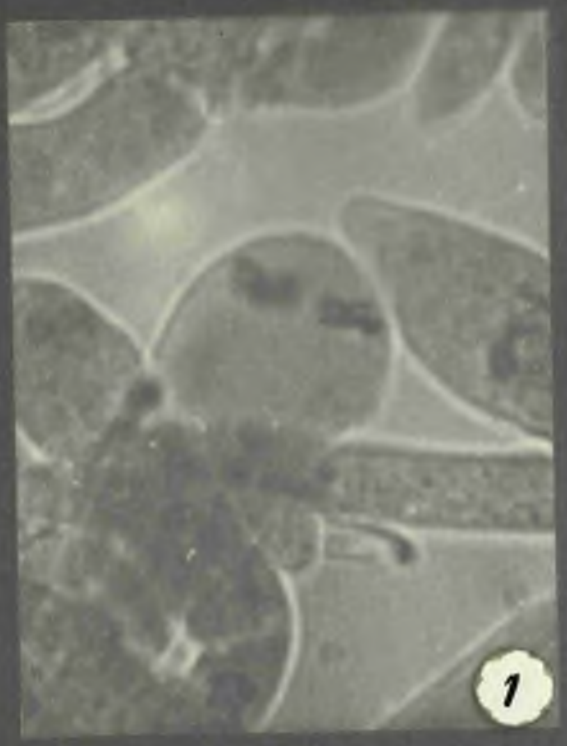

5μ
4

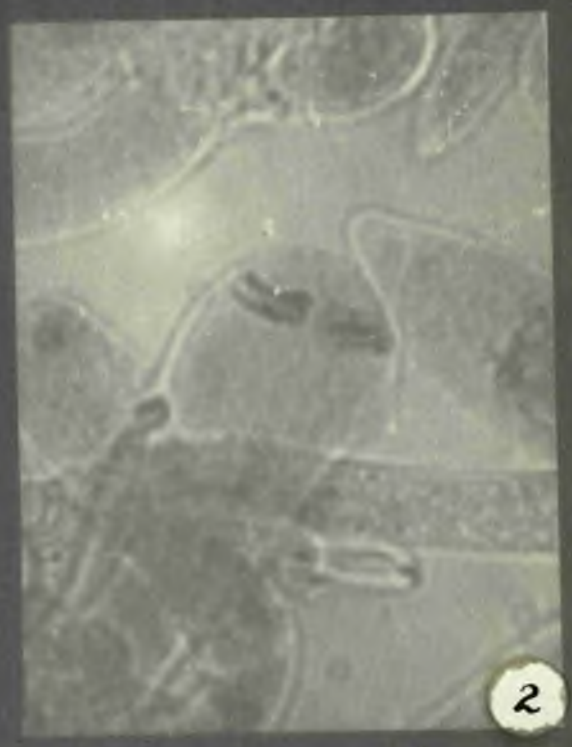
PLATE XL.

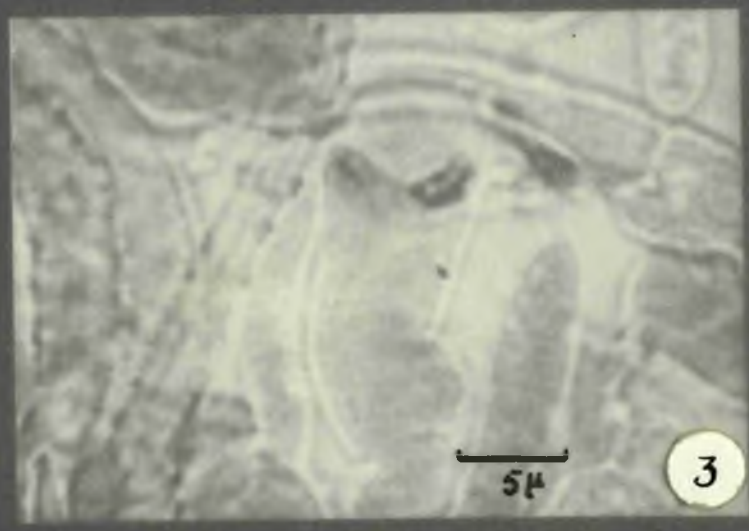
Meiosis II in the basidium of M. androsaceus.

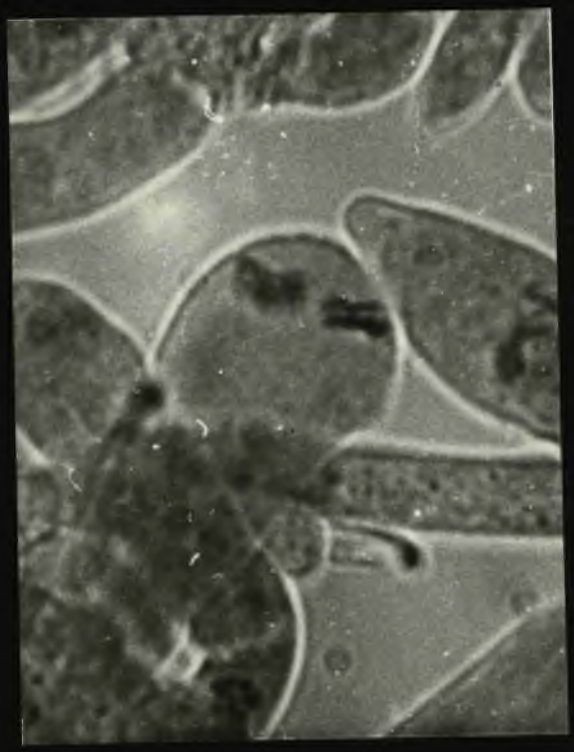
Figs. 1 & 2. Different focuses of nuclei showing 'metaphase' rods of chromatin. Aceto-orcein stained; bright light.

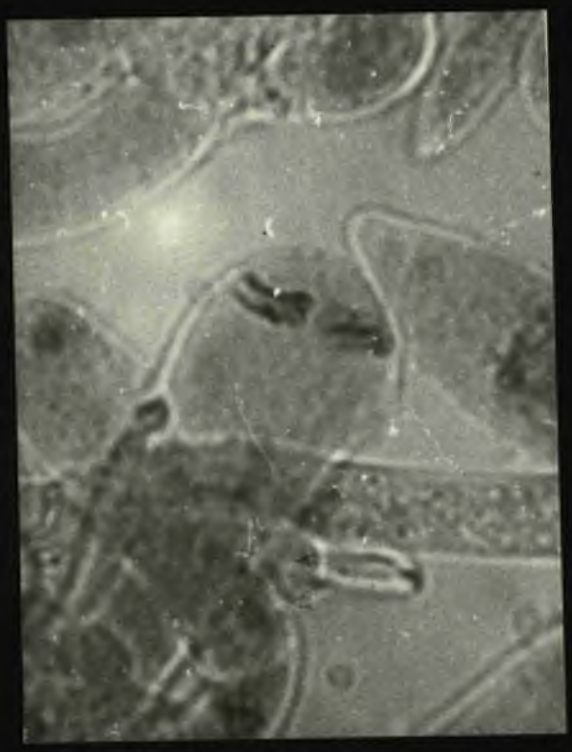
Fig. 3. 'Metaphase' rods of chromatin showing beaded appearance, and seen lying freely in the cytoplasm. Aceto-orcein stained; bright light.


1


2


5μ
3





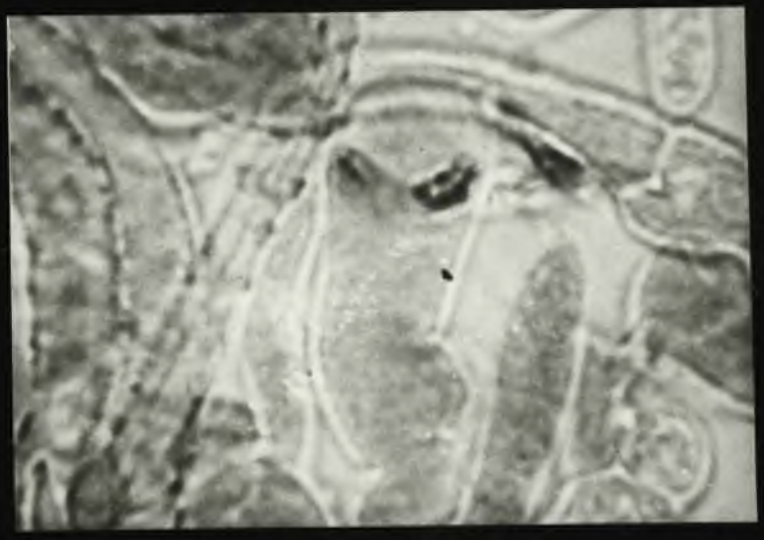
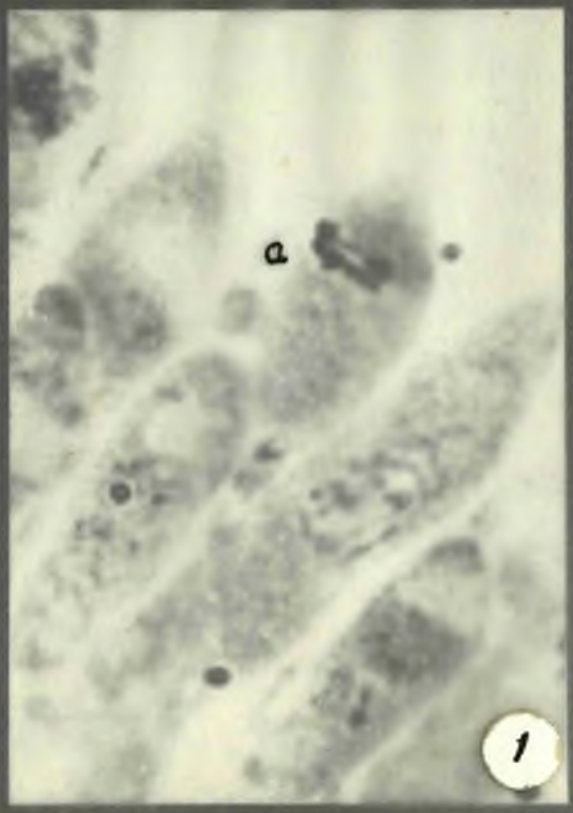
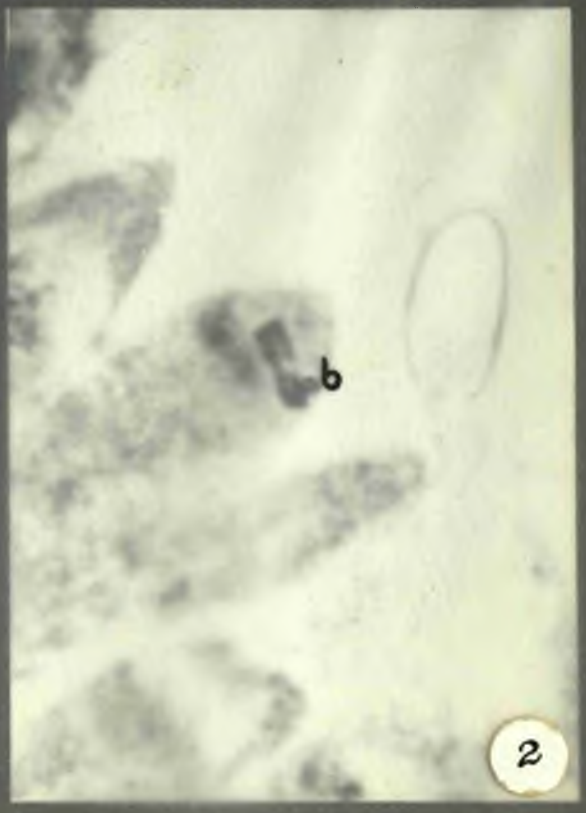


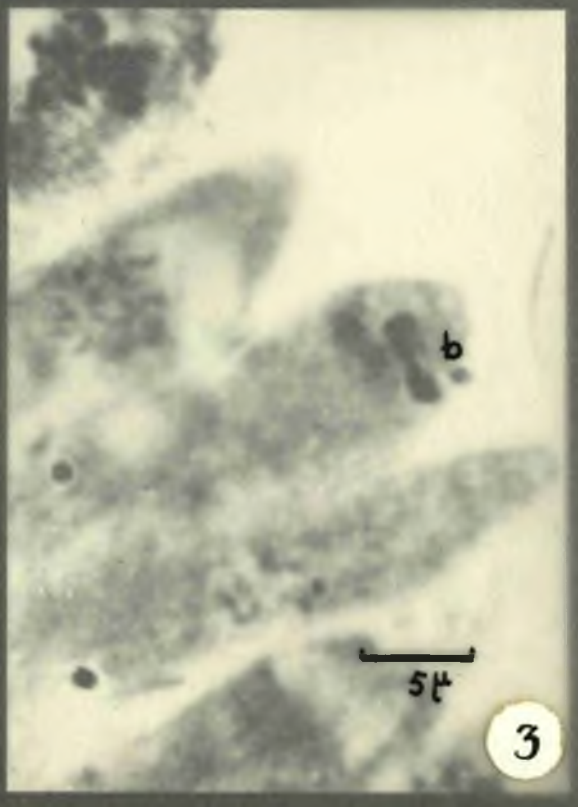
PLATE XLI.

Figs. 1, 2 and 3. Different focuses of same basidium showing nucleus (a); two metaphase rods of chromatin and nucleus (b); double rings of beaded chromatin not yet extended linearly. All lie freely in the cytoplasm. Aceto-orcein stained; bright light.

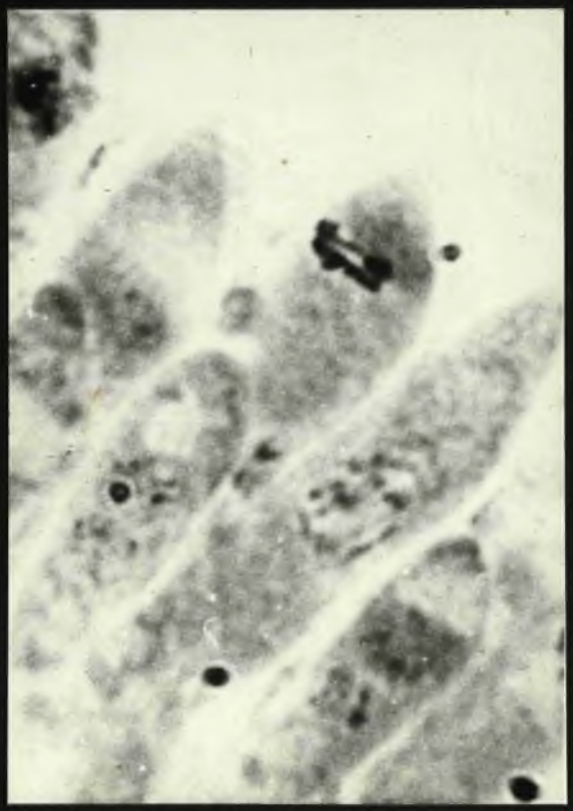
Fig. 4. Drawing of nuclei above.

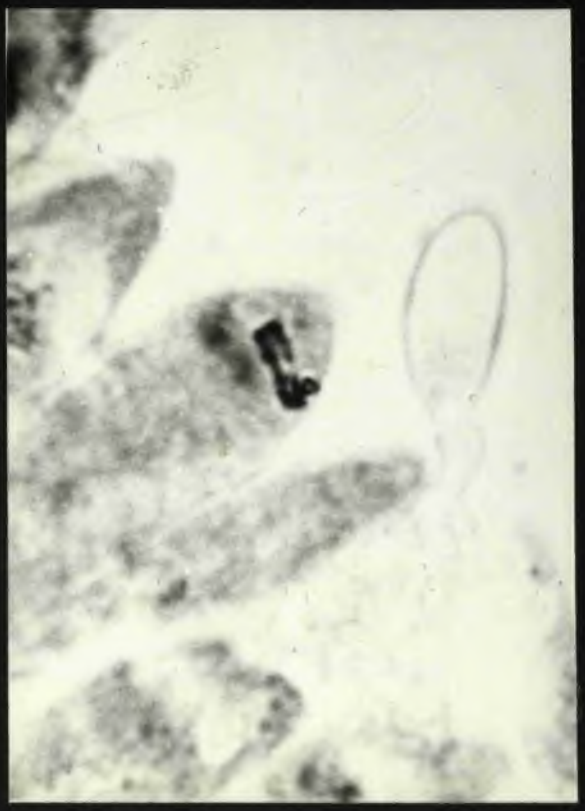

a
1

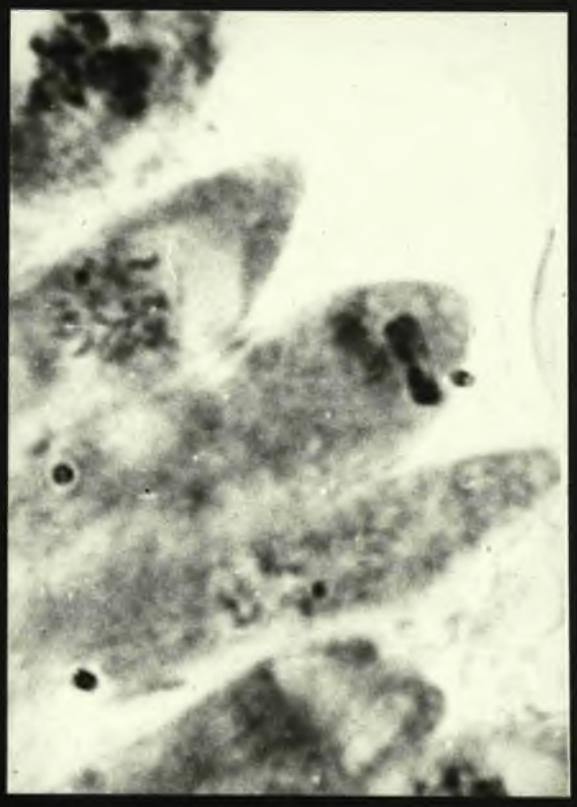

b
2

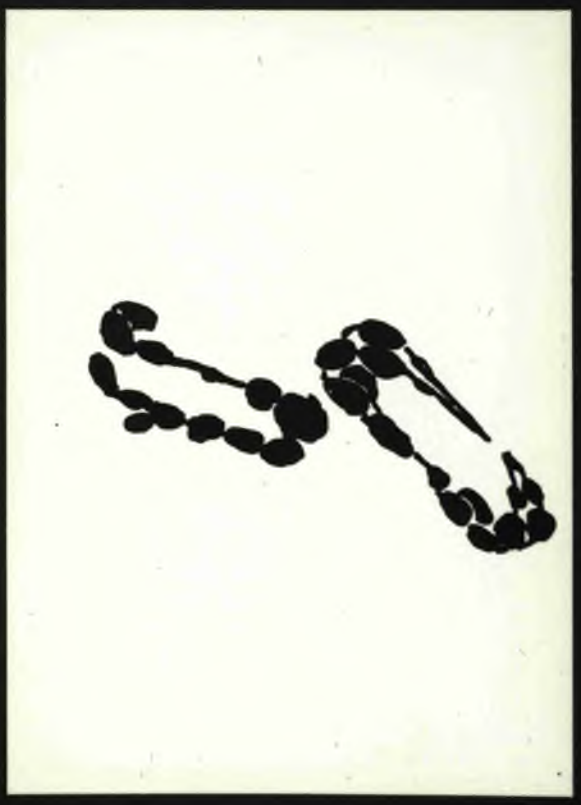

b
5μ
3


a
b
4









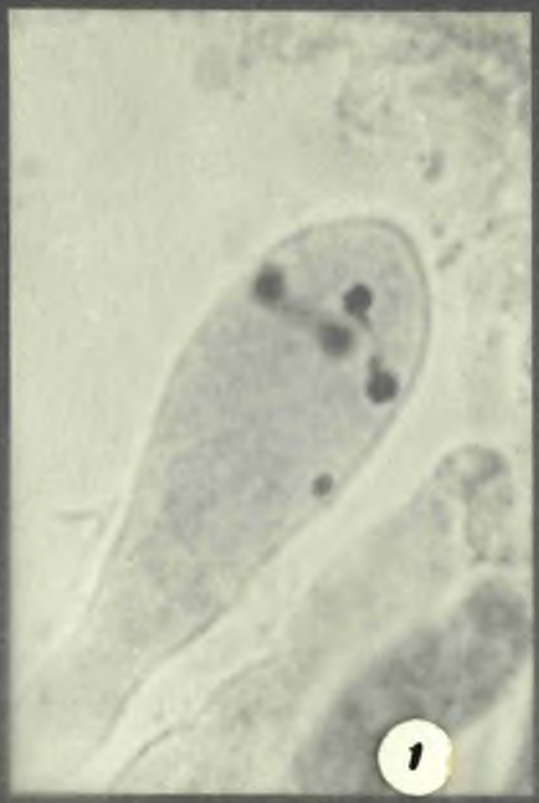
# PLATE XLII.

Meiosis II in the basidium of *M. androsaceus*.

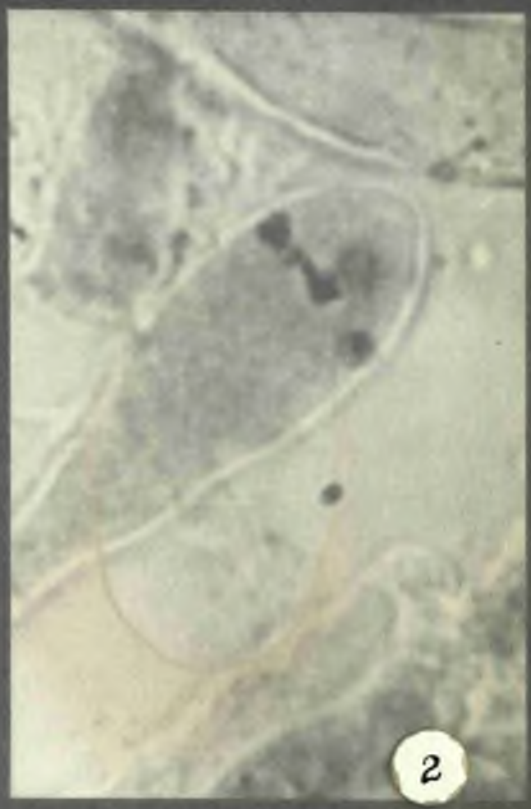
Figs. 1 & 2. Different focuses of same basidium showing nuclei in anaphase. Chromatin bridges are seen. Aceto-orcein stained; bright light.

Fig. 3. Drawing of nuclei above.

Fig. 4. Telophase. Three of the four nuclei are seen; arrowed nucleus shows four rounded chromosomes. Aceto-orcein stained; bright light.



1

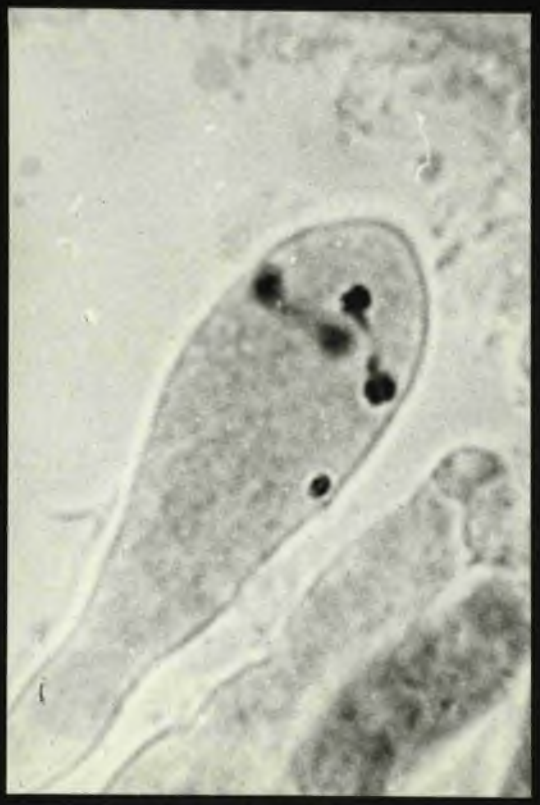


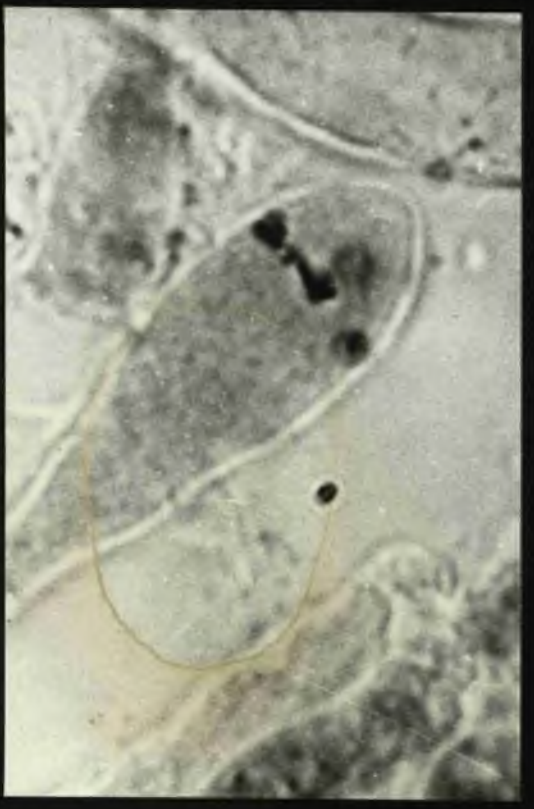
2

3

5μ

4





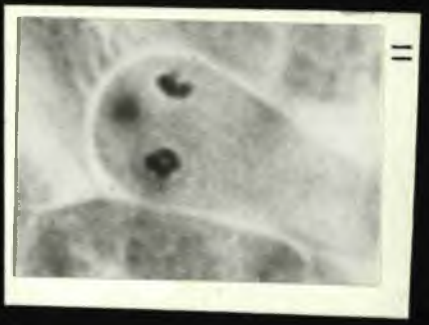
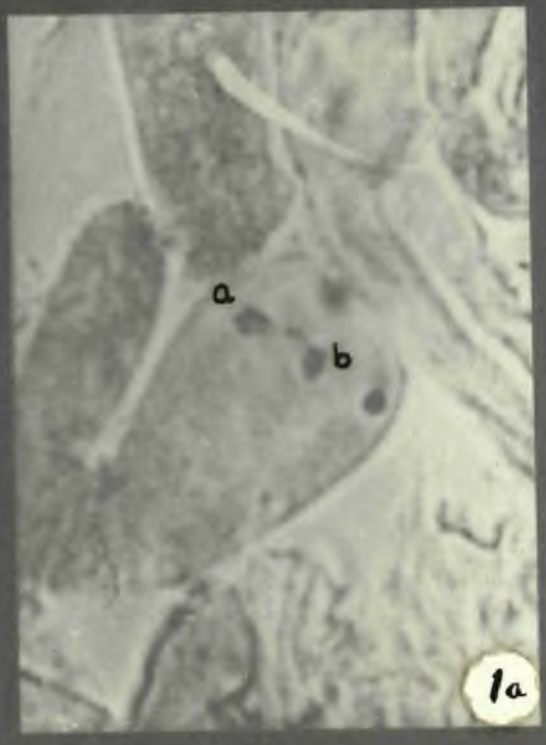


PLATE XLIII.

Meiosis II in the basidium of M. androsaceus.

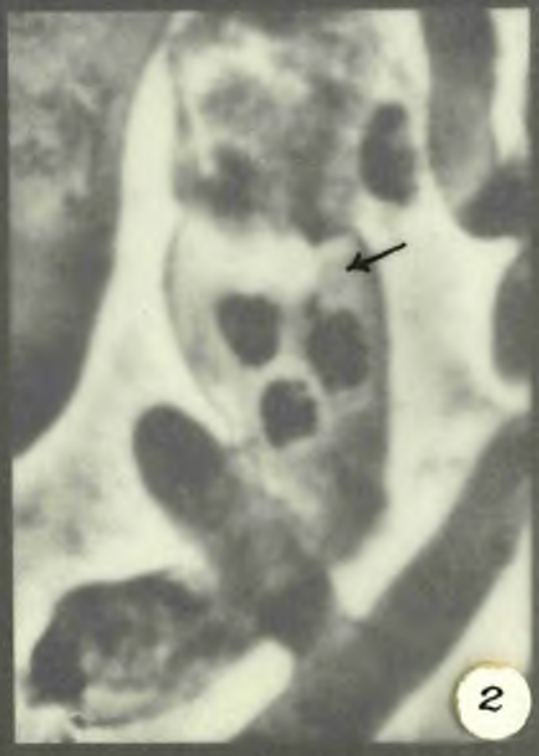
Figs. 1a & b. Late 'anaphase' - early 'telophase'. The bridge between the nuclei on the right has been broken; bridge between nuclei on left still persists, and on it a lagging chromosome belonging to group (b) is seen. The four chromosomes of group (a) are obvious. Aceto-orcein stained; bright light.

Figs. 2 & 3. Basidia showing the reorganised nuclei at the end of meiosis. Nucleoli (arrows) with attached chromatin strands. Aceto-orcein stained; bright light.




1a


1b



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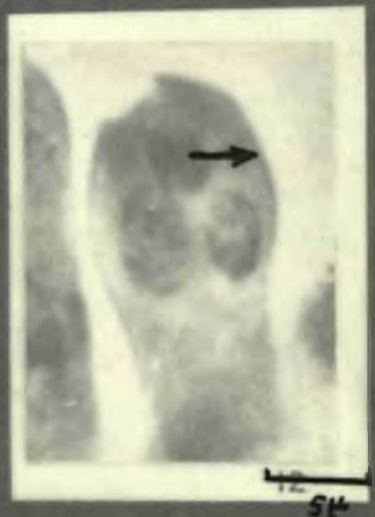
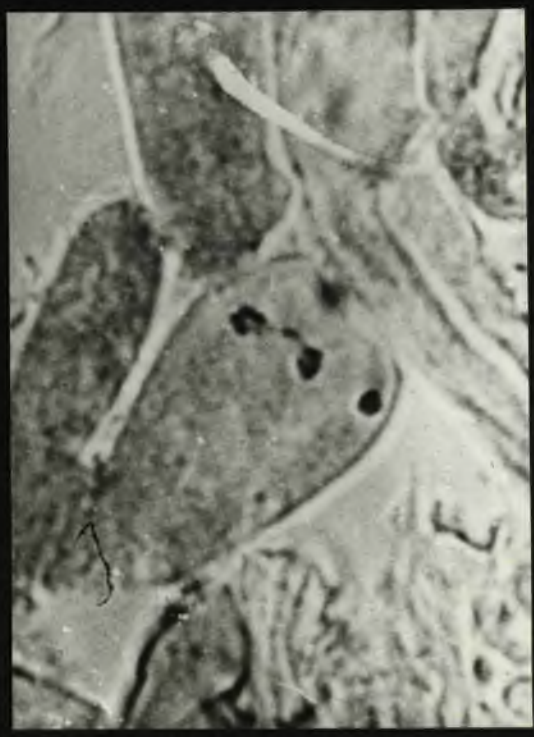
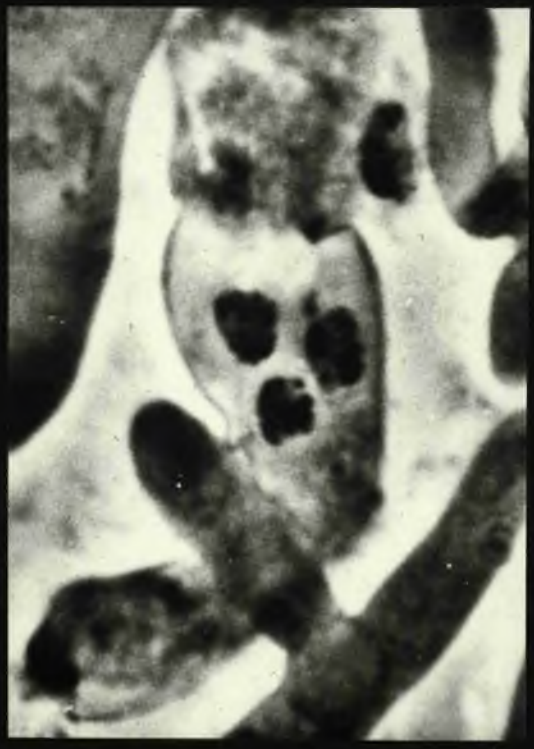
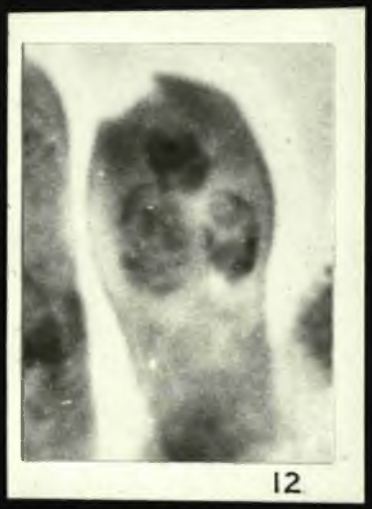



3







12

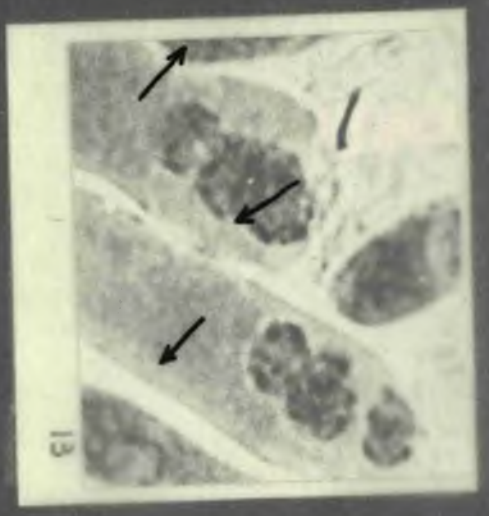
PLATE XLIV.

Fig. 1. Resting nuclei in the basidium (nuclei arrowed). Aceto-orcein stained; bright light.

Fig. 2. Basidium in which two of the four nuclei formed as a result of meiosis in the fusion nucleus have undergone an additional mitotic division. There are thus 6 nuclei in the basidium. Aceto-orcein stained; bright light.

Fig. 3. Spores showing nuclei: (a) highly contracted hairpin shaped nucleus migrating into spore; (b) spore possessing two nuclei. All nuclei in highly contracted form; no obvious nucleoli. Aceto-orcein stained; bright light.

Fig. 4. Spores showing resting nuclei. Aceto-orcein stained; bright light.



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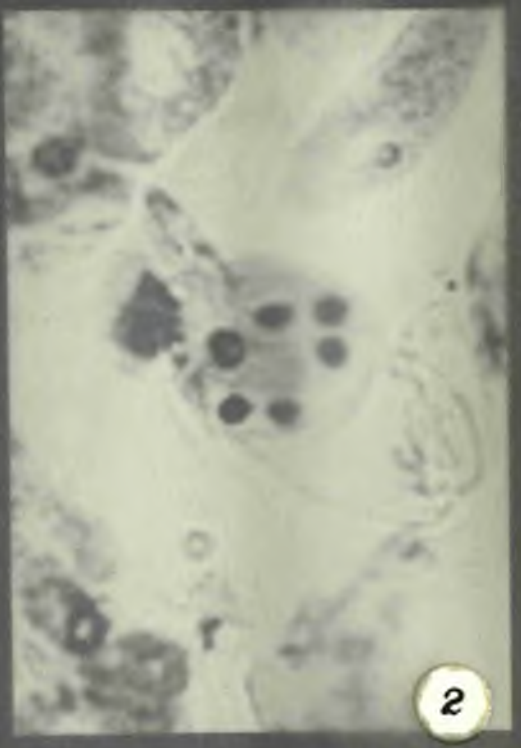

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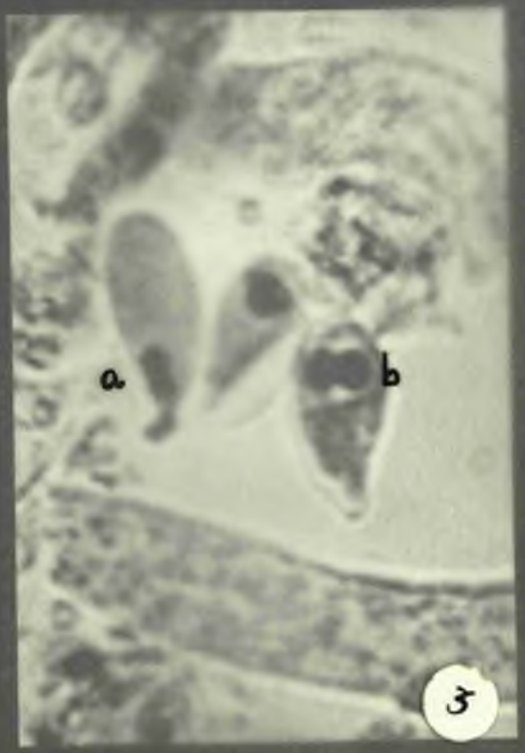


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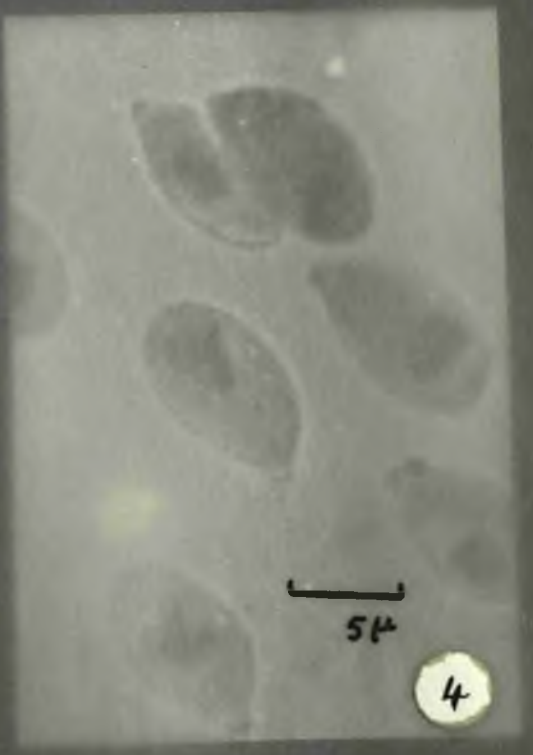


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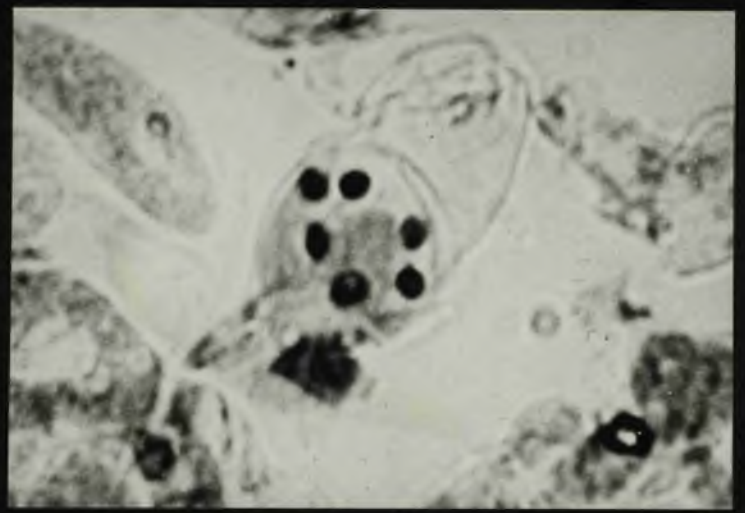

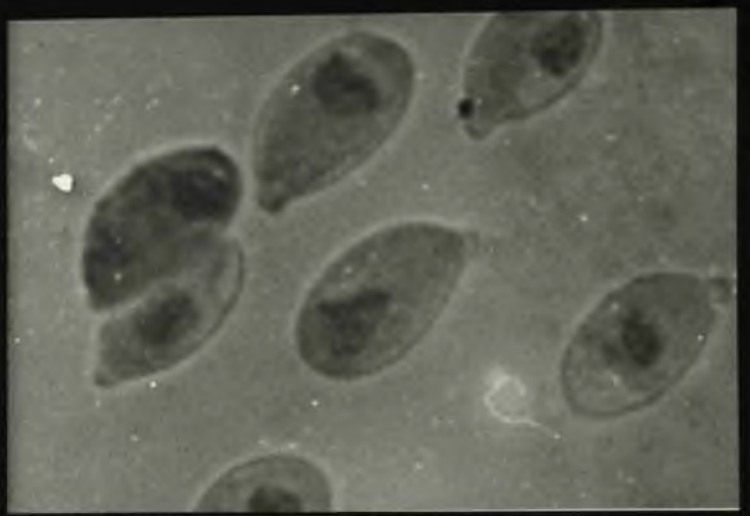

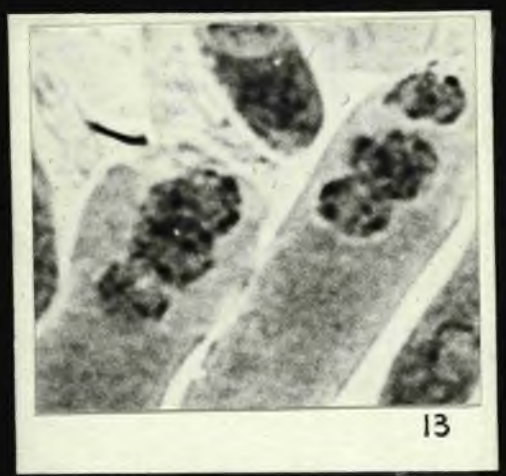

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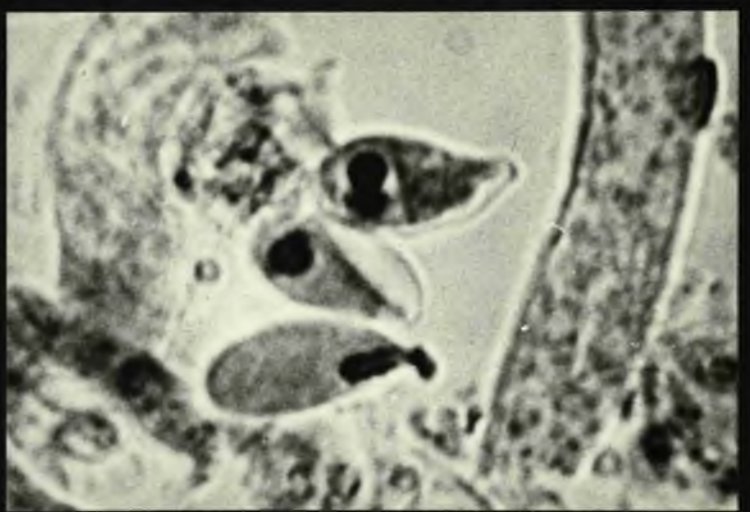


PLATE XLV.

Division in the ultimate clamp of M. rotula.

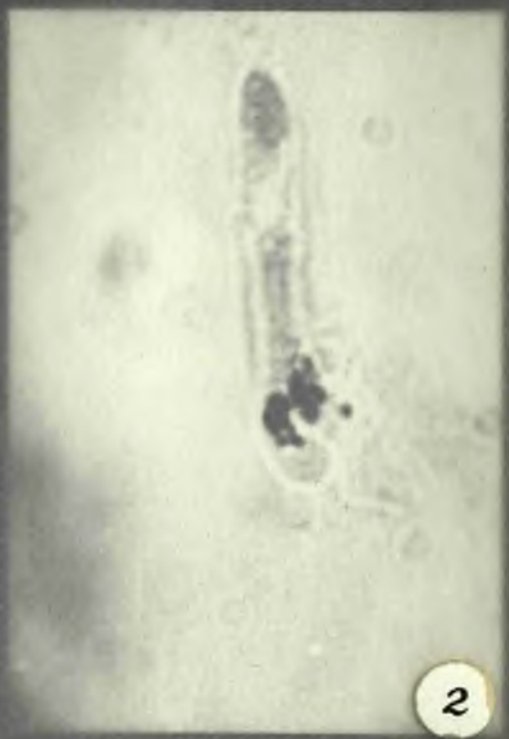
Fig. 1. Prophase. Four deeply stained bodies are seen on chromatin ring. Aceto-orcein stained; bright light.

Fig. 2. 'Metaphase'- arms of beaded chromatin rings opening out linearly. Aceto-orcein stained; bright light.

Fig. 3. Arms extended linearly in (a); not fully extended in (b). Beaded nature obvious. Aceto-orcein stained; bright light.

Fig. 4. Anaphase. Aceto-orcein stained; bright light.


1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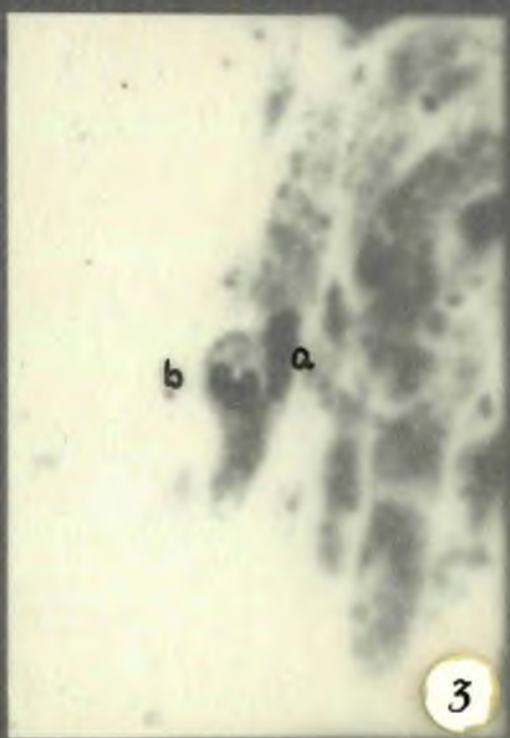
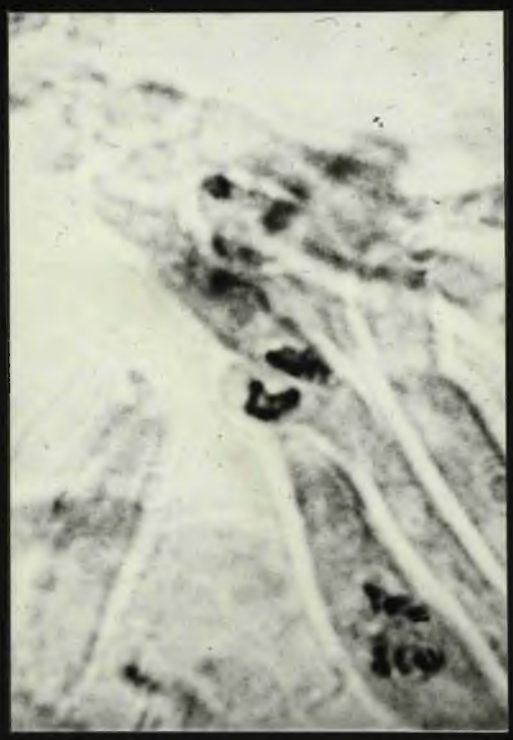
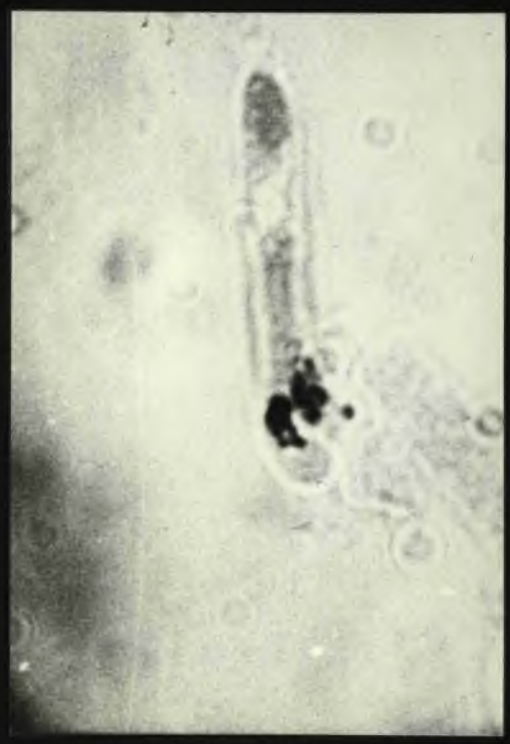
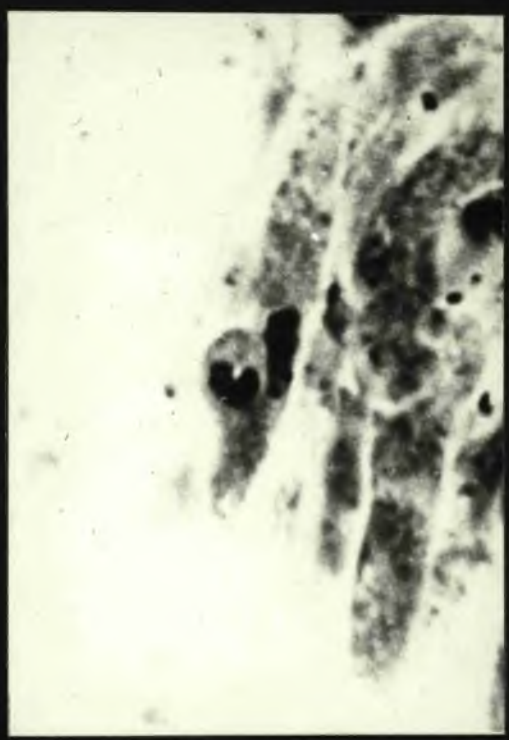

b
a
3


5μ
4







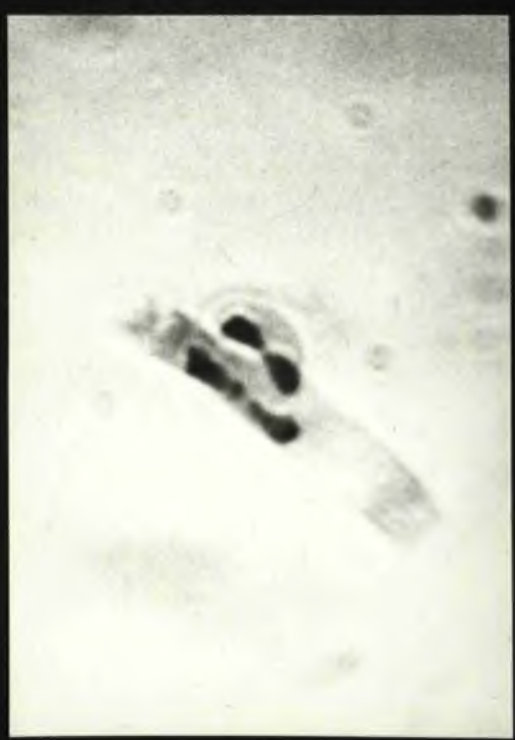
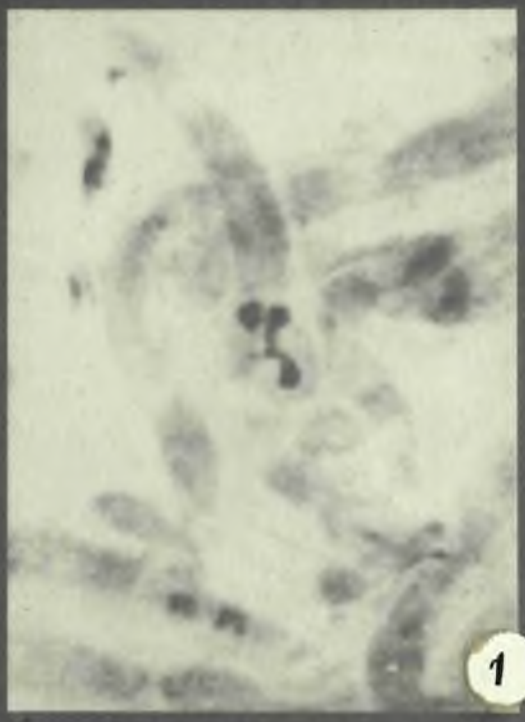


PLATE XLVI.

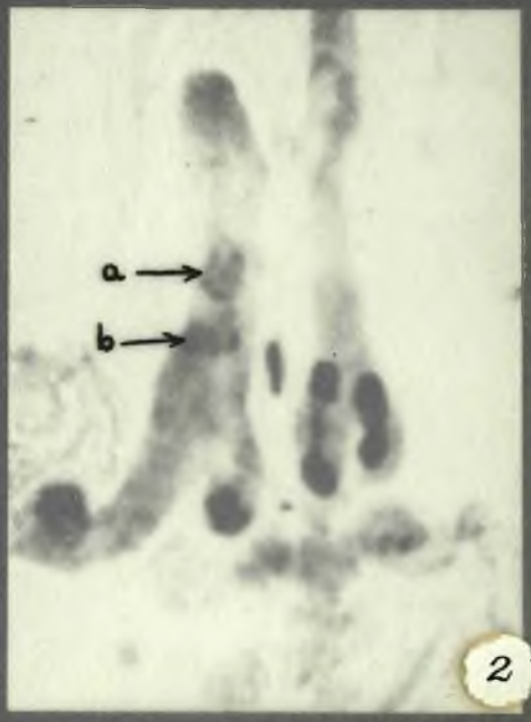
Division in ultimate clamp of *M. rotula*.

Figs. 1 & 2. Late anaphase - early telophase. Bridges between sister groups of chromosomes obvious; four chromosomes seen in arrowed nucleus. Arrowed nuclei (a) and (b) fuse at nucleolar ends. Aceto-orcein stained; bright light.

Fig. 3. Fusiform element of hymenium of *M. rotula*; prophase-like chromosomes seen in large fusion nucleus. Aceto-orcein stained; phase contrast.

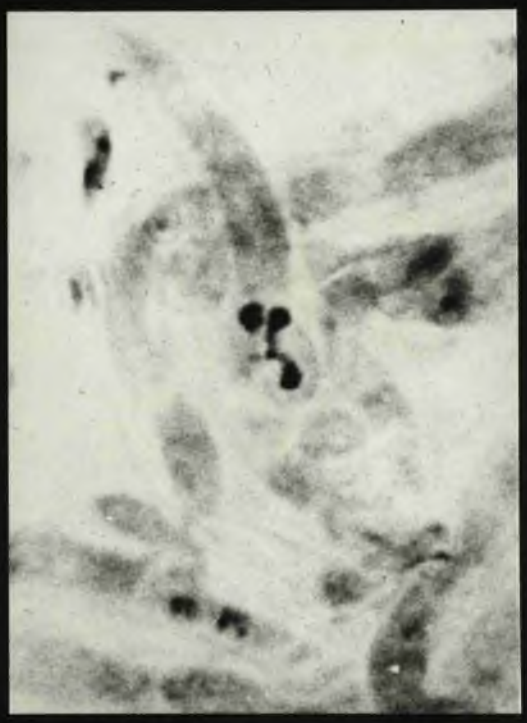


1




2


3



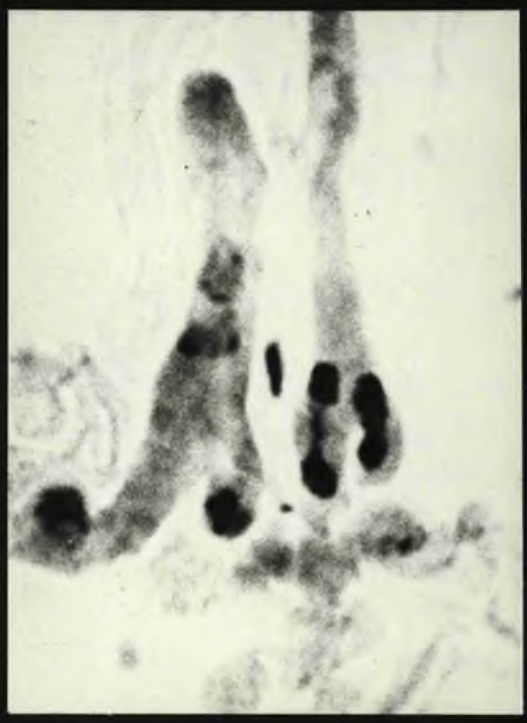


PLATE XLVII.

Division in the basidium of *M. rotula*.

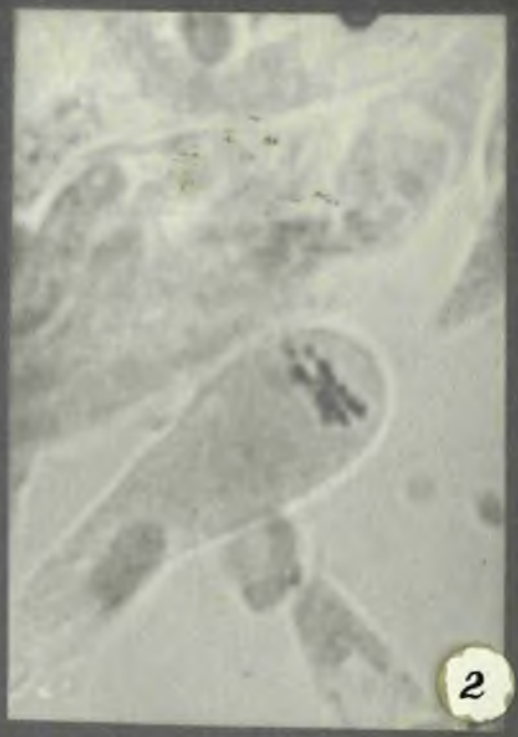
Fig. 1. Leptotene.

Fig. 2 & 3a. Pachytene; beaded bivalents seen lying free in the cytoplasm parallel to each other.

Fig. 3b. Drawing of 3a to show separate bivalents.

Figs. 1, 2, 3a. Aceto-orcein stained; bright light.


1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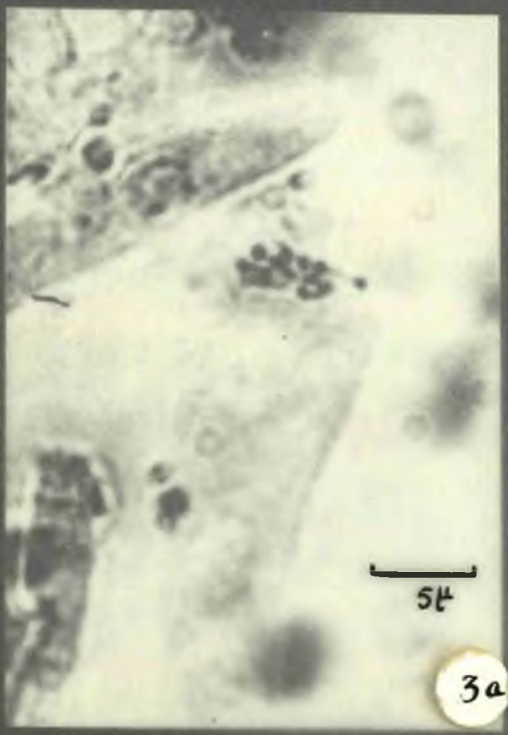

5μ
3a


4
3
1
2
3b

4
3
1
2

PLATE XLVIII.

Meiosis in the basidium of *M. rotula*.

Figs. 1a, 1b & 2. Pachytene. Bivalents I & II show incomplete synapsis. Beaded appearance still evident. Aceto-orcein stained; bright light.

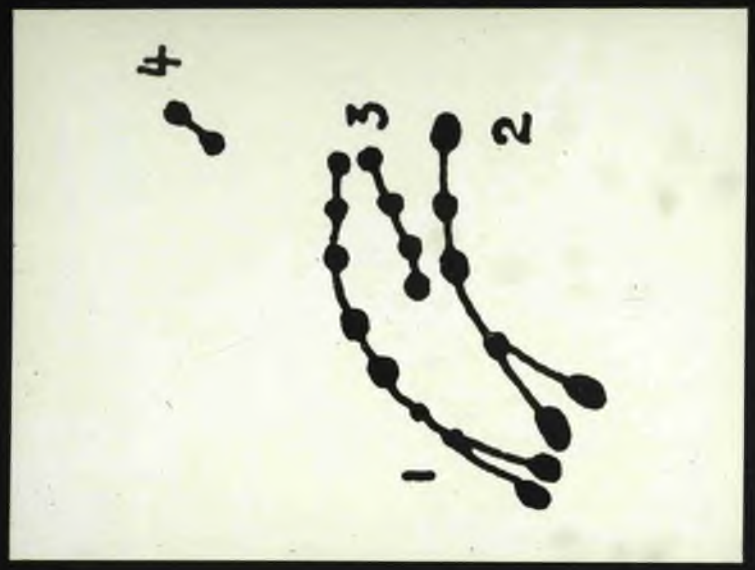
Fig. 3. Drawing showing the length and morphology of chromosomes at pachytene.

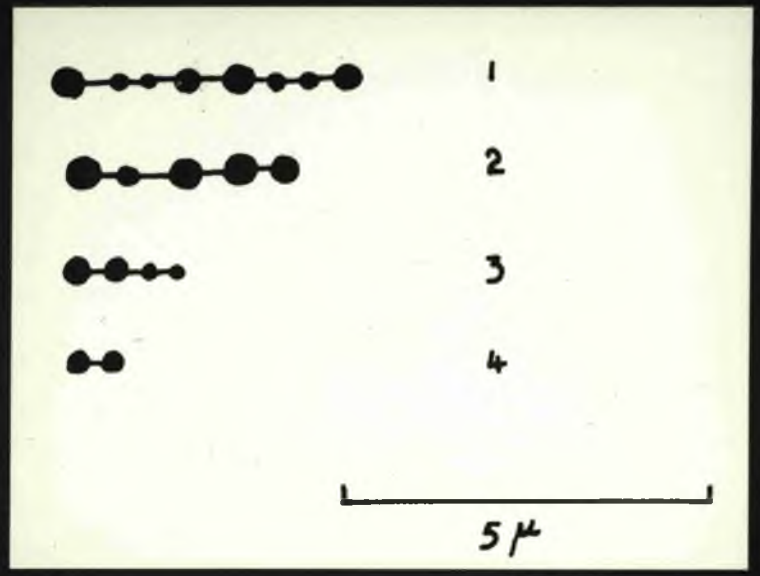
1a

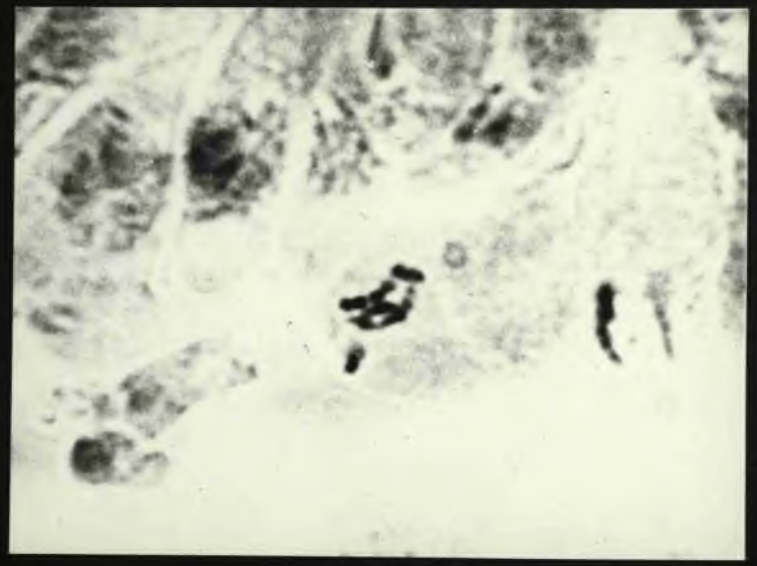

4
3
1
2
1b


5μ
2
5μ


4
3
2
1
5μ
3


4
3
2
1


1
2
3
4
5μ



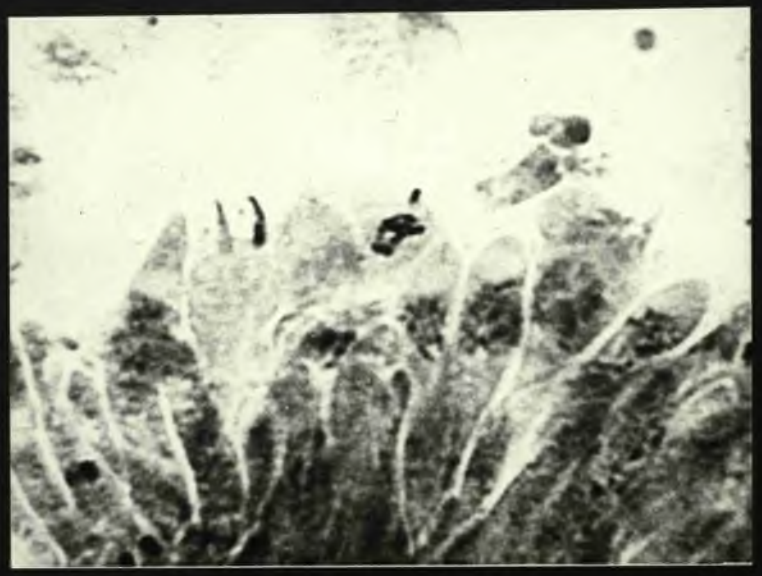


PLATE XLIX.

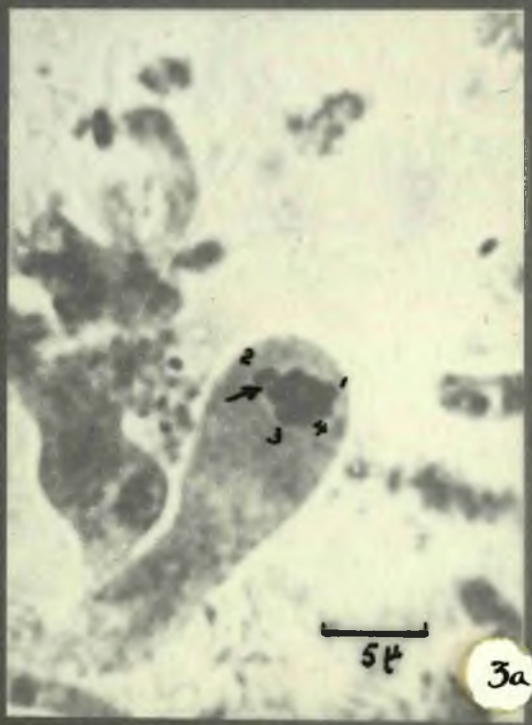
Fig. 1. Bivalents seen thrown into large coils; bivalents still lie parallel to each other. Aceto-orcein stained; bright light.

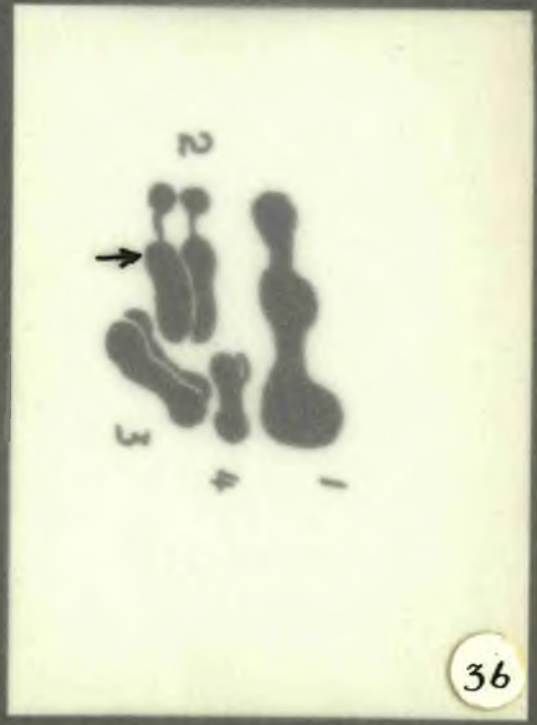
Fig. 2. Three of the four coiled bivalents joined to one another. Satellited bivalent arrowed. Aceto-orcein stained; bright light.

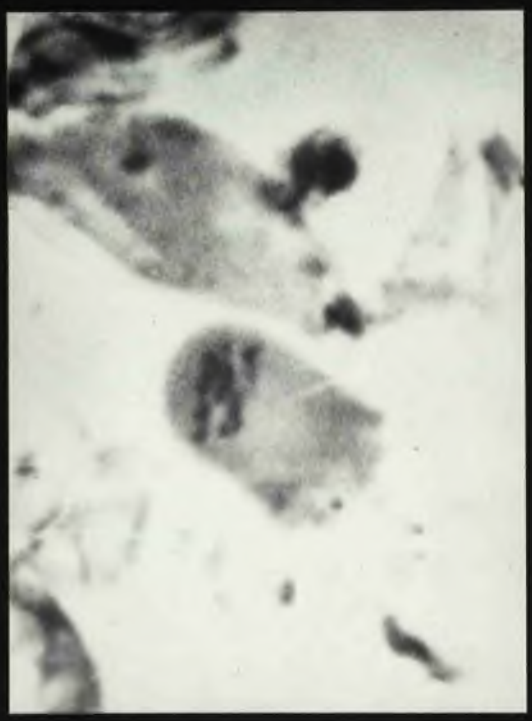
Fig. 3a & b. Diakinesis. Contraction probably at its maximum; duality of bivalents seen (arrows). Aceto-orcein stained; bright light.

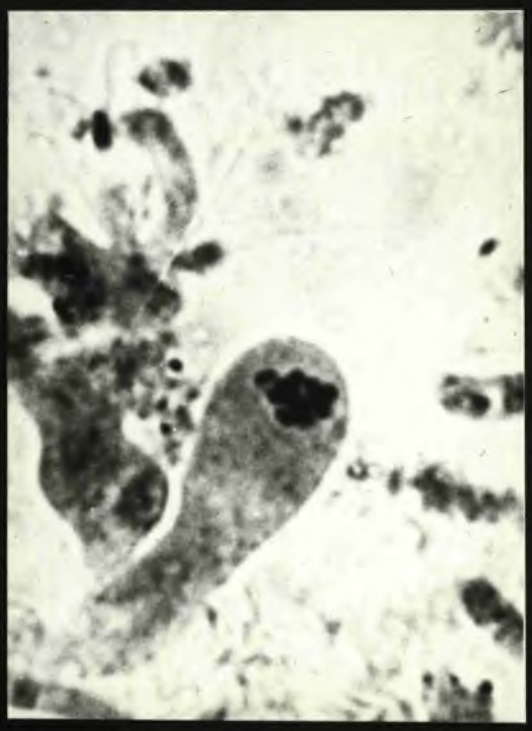

1


2


2
1
3
4
5μ
3a


2
3
4
1
3b






2
3
4
1

PLATE L.

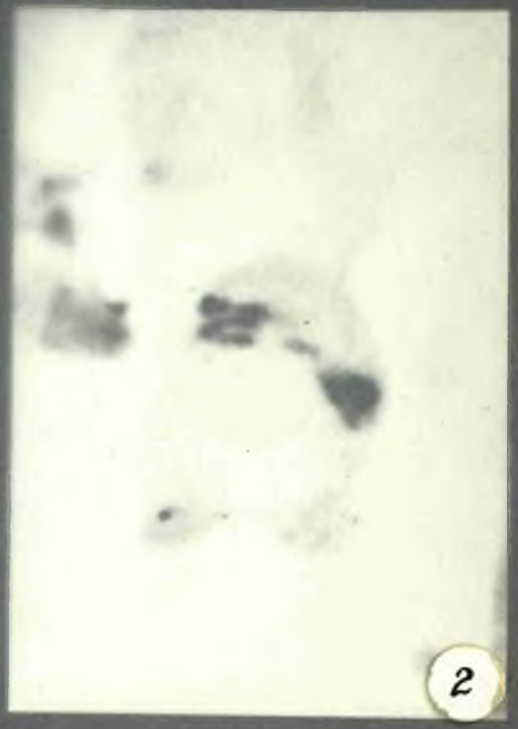
Meiosis in the basidium of M. rotula.

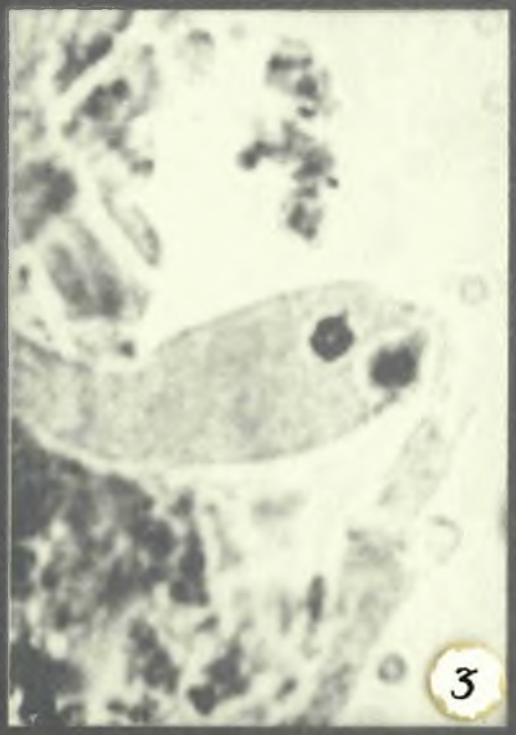
Fig. 1. Bivalents joined one to the other by a faintly stained thread. Pre-metaphase. Aceto-orcein; bright light.

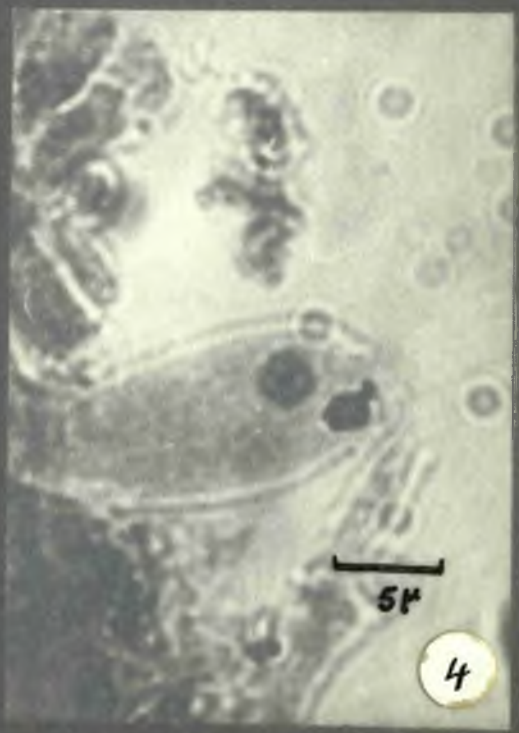
Fig. 2. Anaphase separation. Chromosomes seen joined one to the other by a faint thread which forms a bridge between the groups. Aceto-orcein stained; bright light.

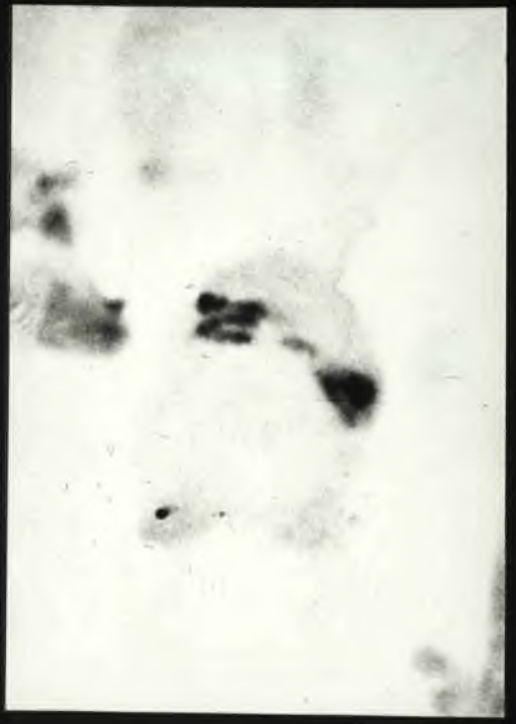
Figs. 3 & 4. Different focuses of same basidium showing ring formed by chromosomes at telophase. Aceto-orcein stained; bright light.

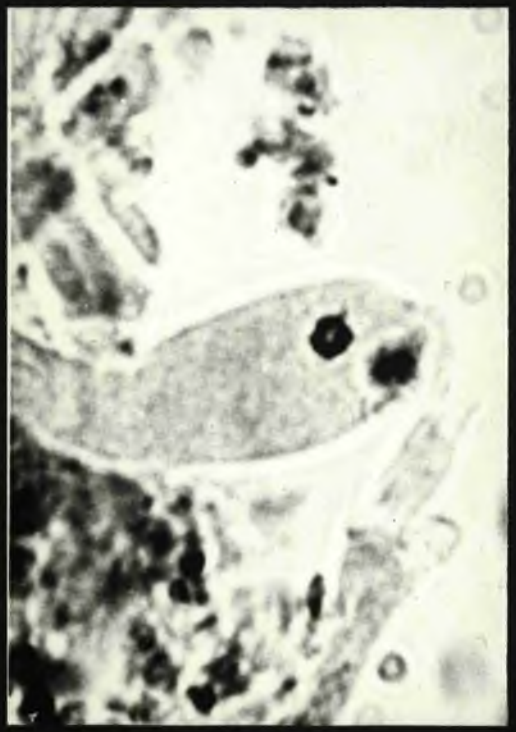

1

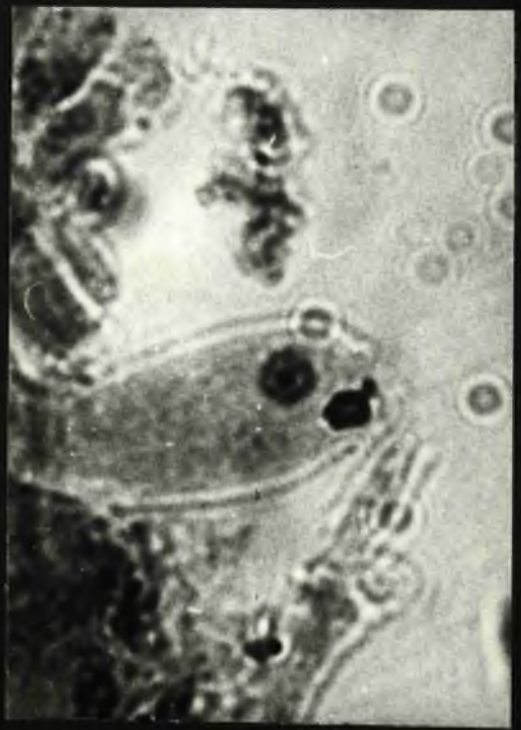

2


3


5μ
4







# PLATE LI.

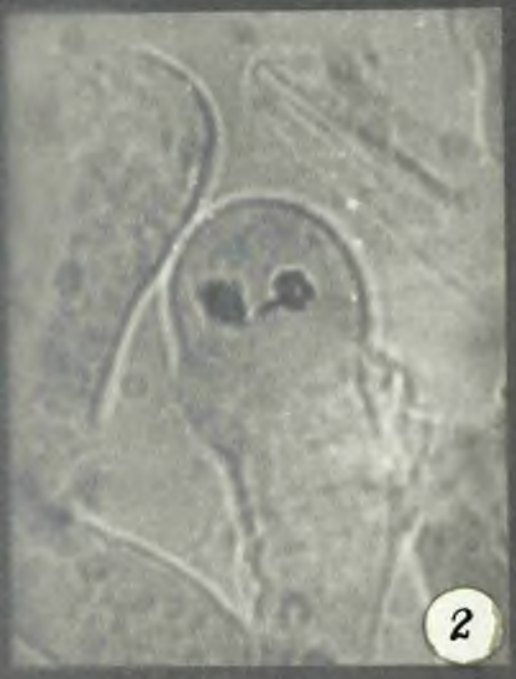
Meiosis II in the basidium of *M. rotula*.

Figs. 1 & 2. Four chromosomes in ring at early telophase; bridge is seen in fig. 2. Aceto-orcein stained; bright light.

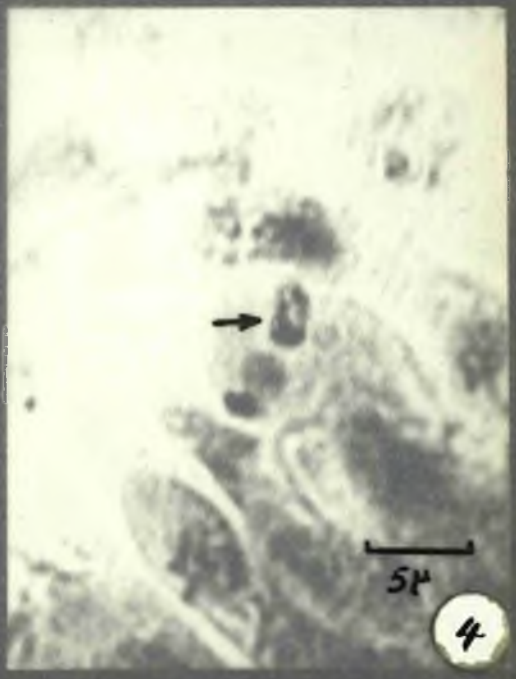
Fig. 3. Prophase II. Double strands of beaded chromatin are seen. Nucleoli are not evident. Aceto-orcein stained; bright light.

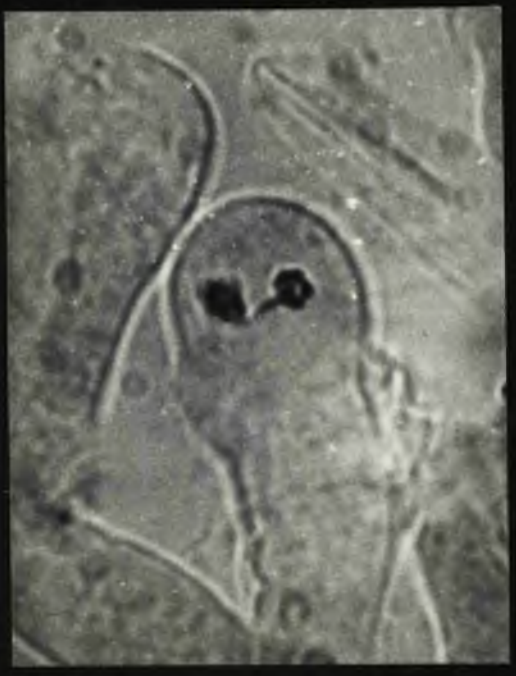
Fig. 4. Metaphase II. Double rings of chromatin (arrows). Aceto-orcein stained; bright light.

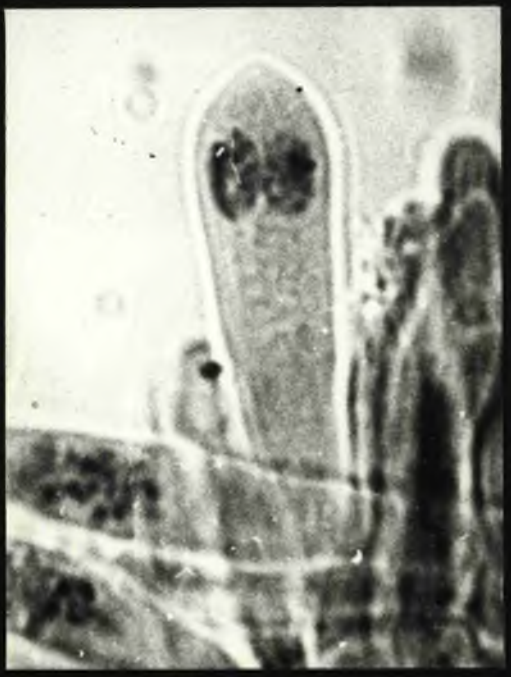

1


2


3


5μ
4





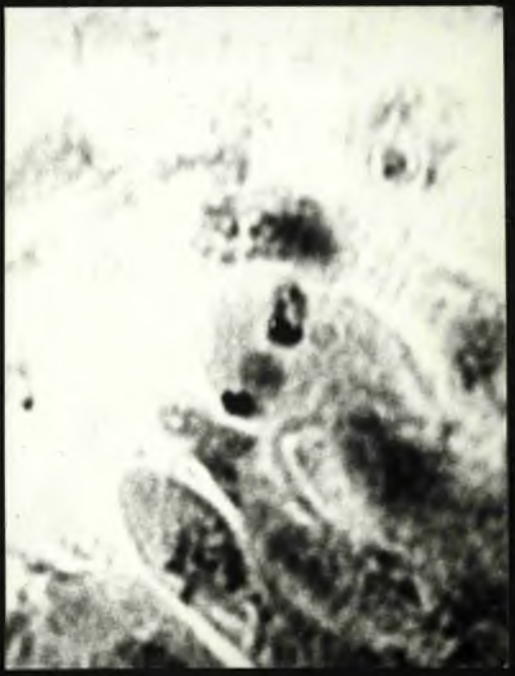


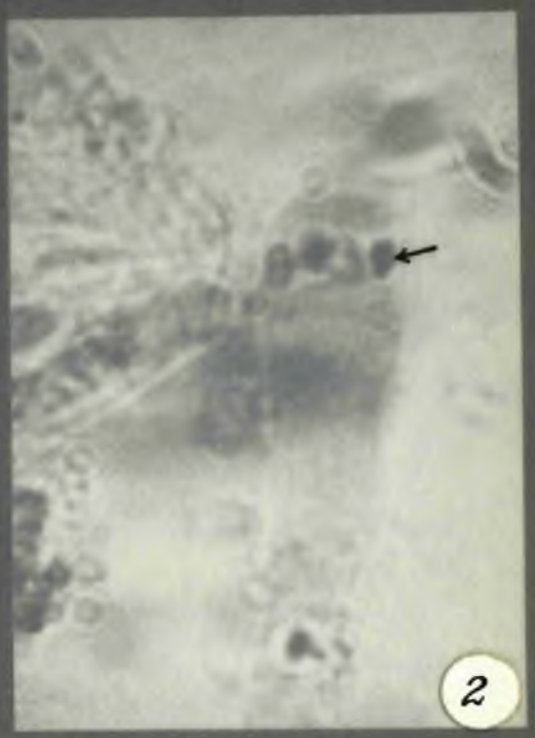
PLATE LII.

Meiosis II in the basidium of *M. rotula*.

Figs. 1 & 2. Different focuses of the same basidium showing the four nuclei formed as a result of Meiosis. Arrowed nuclei show four rounded chromosomes. Aceto-orcein stained; bright light.

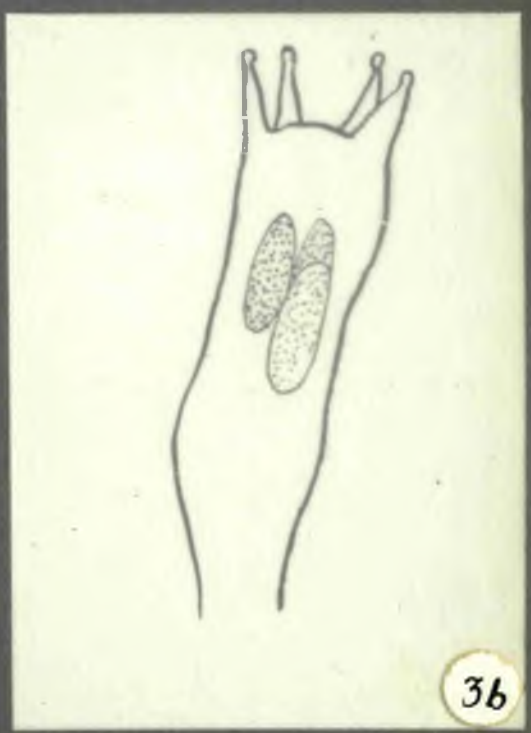
Figs. 3a & b. Four sterigmata on basidium in which three of the four resting nuclei are seen. Aceto-orcein stained; bright light.

1

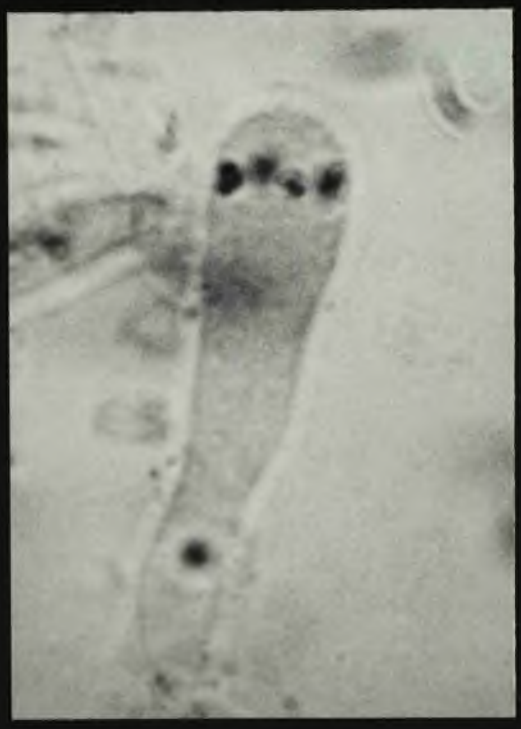


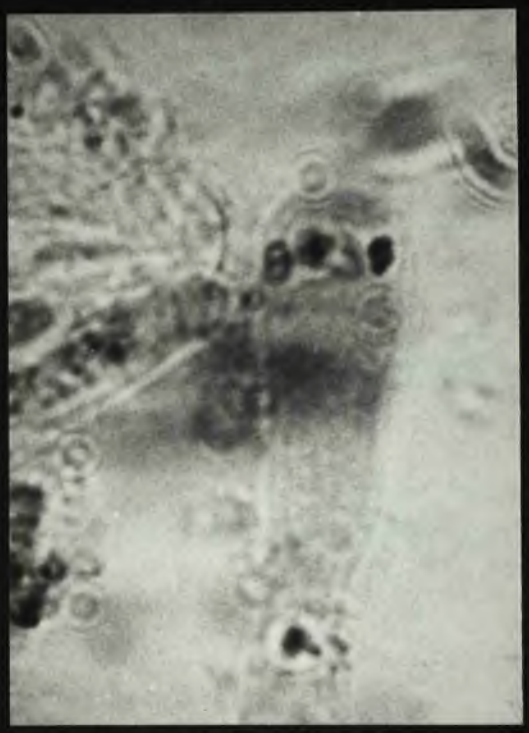
2


3a



3b





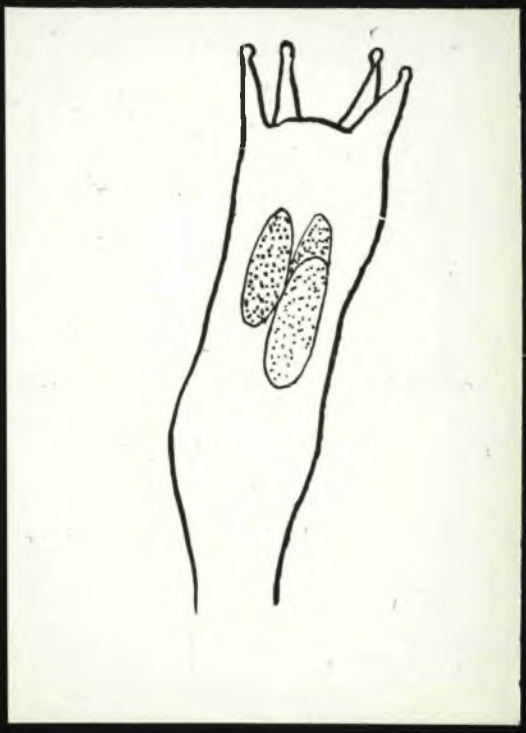


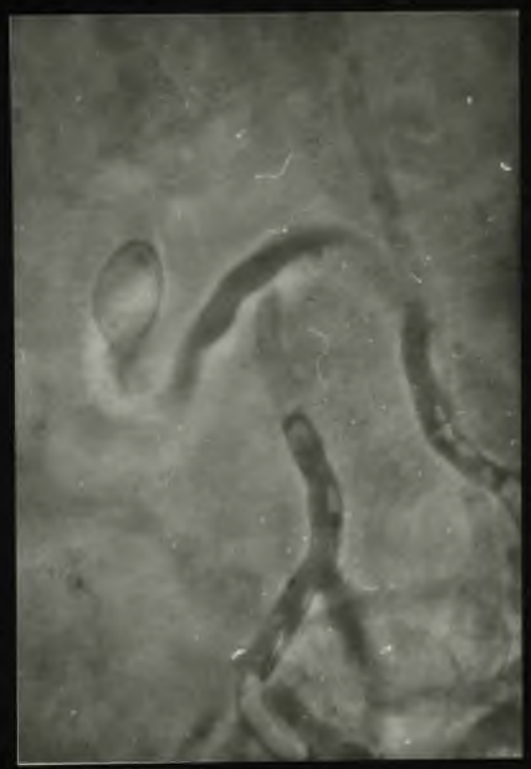
PLATE LIII.

Fig. 1. Germinating spore of *M. rotula*.

Fig. 2. 'Monokaryon' hypha of *M. rotula* showing two nuclei in one cell (arrow) and one in another (x).

1


X
5μ
2



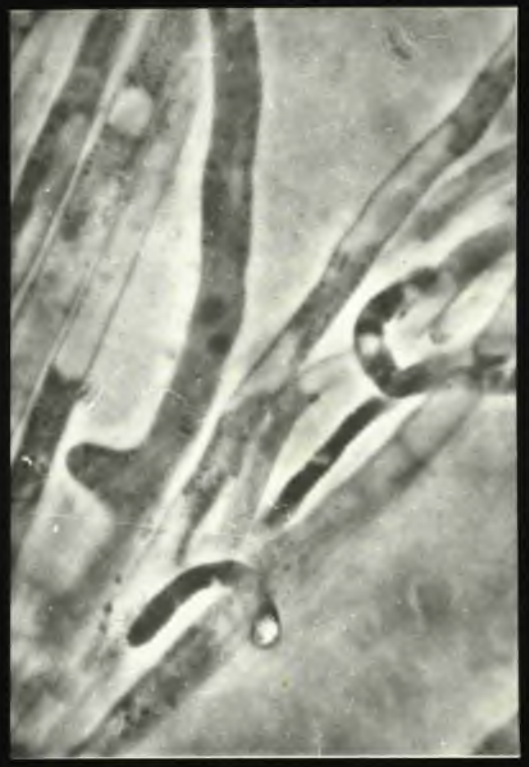
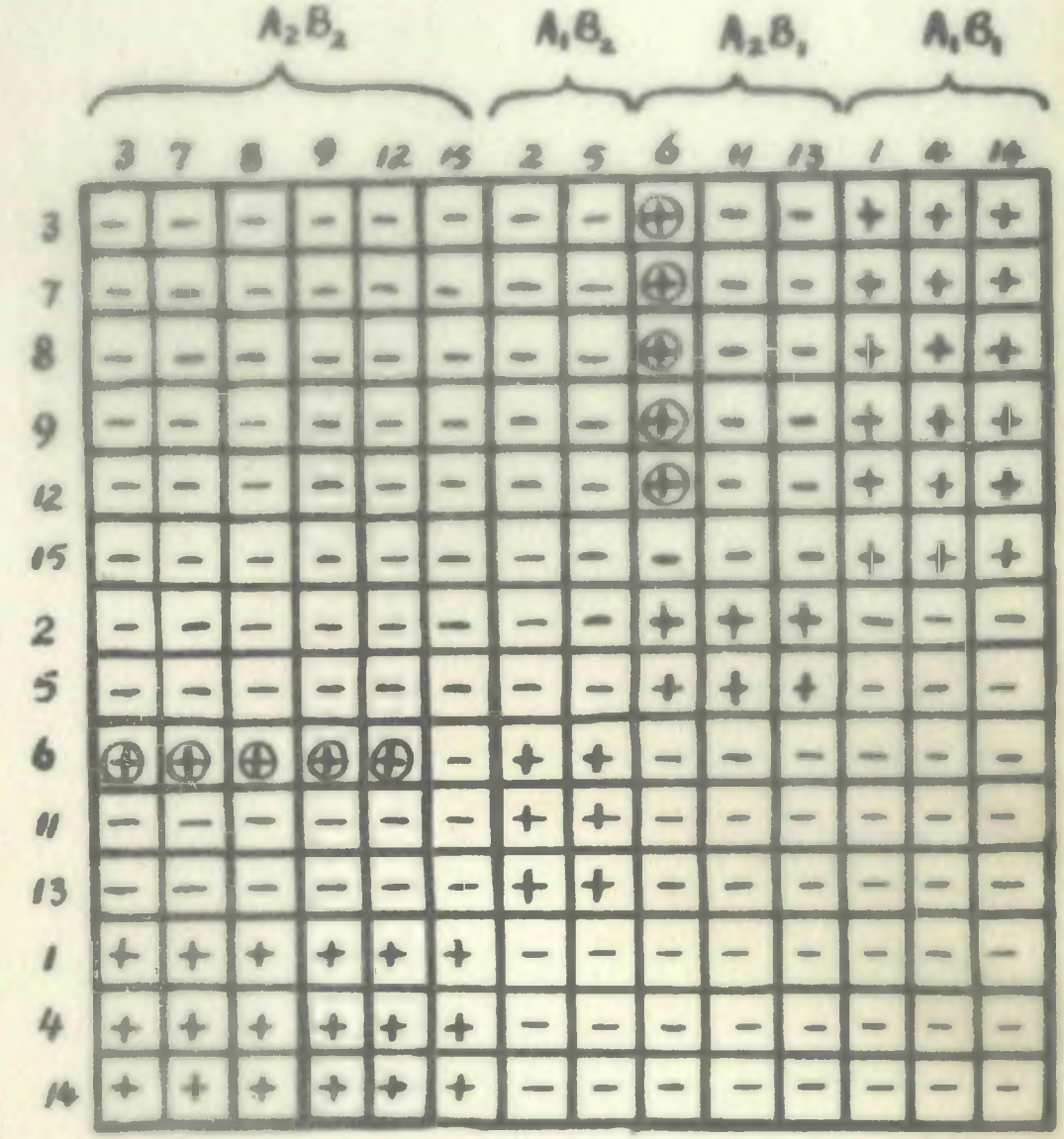


PLATE LIV.

Table showing the results of all possible pairings of fourteen monosporous mycelia derived from fourteen spores of a single fruit body.


A2B2
A1B2
A2B1
A1B1
3 7 8 9 12 15 2 5 6 11 13 1 4 14
3 7 8 9 12 15 2 5 6 11 13 1 4 14

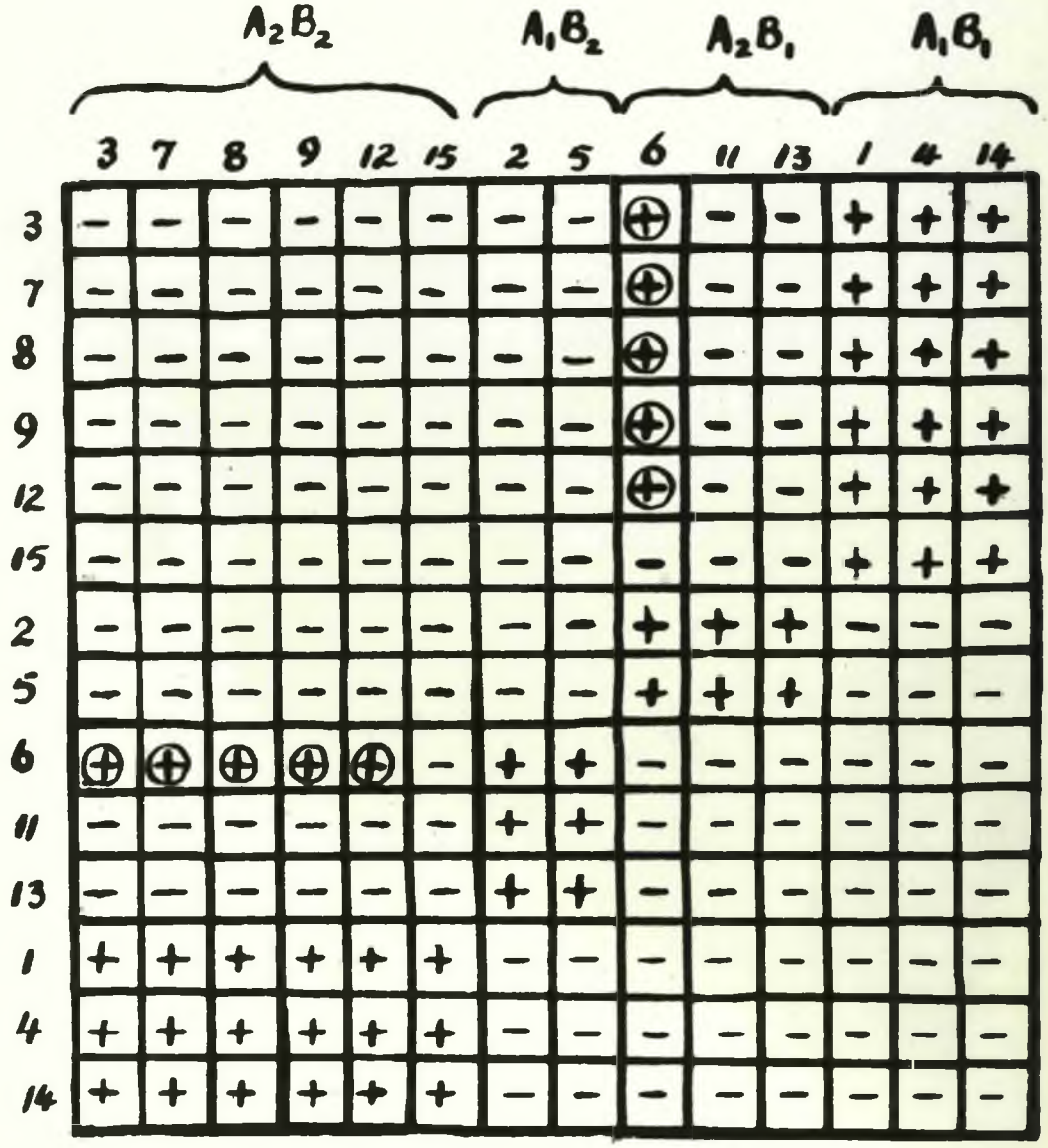
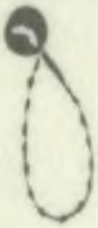

$A_2B_2$
$A_1B_2$
$A_2B_1$
$A_1B_1$
3 7 8 9 12 15 2 5 6 11 13 1 4 14
3 − − − − − − − − ⊕ − − + + +
7 − − − − − − − − ⊕ − − + + +
8 − − − − − − − − ⊕ − − + + +
9 − − − − − − − − ⊕ − − + + +
12 − − − − − − − − ⊕ − − + + +
15 − − − − − − − − − − − + + +
2 − − − − − − − − + + + − − −
5 − − − − − − − − + + + − − −
6 ⊕ ⊕ ⊕ ⊕ ⊕ − + + − − − − − −
11 − − − − − − + + − − − − − −
13 − − − − − − + + − − − − − −
1 + + + + + + − − − − − − − −
4 + + + + + + − − − − − − − −
14 + + + + + + − − − − − − − −

PLATE LV.

Diagramatic representation of the stages of division in the ultimate clamps, Meiosis II and in the vegetative hyphae.

Prophase 1 - 3; Metaphase 4 - 5; Anaphase 6a, 6b & 7; Telophase 8.



1

2

3

4

5

6a

6b

7

8

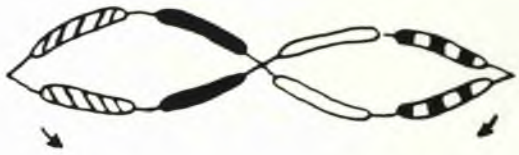
PLATE LV.

Diagramatic representation of the stages of division in the ultimate clamps, Meiosis II and in the vegetative hyphae.

Prophase 1 - 3; Metaphase 4 - 5; Anaphase 6a, 6b & 7; Telophase 8.

1

2



3

4

5

6a

6b

7

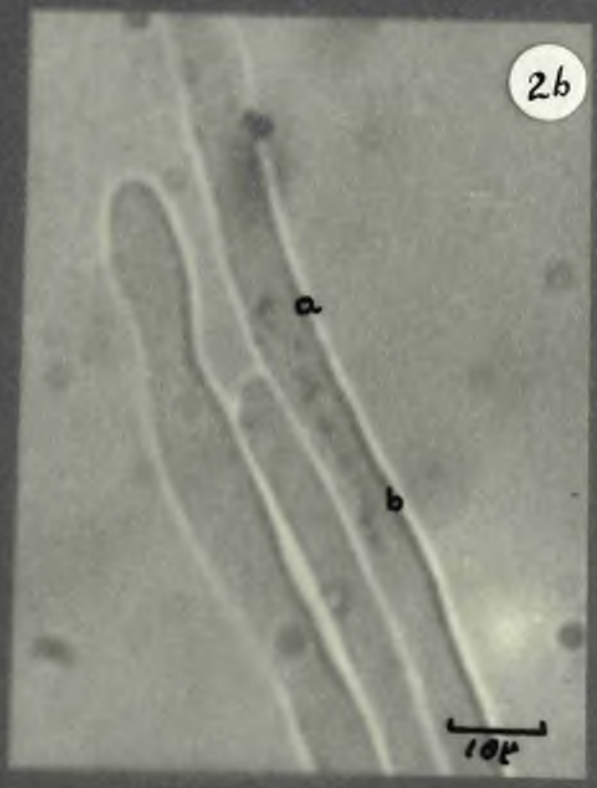
8

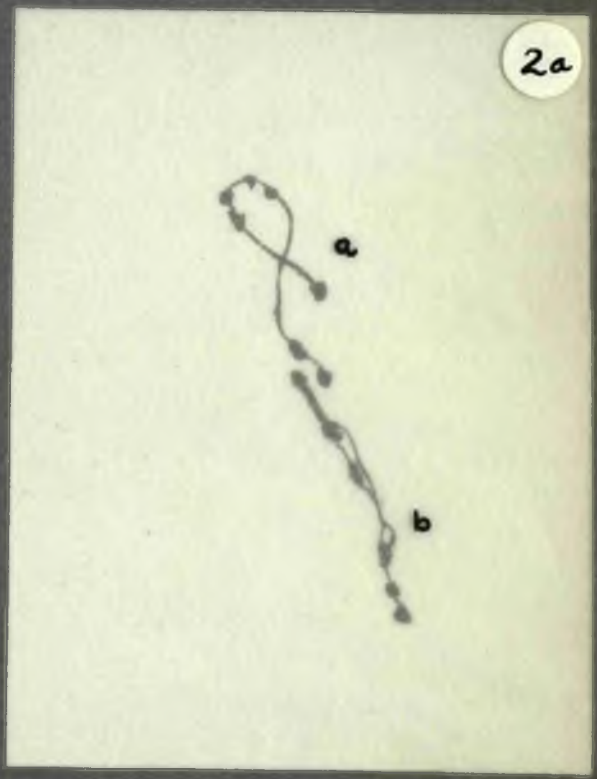
PLATE LVI.

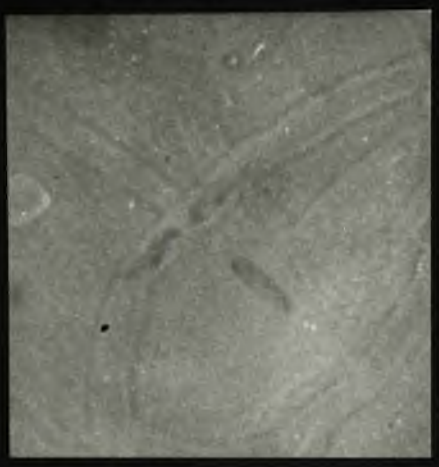
Fig. 1. Resting nuclei in the vegetative hyphae of M. rotula. Feulgen - Fast Green stained; bright light.

Figs. 2a & b. Nuclei a & b in vegetative hyphae of M. androsaceus undergoing great elongation. Feulgen - Fast Green stained; bright light.


10μ
1


2b
a
b
10μ


2a
a
b



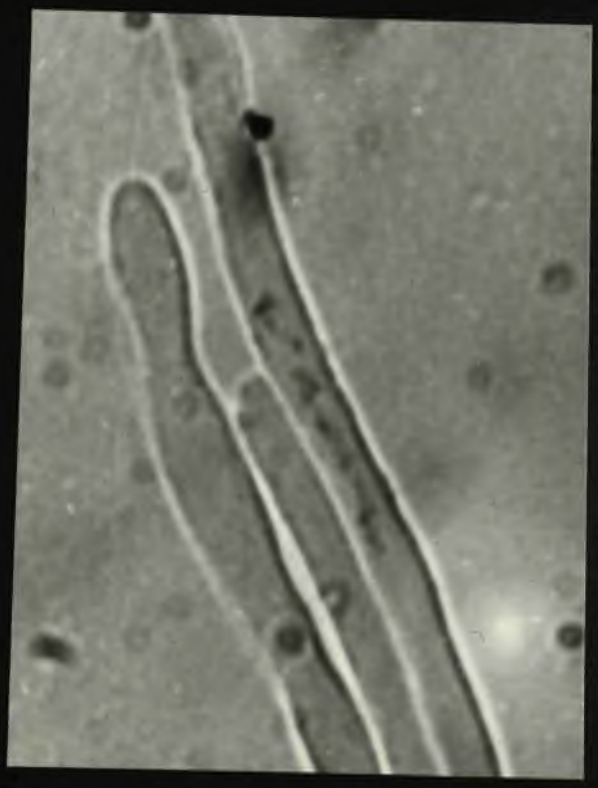


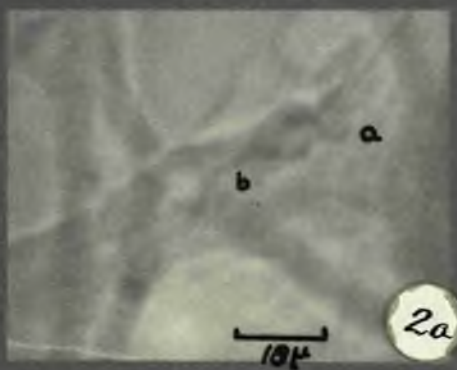
PLATE LVII.

Division in the vegetative hyphae.

Fig. 1. Nucleus seen as a double structure of chromatin; early prophase. Feulgen-Fast Green stained; bright light.

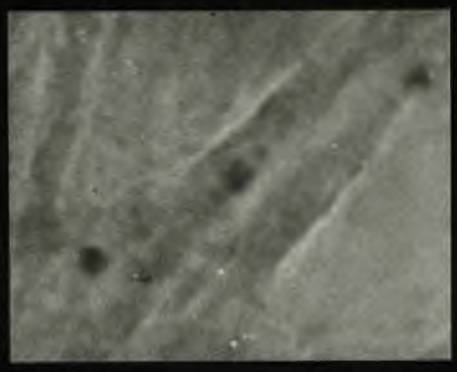
Figs. 2 a & b. Nuclei greatly elongated. Nucleus (b) twisted; the strands of nucleus (b) and (a) apparently joined at one end only. Feulgen-Fast Green stained; bright light.

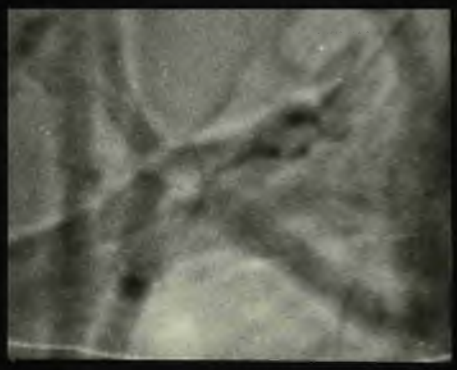
1




2a


2b





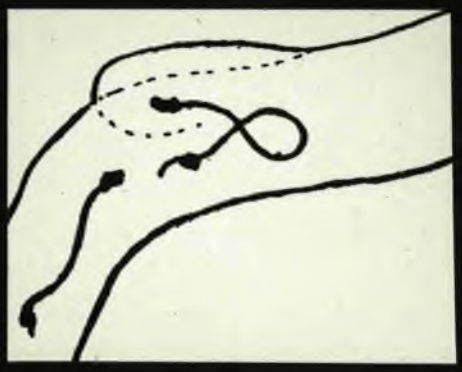


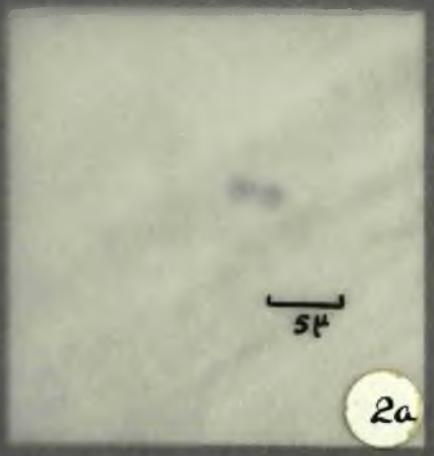
PLATE LVIII.

Division in vegetative hyphae.

Fig. 1. Drawing of nuclei in M. rotula seen undergoing the twist (late prophase).

Figs. 2a & b. Metaphase - double rings of chromatin formed as a result of the bending over of the loops of the figure of eight. Feulgen - Fast Green stained; bright light.


1


5μ
2a


2b

PLATE LIX.

Division in the vegetative hyphae.

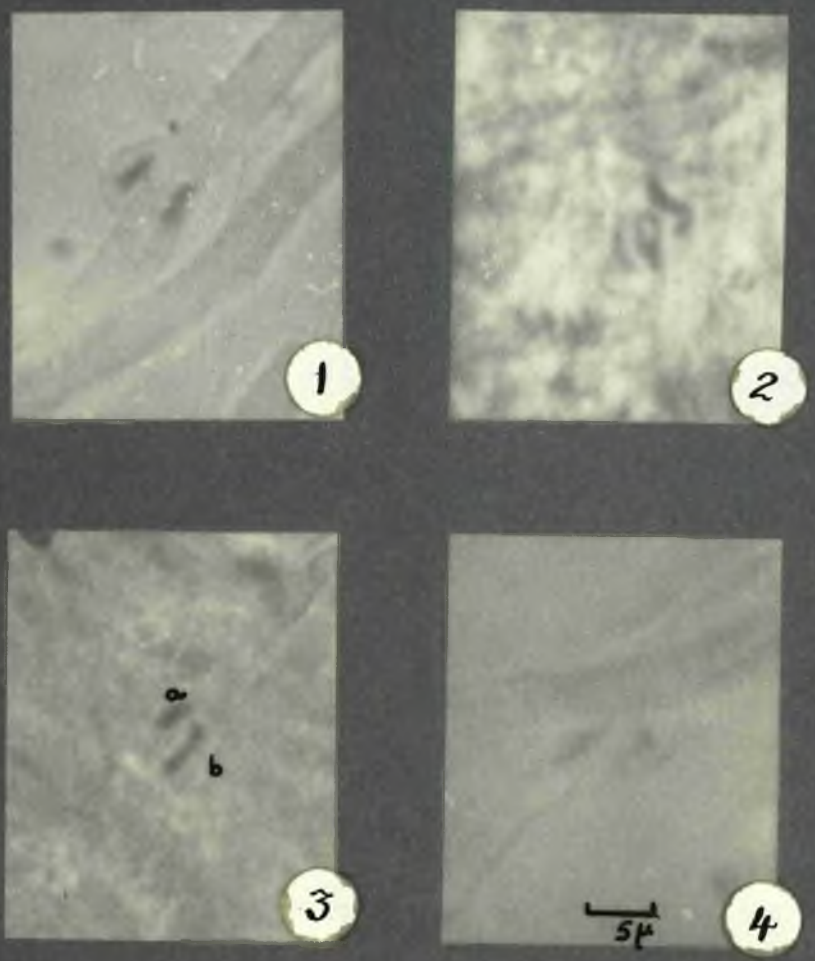
Metaphase.

Fig. 1. M. androsaceus - double rods of chromatin seen in each nucleus. Feulgen-Fast Green stained; bright light.

Fig. 2. M. rotula - arms of double rings of chromatin seen opening out. Feulgen-Fast Green stained; bright light.

Fig. 3. M. rotula - nucleus (a) double rods of chromatin. Nucleus (b) beginning of anaphase separation. Feulgen-Fast Green stained; bright light.

Fig. 4. M. androsaceus - double rods of chromatin seen in each nucleus.


1
2
a
b
3
5μ
4

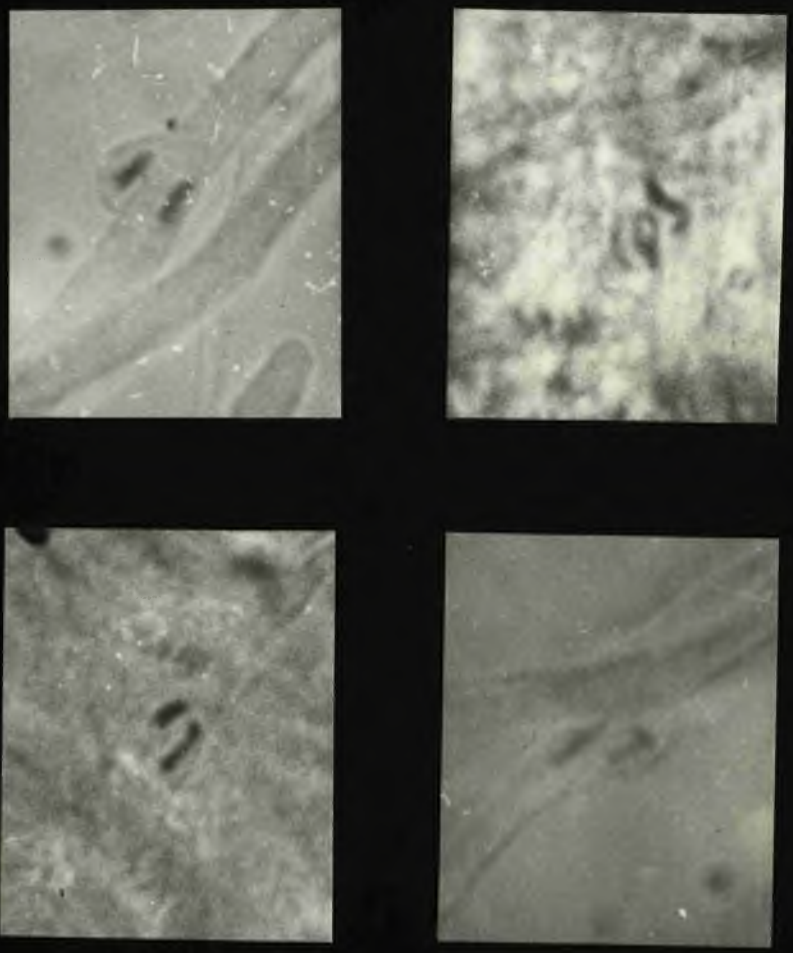


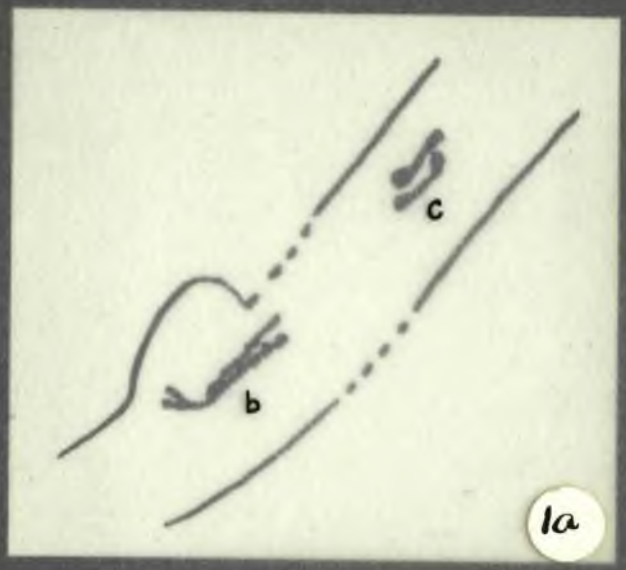
PLATE LX.

Division in the vegetative hyphae.

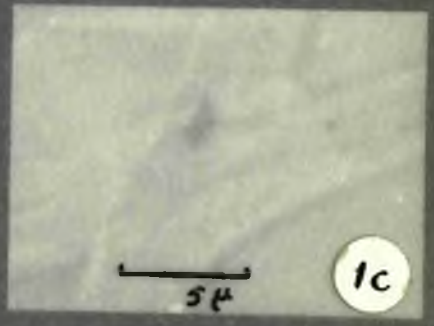
Fig. 1 a) Drawing of b & c.

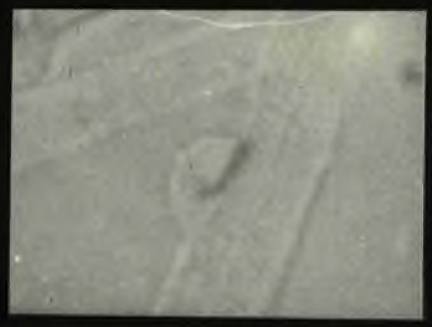
b) Nucleus in clamp showing two rods of chromatin in early anaphase.

c) Nucleus showing two rods of chromatin in metaphase. Both nuclei are within the same hypha. Feulgen-Fast Green stained; bright light.


c
b
1a


1b


5μ
1c



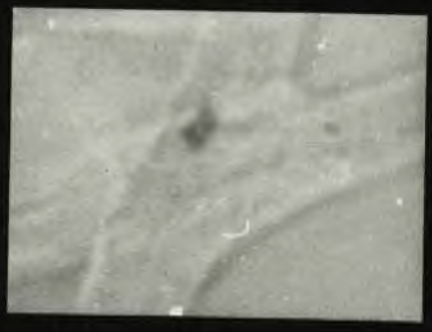


PLATE LXI.

Division in the vegetative hyphae.

Anaphase.

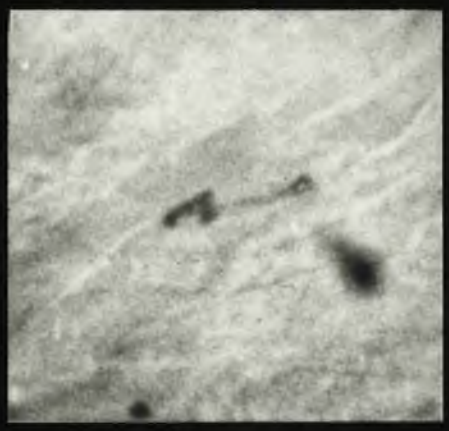
Fig. 1. M. androsaceus.

Fig. 2. M. rotula.

Figs. 1 & 2 - Feulgen-Fast Green stained; bright light.

1


5μ
2



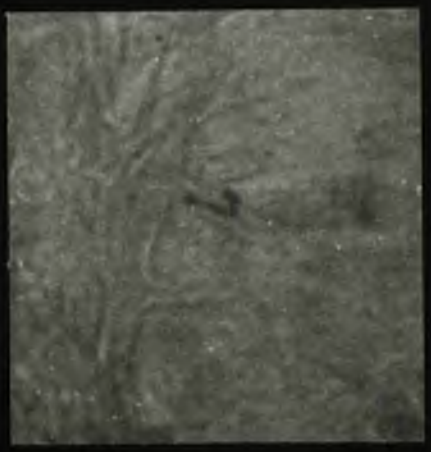


PLATE LXII.

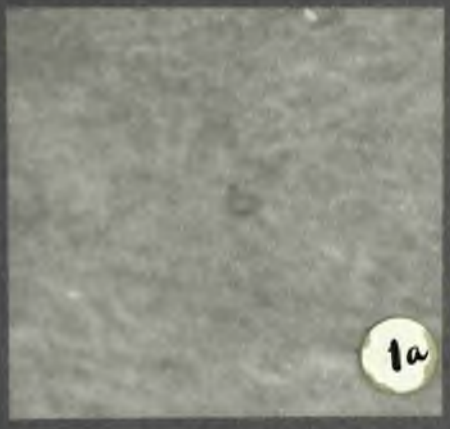
Division in the vegetative hyphae.

Anaphase.

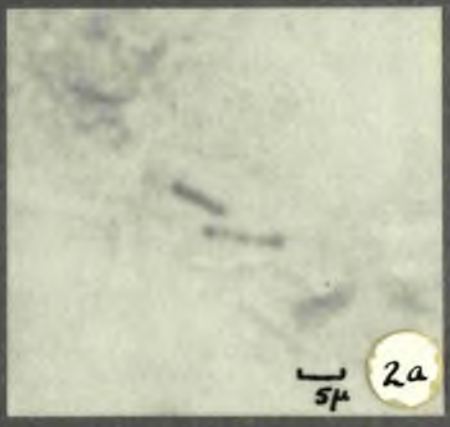
Figs. 1a & b. M. rotula.

Figs. 2 a & b. M. androsaceus. Nucleus (a) early anaphase; nucleus (b) late anaphase.

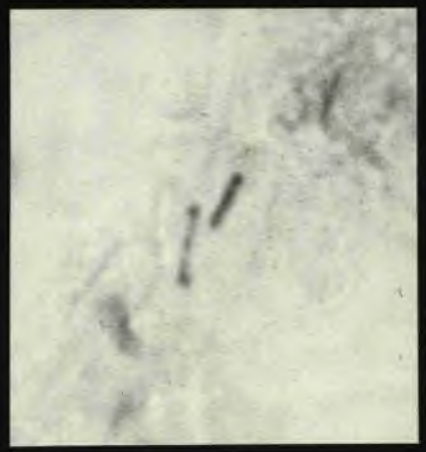
Feulgen-Fast Green stained; bright light.


1a


1b


5μ
2a


2b



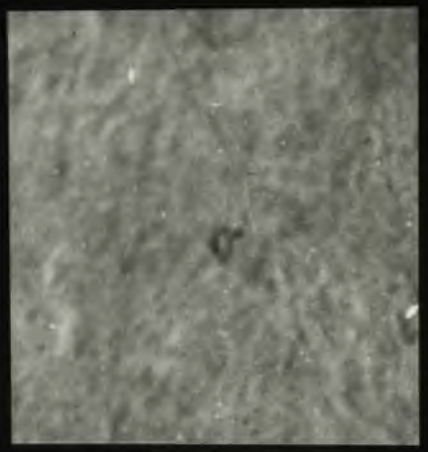


PLATE LXIII.

Division in the vegetative hyphae.

Anaphase.

Fig. 1 a & b. M. rotula.

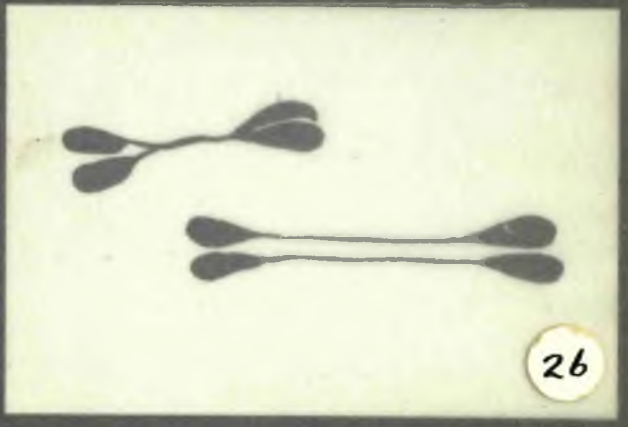
Fig. 2 a & b. M. androsaceus; both nuclei groups show connecting bridges.

Feulgen-Fast Green stained; bright light.


1a


1b


2a
5μ


2b

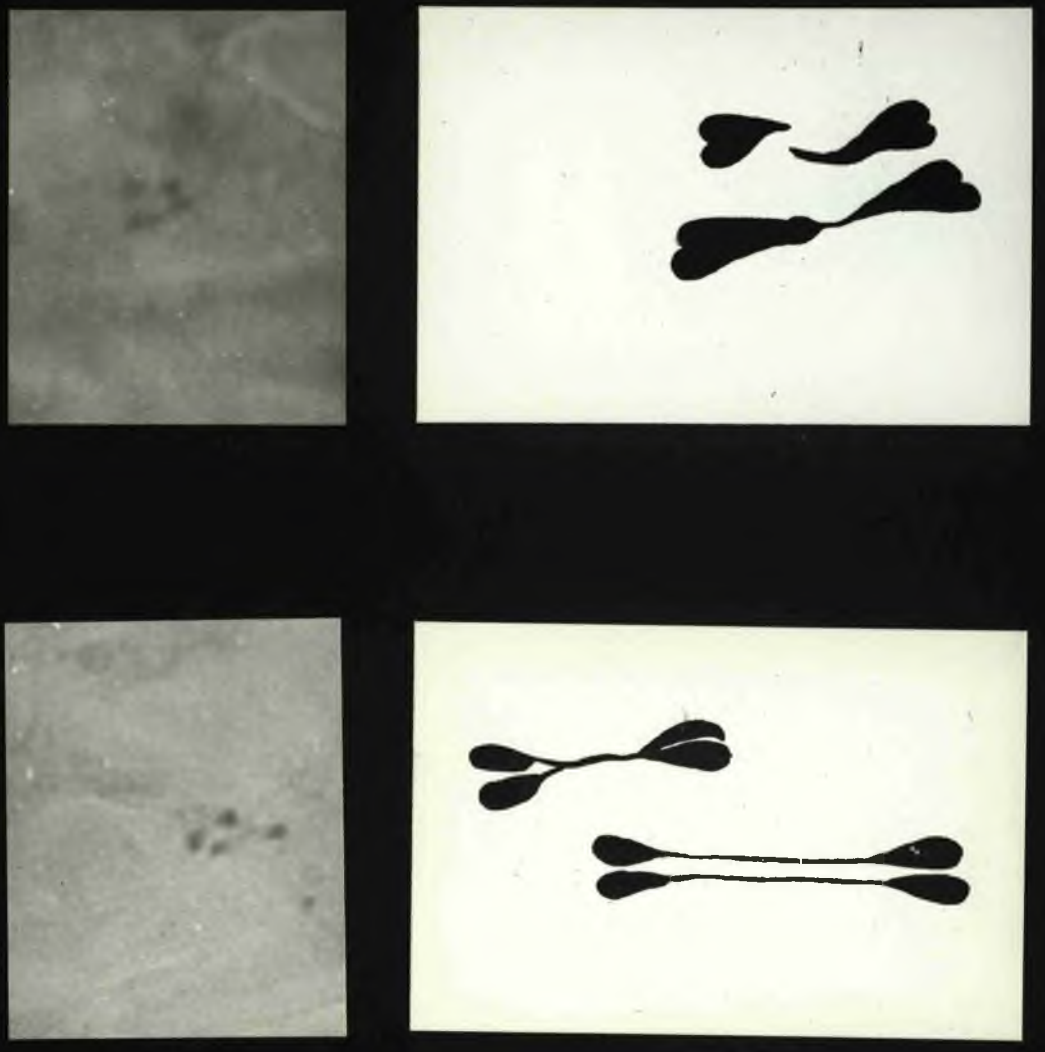


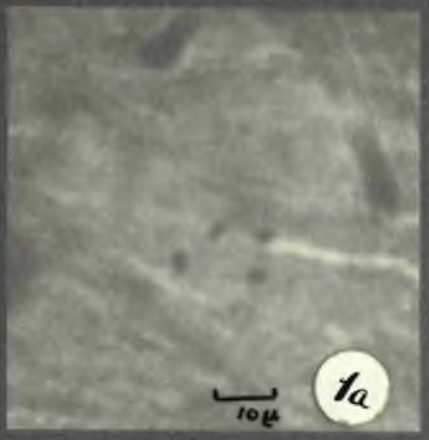
PLATE LXIV.

Division in the vegetative hyphae.

Figs. 1 a & b. Late anaphase in M. rotula.

Figs. 2 & 3. Drawings of anaphase in M. androsaceus.

Feulgen-Fast green; bright light.




1a

1b

2a

2b

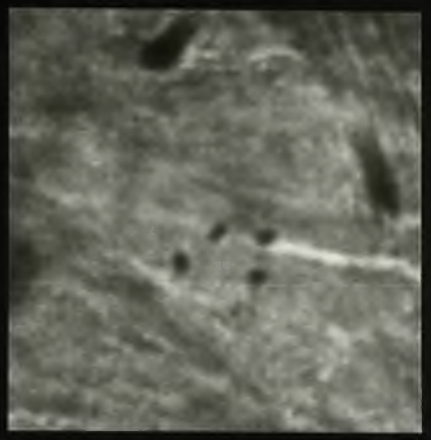


PLATE LXV.

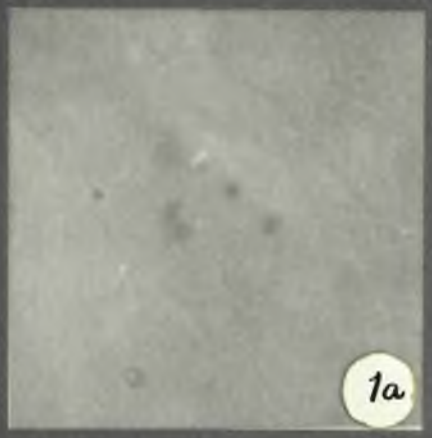
Division in the vegetative hyphae.

Late anaphase - telophase.

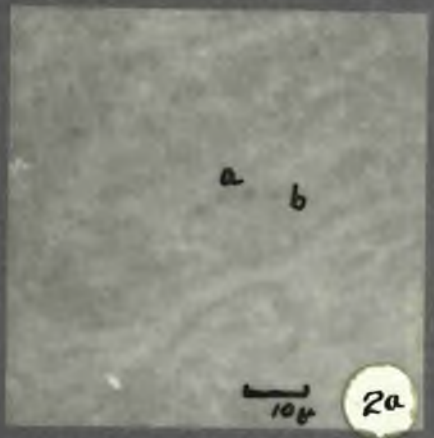
Figs. 1 a & b. Telophase in M. androsaceus. Arrowed nucleus shows four rounded chromosomes.

Figs. 2 a & b. Anaphase (nucleus a); telophase (nucleus b) in M. rotula.

Feulgen-Fast Green stained; bright light.


1a


1b

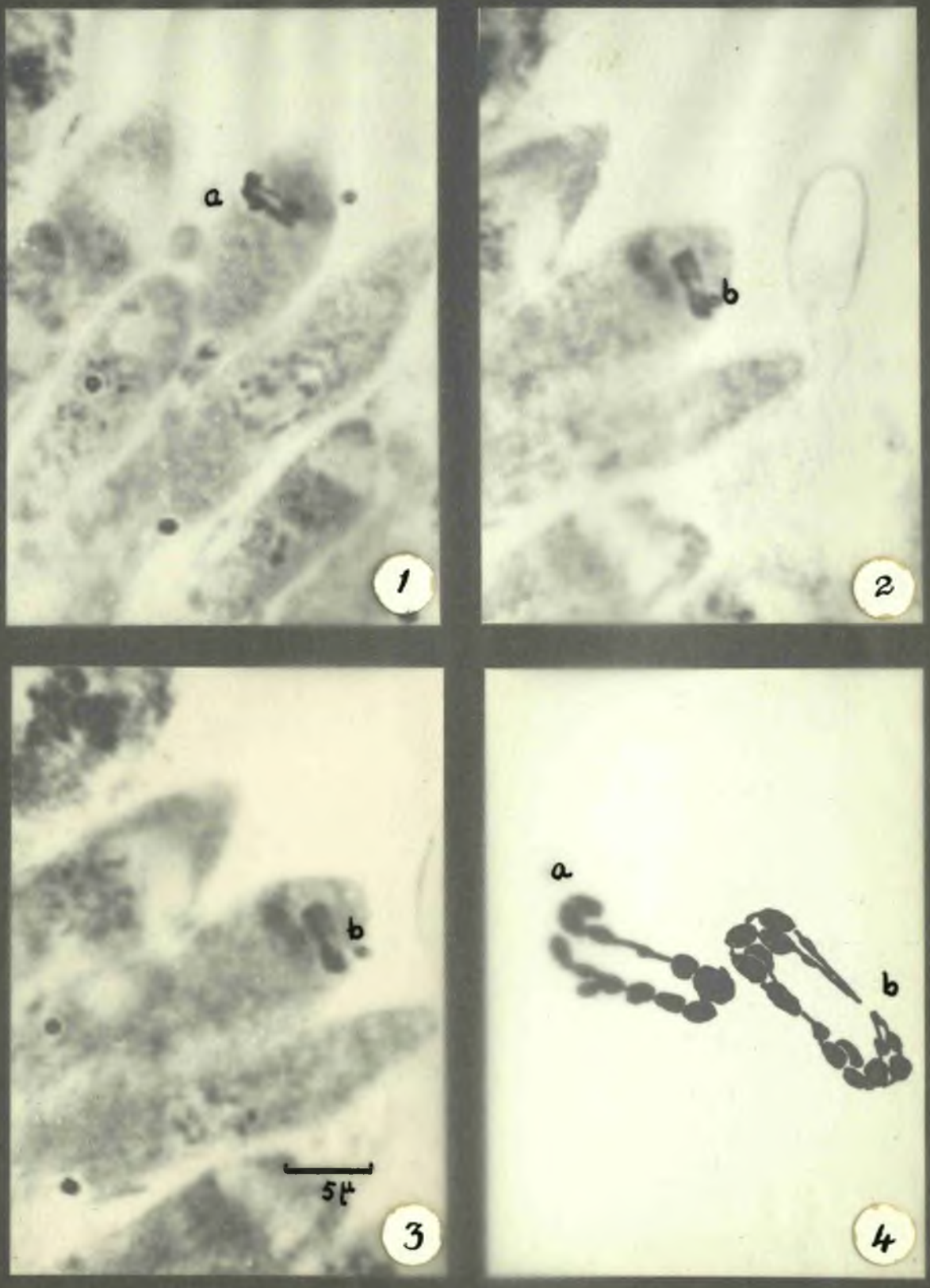

a
b
10 µ
2a


a
b
2b

PLATE XLI.

Figs. 1, 2 and 3. Different focuses of same basidium showing nucleus (a); two metaphase rods of chromatin and nucleus (b); double rings of beaded chromatin not yet extended linearly. All lie freely in the cytoplasm. Aceto-orcein stained; bright light.

Fig. 4. Drawing of nuclei above.


a
1
b
2
b
5μ
3
a
b
4