OBSERVATIONS ON CELL GROWTH, MITOSIS, AND DIVISION IN THE FUNGUS *BASIDIOBOLUS RANARUM*

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ABSTRACT

The paper describes the forward streaming, growth, and division of the vegetative cell of *Basidiobolus ranarum*. The cell is several hundred microns long and has a single large nucleus. Mitosis is invariably followed by cell division. Both processes have been studied in the living cell by ordinary and phase contrast microscopy. Mitosis is accompanied by a temporary coarsening of the organisation of the cytoplasm and a considerable slowing down of the rate of growth of the cell wall tube. Fixed and stained preparations have shown that there is a large number of small chromosomes and that the mitotic spindle is formed from the nucleolus. *Basidiobolus* appears suitable for observations on the cell duplication cycle and the physiology of mitosis.

INTRODUCTION

The purpose of this paper is to reintroduce into experimental biology an interesting cell which was well known to botanists sixty years ago but which since that time has received less attention than it deserves. *Basidiobolus*, to give it its name, may be described as a colonial organism. The individual is a long cylindrical cell within a tube or hypha and has a single, large nucleus with a voluminous nucleolus. The walls of the hyphae are continuous with each other but the protoplasts inside have no neighbours. Each inhabits the terminal chamber of a branch of its own. The fungus is easily maintained and handled and provides rewarding material for the study of cytoplasmic streaming and the morphology and physiology of mitosis and cell division. *Basidiobolus* is not suitable for studies of single chromosomes with the light microscope but an interesting feature of its mitosis is the spindle apparatus which the metaphase chromosomes generate in the rearranged substance of the former nucleolus.

Basidiobolus is a member of the family *Entromophthoraceae* in the order Phycomycetes of the fungi. It is a regular parasite of frogs from whose intestines it is readily isolated. This was first done by Eidam in 1887 (5), who gave a good general description of the fungus and its remarkable conidia which are shot from the mycelium with the aid of an explosive device fashioned by the conidium-to-be out of its own substance before it transforms the rest of itself into the conidium proper. Eidam also described the zygospores of the new fungus which arise from the fusion of adjoining cells and saw something of the nuclear events accompanying this process. More informative accounts of zygospore-formation were later

provided by several of Eidam's contemporaries (6, 18) whose illustrations are still to be found in most textbooks of mycology. Rarely emphasised and little studied since Eidam's time, despite its many remarkable features, is the large, mitotically dividing nucleus of the *vegetative* cells of *Basidiobolus*.

Many accurate observations on the growth and division of living cells of this organism were made by Raciborski (20–22) who rightly thought that it held promise for experimental work on mitosis. Raciborski published drawings of dividing nuclei seen in stained preparations (21). They show the half spindles of anaphase very clearly but in other respects are inferior to the illustrations of mitosis in the papers by Fairchild (6) and Olive (18) referred to above, whose specimens were better preserved than those examined by Raciborski. Several papers on nuclear behaviour during zygospore formation appeared in the course of the next fifty years (16, 29), but they added no new features to the established picture of mitosis in *Basidiobolus*. Drechsler (4), an authority on the taxonomy of *Basidiobolus*, recently drew attention to the fact that the nucleus of this fungus can be recognised in the living cell with ordinary microscopy, but he did not concern himself with its division. A solitary profile of a Feulgen-stained metaphase plate is in a paper by Soudek and Stranska (27) and a photograph of a resting nucleus, starkly conveying its exceptional size, was recently published by Girbardt (7). The last two pointers, reinforced by recollections of the cytology of *Basidiobolus* communicated to the writer by Dr. Laura Garnjobst, led to the undertaking of the present investigation.

MATERIALS AND METHODS

This work was begun at a time when frogs were not available in the open and was mainly done on a strain of the fungus obtained from the Centraalbureau voor Schimmelcultures (C.B.S.) at Baarn, Holland. Under the conditions under which it is maintained in this laboratory, this culture forms neither conidia nor zygospores and resembles in this respect the old laboratory strain used by Soudek (26) in his work on the nucleolus of *Basidiobolus*. More recently the writer has isolated a fresh strain of the fungus from insect remains from the intestines of a frog caught in the vicinity of London, Ontario. Two other frogs caught at the same time also harboured the fungus. This strain is growing well and produces both conidia and zygospores but its cells and their nuclei are somewhat smaller than those of the C.B.S. culture which they closely resemble in their mode of division. A related species, *B. haptosporus*, first isolated by Drechsler from forest litter, has also been examined. It readily forms and shoots off conidia. Its cells and nuclei are intermediate in size between those of the C.B.S. strain and the fresh isolate of *B. ranarum*. Details of the technique of isolation are given by Drechsler (4).

The three strains were maintained on a Sabouraud-type medium composed of 4 per cent glucose, 1 per cent proteose peptone, and 1.5 per cent agar to which 1 per cent yeast extract had been added. The pH of this medium is 5.6. A less rich medium, 0.5 per cent yeast extract–2 per cent glucose agar, was used equally often. The writer has confirmed that *Basidiobolus* will grow in a solution of ammonium sulfate plus glucose and salts (20). The work to be reported was only concerned with events in growing cells but it might be mentioned in passing that conidia and zygospores were abundantly formed on agar containing 1 per cent of "Bacto peptone" or "soytone" (a digest of soy beans), both products of Difco Laboratories Inc., Detroit.

The size and the proportions (oblong or spherical) of the cells of *Basidiobolus* can be varied by altering the composition of their environment (20). However, in fresh source material, kept moist but not supplied with artificial food, as well as at the advancing edge of cultures growing on ordinary media, a certain reproducible sequence of cell growth, mitosis, and cell division can be observed which deserves to be called the "natural" growth habit of the fungus and which has greater cytological interest than the experimentally induced other forms of growth of which this fungus is capable. The principal growth habit of *Basidiobolus* was well known to Raciborski (22). It is the main subject of the present paper and was studied both in living cells and in fixed and stained whole mounts of growing cultures. The events in living cultures will be described first.

A. *Methods Used in the Study of Living Cells*

It is possible to study the behaviour of growing cells of *Basidiobolus* by focusing on the edge of a colony in a Petri dish, but the closeness of the phalanx of hyphae advancing through the agar at many different levels makes it difficult to keep a chosen cell in focus for long. It is more rewarding to start from a small inoculum cut from a sparsely grown culture from a diluted medium (*e.g.*, ⅕ normal strength yeast extract-glucose agar) or from a wisp of hyphae fished out of a liquid culture. Thin sheets of hyphae with sides of about 1 mm but only a fraction of a mm thick were cut from the edge of growing cultures under a dissecting microscope

using cataract knives. Transplants will grow on a wide range of media, but Sabouraud's was usually chosen because on this rich medium the cells tend to be large. Migration of cells from the edges of small inocula is rapid, and 4 to 5 hours later many of them will be found in division.

The behaviour of living cells emerging from the edges of small explants in agar media was followed in Petri dishes or, when higher magnification was desired, in slide cultures. The two kinds of cultures were set up as follows:

1. Petri Dish Cultures for Observation at Low Magnifications

Koehler illumination through a green interference filter was employed to reduce glare. The necessary conditions for this illumination were obtained by removing the top element of the substage condenser (Zeiss, achromatic, aplanatic NA 1.4) and by placing the dish upside down on the microscope stage. The lid of the dish was replaced by a flat glass plate that had been lightly warmed to prevent it from being frosted over with water of condensation from the agar. Irregularities in the bottom surface of the dish (now the surface nearest to the objective) were cancelled out by placing a drop of oil over the area to be examined and covering it with a No. 1 glass slip. To avoid the broad haloes of diffraction along the sides of hyphae growing on the surface of the medium, the explant was covered with a thin blanket of agar. This was cut from a film obtained by dipping an object slide twice into molten Sabouraud and agar and by allowing the agar to set between the deposition of first and second coat. To increase the supply of air, gutters were cut in the agar close to the explant. The arrangement described is illustrated in Fig. 1.

2. Slide Cultures

A two-dip film of agar was prepared on an ordinary object slide. All but an area 4 to 6 mm square in the centre of the slide was cut away. An explant about 1 mm^2 in area, but thinner than 1 mm, was placed on the agar, covered with a No. 0 glass slip, and sealed with wax. Illumination was arranged as for Petri dish cultures. To hold the slide at a sufficiently long distance from the substage condenser (minus top element) it was fixed with immersion oil to the upper surface of the lid of a Petri dish. In this way satisfactory and relatively glare-free illumination was achieved at high magnification. Observations were made with a Zeiss x35 apochromatic oil immersion objective of NA 0.85 in conjunction with a x15 compensating eyepiece. This objective is no longer obtainable, but several similar medium power immersion lenses of high numerical aperture are being offered by various firms.

3. Phase Contrast Microscopy

This is the method of choice for the study of detail in living cells. To prepare cultures for phase contrast microscopy a pattern of four drops of gelatin not exceeding the area of an ordinary square coverslip was placed in the centre of an object slide in such a way that three of the drops corresponded to the corners and one to the centre of the coverslip. The gelatin, which contained 1 per cent yeast extract and 1 per cent glucose, was inoculated and covered with a No. 0 glass slip. The culture was then placed on the lid of a Petri dish filled with water warmed to 45°C. When the gelatin began to spread the culture was instantly transferred to the lid of a dish filled with water that had been cooled in the refrigerator. A thin film of gelatin of irregular shape indented by ample air spaces was thus produced under the coverslip. The preparation was sealed with wax from a small birthday candle (safer than electrical hot-plates) and was examined under a high-dry phase contrast objective (Phase 2, Zeiss) in conjunction with a x16 compensating eyepiece.

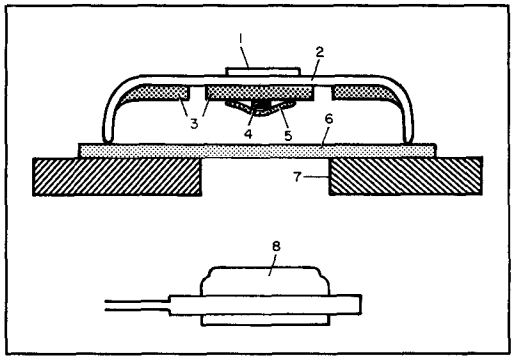


Figure 1

Arrangement for the observation of growing hyphae at low magnification. Dimensions and proportions not to scale.

1, coverslip. *2*, Petri dish. *3*, Sabouraud (or other) agar. *4*, explant. *5*, thin blanket of agar. *6*, glass plate. *7*, stage of microscope. *8*, substage condenser with top element removed.

Instructive living preparations may also (and more directly) be started by exposing agar in dishes or on slides for a short while to a bombardment with conidia from a sporing culture.

B. *Methods of Fixation and Staining*

Cultures that were to be stained were grown on squares cut from dialysing tubing made from very thin cellulose and slit open after moistening to provide a single thickness. The cellulose was boiled for 10 minutes in distilled water and placed on the surface

of yeast extract–glucose agar, diluted with water agar to 1/5 or 1/10 of its normal strength to reduce crowding of hyphae. After having been drained and dried on the agar at 37°C, the celullose was inoculated with explants measuring about 1 mm², cut with cataract knives from the edge of a growing culture. Cultures on cellulose were incubated for 18 to 24 hours at 20°C or on the bench. Growth under these conditions was excellent and of normal morphology.

Most cultures were fixed with Helly's fluid, some with Benda's modification of Flemming's fixative or with Zenker-formol-osmium, favoured by Maximow, (12). As was to be expected, the osmium-containing fixatives preserved the organisation of the cytoplasm better than Helly's fluid, but the latter also preserved the nuclei, nucleoli, spindles, and chromosomes well and left them in a condition in which they were easily stainable. Acetic acid–alcohol, the fixative of choice in chromosome studies, could not be used since it caused the cells to burst. Fixed cultures were washed several times with 70 per cent alcohol and either dealt with immediately or stored in the refrigerator in Newcomer's preservative. The chromosomes were made visible by the Feulgen procedure; the nuclear envelope, nucleoli, and spindle fibres, by iron alum haematoxylin. The Feulgen reaction and haematoxylin staining were carried out as previously described (23) and, as recommended there, Feulgen preparations were examined in aceto carmine (15). Colonies cannot be relied upon to remain attached to the cellulose unless they are coated with collodion (23). Nine minutes' hydrolysis in N/1 HCl at 60°C followed by 3 hours in Schiff's reagent prepared with "Diamant Fuchsin" (Chroma G.M.B.H., Stuttgart, Germany) proved satisfactory. The haematoxylin was prepared according to Baker and Jordan (1). Fixed cultures were mordanted in 2 per cent iron ammonium sulfate for 6 to 18 hours and stained for an equal length of time. Differentiation was started in a saturated watery solution of picric acid and finished in the mordant. This procedure leaves a minimum of stain in the cellulose. The progress of differentiation was watched with a x40 water immersion objective. Some of the haematoxylin preparations were dehydrated with alcohol, cleared in xylene, and mounted in polystyrene resin (11) available under the name of D.P.X. (from George Gurr, London, S.W. England) and others were examined in water. These proved best for photography.

The changing states of the nucleolus in the course of mitosis were brought out with particular clarity by the mercuric bromphenol blue technique of Mazia *et al.* (14) and by acid fuchsin. This dye was used at a concentration of 0.1 per cent dissolved in 2 per cent acetic acid in distilled water. The pH of the staining solution was 3.1. Helly-fixed cultures were stained for one minute and differentiated in distilled water which had been given a pH of 10.3 by the addition of 5 per cent sodium carbonate, five drops per Petri dish being the usual proportion. Finished preparations were returned to 2 per cent acetic acid water and examined in this solution. Photographs were taken with a Zeiss, x60 apochromatic objective, NA 1.4, in conjunction with a x15 compensating eyepiece. A Zeiss achromatic-aplanatic substage condenser of NA 1.4 was used, oiled to the slide, and illumination was arranged according to the Koehler principle.

RESULTS

A. *Observations on Living Cells with Ordinary Microscopy*

Four kinds of phenomena, all commented on by Raciborski (21, 22), may be studied in living cell

FIGURE 2

Time lapse photographs of some steps in two episodes of expansion and contraction of a growing cell of the CBS strain of *Basidiobolus ranarum*. All the remaining illustrations are of cells of the same strain except Figs. 12*a* to *c*.

Impurities in the agar provide points of reference. Panel *g* had to be moved down 1¼-inch relative to panel *f* in order to fit it into the available space.

The cells in panels *a* and *g* are in comparable states of contraction and are in the process of laying down a transverse septum.

Figures indicate hours and minutes. Microns give the length of the protoplast at comparable stages of contraction. It measured only 220 microns at the beginning of the experiment (not shown here). Note how during the interval between *a* and *e* a length of hypha is evacuated by the cytoplasm and later sealed off to form another link in an already long chain of empty cell chambers. For a closer view of this process see Fig. 3.

Petri dish culture. Magnification, 334.

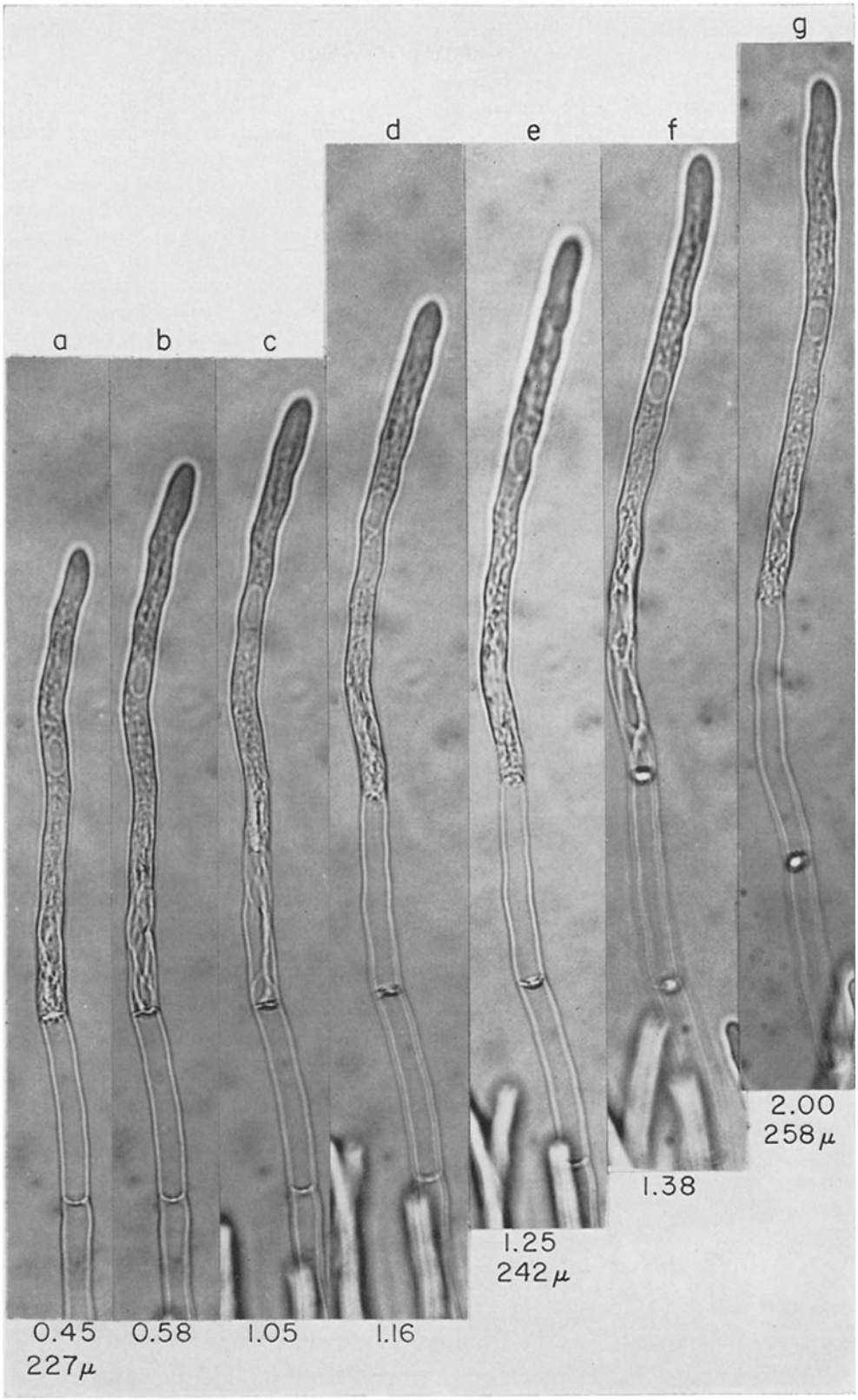


FIGURE 2

of growing cultures of *Basidiobolus*. 1. Differences in the organization of the cytoplasm at the front and the rear of the cell. 2. Alternating periods of expansion and contraction of the forward creeping protoplast. 3. Growth and mitosis. 4. Cell division and the separation of sister cells. They will now be described.

1 and 2. Under the conditions of cultivation described above, the protoplast of the C.B.S. strain of *B. ranarum*, 200 to more than 400 microns long, creeps forward in its continuously growing cell wall tube at a rate of about 4.4 microns per minute. The nucleus, close to 25 microns in length, lies at a variable, but always considerable, distance from the tip of the cell (Fig. 2). The cytoplasm in front of it is dense and filled with numerous, small, rod-shaped mitochrondria and other fine particles which move rapidly towards the tip and back again along invisible channels, frequently passing each other in opposite directions. The cytoplasm to the rear of the nucleus is turbulent and lacerated by large, continously shifting and changing lacunae, (Fig. 3 *a*). With time, the lacunae get increasingly larger and it becomes obvious that the cytoplasm is being withdrawn from this part of the cell. When a length of about 100 microns has been nearly cleared of cytoplasm, a transverse septum is laid down across the cell along the lower edge of the contracted protoplast, separating the inhabited from the empty portion of the cell wall tube. A plug of protoplasm remains for some time closely applied to the proximal side of the septum; ultimately it may disappear. Sequences of photographs illustrating this process are shown in Figs. 2 and 3. Meanwhile, at the far side of the septum, the cytoplasm moves forward again, diluted and broken up by longitudinal lacunae which change their shape with the swiftness of a flame. The cell creeps steadily forward and the process of stretching out and pulling together again, the "stepwise growth" of Raciborski (22), continues, usually for several hours and at remarkably regular intervals of time. A single moving protoplast thus leaves in its wake a long chain of empty cell chambers.

3. At first glance it seems that the protoplast does nothing but move forward and make more cell wall and that the function of the regularly repeated laying down of septa is to prevent the cytoplasm from becoming stretched out over too large an area of cell wall. In reality, the protoplast itself is steadily growing for most of the time, though at a very slow rate compared with the rate of growth of the cell wall tube in which it lives.

When the length of a cell at the moment of its greatest degree of contraction, that is, just after the laying down of a transverse septum, is compared with the lengths attained by the same cell at comparable moments of further episodes of extension and contraction, then it is seen that the protoplast is gradually getting longer (Table I). When it has reached a certain length the cell enters mitosis (Figs. 4 and 5). Most cells growing close to air, uncrowded in a suitable medium divide when they have reached a length of 300 to 400 microns. This was the size attained by four cells from four different cultures whose growth was followed over several hours (see Table I). Most of the first dozen cells in mitosis encountered in a set of three fixed preparations were also close to 330 microns long, and that was true also of a similar number picked out in succession along the

FIGURE 3

Successive photographs of two expansion-contraction episodes (the "stepwise growth" of Raciborski) in the same living cell. In panel *a* the protoplast is in the last stages of withdrawal from the boot-shaped lower part of the cell wall tube. Small, irregularly shaped cells like the "boot" were common in the old liquid culture which was the source of the inoculum for this experiment. In *b* the cell has moved out of the "boot" and has sealed itself off against it with a transverse partition (arrow). A repetition of this process starts in *c* and again in *e*. The first formed septum, close to the lower margin of *c* is now concave and thicker than it was in *b*. The next septum is under construction at the tip of the arrow in *d*.

The normal differentiation of the cell into a relatively calm forward section and a turbulent rear is well brought out in *a* and *b*. As is also the cap of nucleoplasm which often forms ahead of the nucleolus.

Slide culture. Ordinary microscopy. Figures indicate hours and minutes. Magnification, 870.

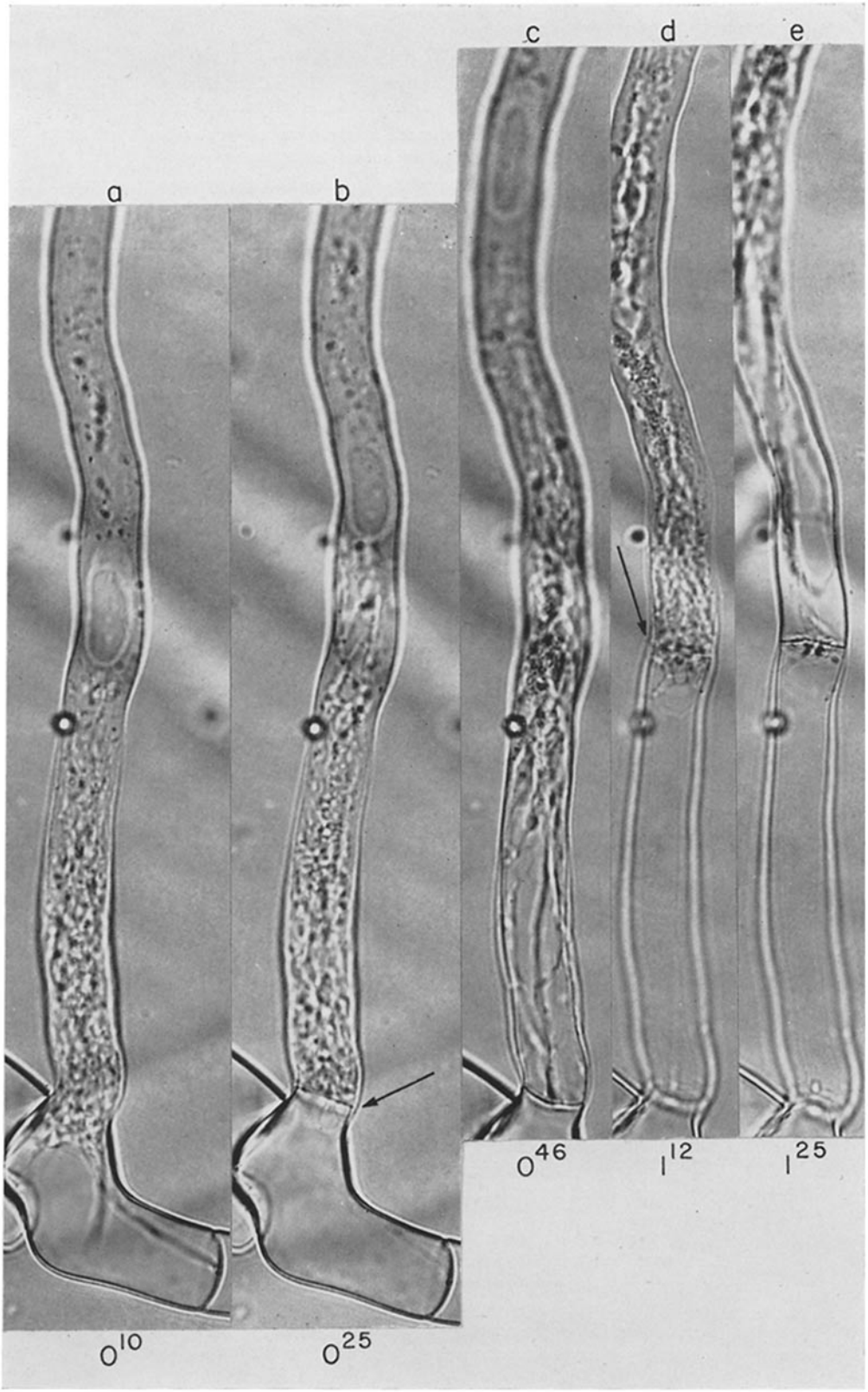


FIGURE 3

TABLE I

Lengths in Eyepiece Micrometer Scale Divisions of Four Different Living Cells Measured at the Moment of Septum Formation in the Course of Several Successive Episodes of Extension and Contraction

The last entry in each column is the length of the cell at the moment when mitosis was first recognised. Time is recorded in hours and minutes.

One scale division equals 11.0 microns. Note that there is a remarkably constant interval of about 30 minutes between successive instants of maximum contraction. Note also that there is no growth between some of the episodes in cells *C* and *D*. *M*, mitosis.

Cell A		Cell B		Cell C		Cell D	
Time	Length	Time	Length	Time	Length	Time	Length
hrs. min		*hrs. min.*		*hrs. min.*		*hrs. min.*	
0.40	20.0	0.35	24.5	0.25	21.0	0.05	18.0
1.10	22.0	1.10	26.5	0.59	22.0	0.30	20.0
1.45	24.0	1.44	28.0	1.30	24.5	1.05	20.0
2.16	25.0	2.05	37.0,*M*	2.00	27.0	1.30	21.5
2.55	27.5			2.28	26.8	2.00	23.0
3.08	32.5,*M*			3.03	26.0	2.29	24.2
				3.36	26.0	3.00	26.0
				3.55	32.0,*M*	3.30	25.5
						4.00	28.0
						4.30	27.5
						5.00	27.5
						5.06	30.0,*M*

edge of a living Petri dish culture. These measurements must not be taken to mean that the cell *has* to attain the length of 300 to 400 microns before it can divide, and that, having reached it, it will invariably do so. It is true, however, that under the conditions prevailing at the edge of a culture set up as described above, cells of the C.B.S. strain of the order of length of 300 microns and over must be regarded as likely candidates for mitosis. That knowledge is of help to the

FIGURE 4

Low power survey picture of mitosis and cell division. Panels *a* and *b* show two stages of the stretching out of the cytoplasm and the dilution of its proximal portion, which is the normal and recurring accompaniment of the movement of *Basidiobolus* through an agar medium. Impurities provide points of reference. In the interval between *b* and *c* the cell drew up its cytoplasm to the level marked with a cross and formed a partition there which can be seen just above the lower edge of panel *c*. The arrow connects identical points in panels *b* and *c*. From here on, all panels are aligned at the same horizontal level.

c. Metaphase, no details clearly visible.

d. Telophase, daughter nuclei visible above and just below the level of the black dot on the left edge of the hypha.

e. A septum now divides the cell. First seen at 1.57 when it was quite straight, it is now continued into a bulge on the left. The distal cell is withdrawing from the site of division.

f to *g*. Development of bud and branch. Distal cell interposes empty cell chamber between itself and its sister. Proximal cell withdraws from lower part of its chamber and closes the empty part with a septum, at arrow in *g*. Sister cells, in this instance, are of equal length but do not inherit equal amounts of cytoplasm, the proximal sister receiving the more vacuolated, attenuated portion of the mother cell. Petri dish culture. Numbers indicate hours and minutes. Magnification, 334.

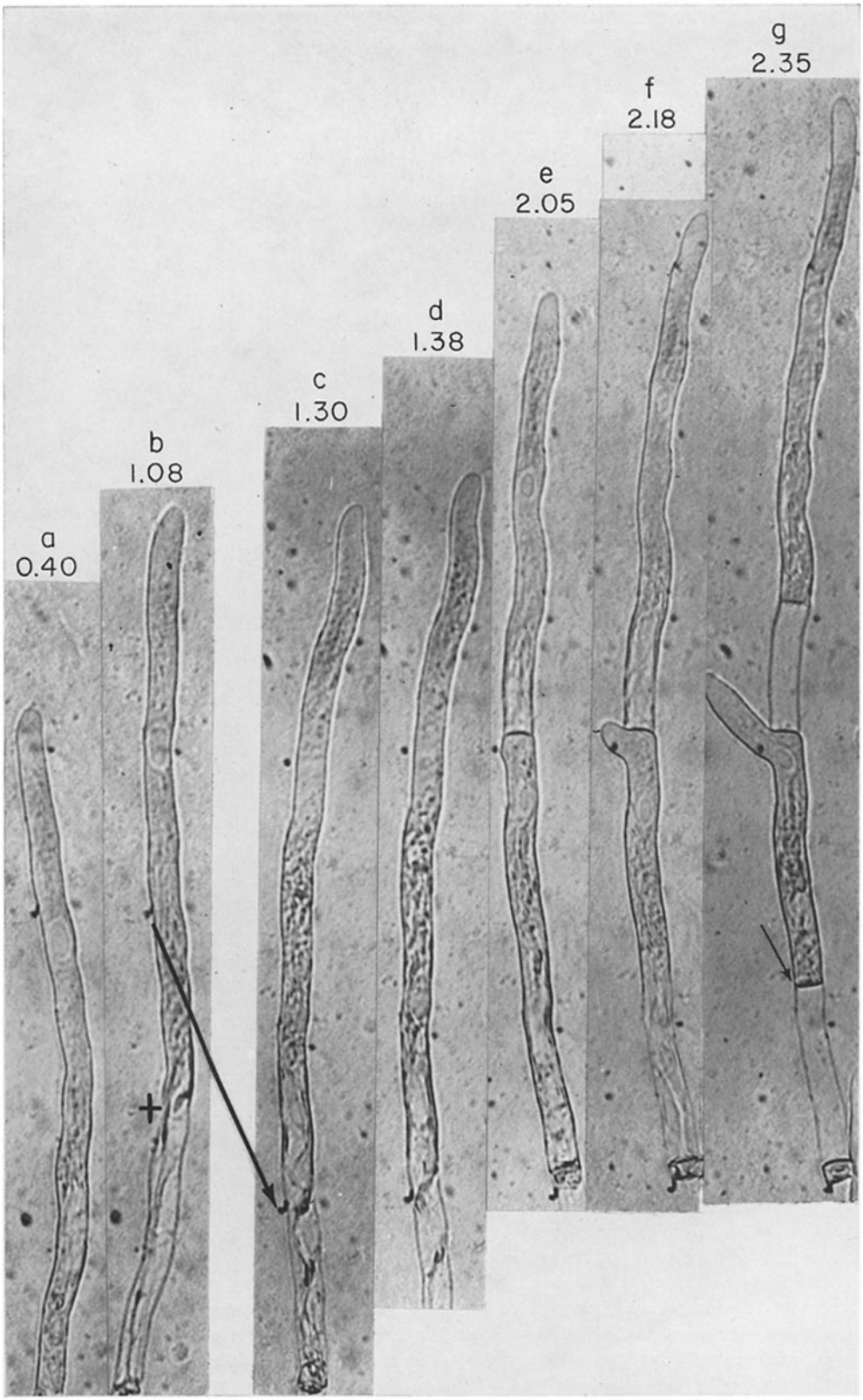


FIGURE 4

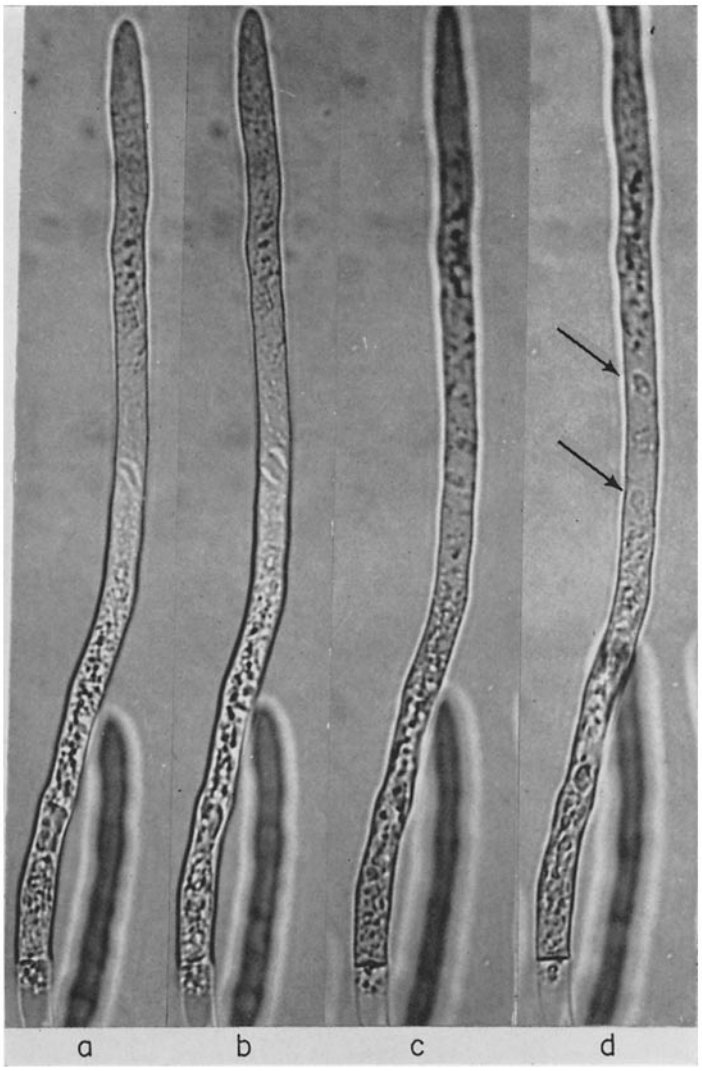


FIGURE 5

Four steps in the division of the nucleus of the same living cell of *Basidiobolus*.

a. *Metaphase*. The dark metaphase plate, seen in profile, is embedded in the remains of the nucleolus which, at this level of focus, appears bright.

b. *Anaphase*. Daughter plates with attached refractile "end-plates" of nucleolar substance. The gap between them contains the lengthening spindle whose fibres are here invisible.

c and *d*. *Telophase*. Arrows point to daughter nuclei in process of reconstruction. The four pictures were taken in the course of 10 minutes. Petri dish culture. Magnification, 334.

observer who wishes to study mitosis from its beginnings.

4. The first visible sign of *mitosis* in the living cell is a swelling and then a fading from sight of the nucleolus. There is also a change of shape of the nucleus from oval to rounded (Figs. 6 and 14 *a*, *b*). In the cell of Fig. 6 the swollen nucleolus, assuming it to be an ellipsoid, had increased its volume by 20 per cent at the end of this first step. At low magnification and with ordinary microscopy (Fig. 5) the metaphase plate and some of the events of anaphase and telophase can be recognised but not the spindle fibres. The latter can sometimes be made out at the higher magnifi-

cation permitted by slide cultures, as in Fig. 7 *b*, but it is likely that the polarising microscope will reveal them more regularly and more effectively. Two phenomena of interest, not mentioned by Raciborski, regularly accompany mitosis. They are: first, the considerable slowing down of the rate of advance of the cell, and, secondly, a temporary coarsening and stiffening of the texture of its cytoplasm. The rate of the forward streaming of the cell (to be distinguished from its rate of *growth*, which is lower by a factor of 10) is reduced to one-half, or a third or even less of its normal value, and does not revert to its former magnitude until some time after cell division. Numerical examples of this regularly observable change are provided in Figs. 8 and 9. More obvious and clearly reflected in the photographs of slide cultures, Figs. 7 and 10, is the gravel-like coarsening of the normally smooth cytoplasm. Cells in this condition give the impression of having dried out or having been killed by a precipitating fixative. Clumps of motionless or only sluggishly moving granules are found throughout the greater part of the cell; only the region of the mitotic figure remains free of them. This effect first becomes clearly noticeable at metaphase. It provides a good demonstration of how mitosis affects the whole of the cell and is reminiscent of similar physical changes accompanying mitosis in plant cells and ova of invertebrates, reviewed by Mazia (13).

Mitosis in *Basidiobolus* seems rigidly linked to cell division. Not one of a large number of cells in which I have watched the course of mitosis failed to divide, and I have rarely seen a cell with two mature nuclei in the same cytoplasm. At the end of telophase the distal daughter nucleus moves, or is moved, rapidly towards the tip of the cell (Fig. 4). The proximal nucleus moves for a much shorter distance in the opposite direction only to reverse its migration shortly after, when a sharply defined transverse septum begins to grow inward from the cell wall at a level close to the plane in which the metaphase plate had first become visible (Figs. 4, 7, 10). After completion of the septum, a process requiring but a few minutes, there is an interval of varying length, at the end of which a bulge forms in the wall directly beneath the new septum. The bulge rapidly grows into a sturdy shoot which is entered by the proximal daughter protoplast and its nucleus. This cell quickly moves into the branch and creeps steadily forward, away from the site of division (Figs. 4, 10). Meanwhile the distal (top) cell has moved ahead and resumed the usual cycle of extension and contraction. It is soon separated from its sister by a sealed, empty segment of cell wall tube. The whole inexorable sequence, first and well described by Raciborski (22), takes about 45 minutes from the first sign of mitosis to the formation of a shoulder beneath the septum. Fifteen to 20 minutes of this are taken up by the division of the nucleus. It seems worth pointing out that mitosis and cell division can be followed in the cells at the edge of an ordinary culture examined through the bottom of an ordinary Petri dish and illuminated with the indifferent light provided by a simple class-room lamp. It is only for studies involving measurements and for photography that the more time-consuming way of setting up cultures, described under A, is recommended.

Division is not always equal. Daughter cells may differ in length and they always inherit different cytoplasms. The dense forward portion of the former single cell goes to the distal daughter while the proximal (lower) one receives the diluted, vacuolated rear. Normally, it is only the lower cell which branches and thus turns away from the forward direction of growth after division. But this seemingly rigid relationship of the daughter cells is maintained only in relation to the usual distribution of untapped resources of food. At the lower margin of explants, that nearest to the centre of the original colony, a remarkable reversal of the direction of growth takes place. Part of it is achieved by growing tips curving sideways or backwards but there is also a true reversal of the streaming of the cytoplasm in some of the cells, and, after division under these conditions, *both* daughter cells may put out branches which point backwards towards the fresh source of food that explantation has made available to them. Observed and illustrated with telling pen drawings by Raciborski (22) and confirmed by the present writer, this swift response of the cell of *Basidiobolus* to gradients of food concentration deserves close examination.

B. *Observations on Living Cells with Phase Contrast Microscopy*

The writer has not yet made much use of this form of microscopy in the study of mitosis in

Basidiobolus.[1] Figs. 10 *a* to *i* are samples of what can be seen in living cells by this means at relatively low magnification. The metaphase complex of chromosomes plus developing spindle cylinder stands out clearly, and so does the coarsening of the texture of the cytoplasm. The formation of the septum between daughter cells is better seen with ordinary microscopy. Not shown in the photographs is the exquisite spectacle of many small mitochondria rapidly moving towards the tip of the cell and away from it at some other level. Still photographs also fail to convey an impression of the swiftly changing pattern of lacunae in the proximal part of the cell.

C. *Observations on the Nucleus at Rest and in Mitosis, made in Stained Preparation*

1. The Resting Nucleus

The periphery of the resting nucleus is filled with a sponge-work of chromatin with little affinity for haematoxylin (Fig. 13 *a*). Except at metaphase, the chromosomes stain weakly and indistinctly with iron haematoxylin—a difficulty also experienced by the first investigators of *Basidiobolus*—and to reveal them clearly also in the remaining phases of mitosis it is necessary to make Feulgen preparations.

In the resting nucleus the chromosomes fill the space between the envelope and the nucleolus except for a gap surrounding the nucleolus which may be filled with liquid. The gap is often visible in stained preparations in which it was first regarded as being due to shrinkage. However, at high magnification it is also visible as a zone of particular brightness surrounding the nucleolus of living nuclei examined in the phase contrast microscope.

The most striking feature of *Basidiobolus* nuclei is their huge nucleolus (Figs. 11 to 13). It often contains a number of vacuoles of different sizes, and Soudek (26) has provided quantitative evidence that under the conditions under which he worked the number and volume of the nucleolar vacuoles is directly related to the growth activity of the cell. He has followed the growth of nucleolar vacuoles and watched them being emptied into the nuclear cavity. In other instances, large nucleolar vacuoles remained unchanged for hours. Both kinds of vacuoles are explained as secretions of the nucleolus. When diffusion keeps pace with secretion the vacuole remains intact, when it lags behind secretion the vacuoles are emptied. Striking nucleolar vacuoles are also shown in a photomicrograph of a nucleus of *Basidiobolus* published by Girbardt (7). In my material, vacuoles were often seen in the nucleoli

[1] A phase contrast motion picture of mitosis in *Basidiobolus* has been made by Dr. M. Girbardt, Jena. It is to be hoped that it will soon become available on this side of the Atlantic.

FIGURES 6 *a* TO *c*

Three successive photographs, taken less than a minute apart, of a living nucleus illustrating the behaviour of the *nucleolus* at the beginning of mitosis. In *a* the nucleolus is oval and there is a clear space between it and the nuclear membrane. In *b* and *c* the nucleolus has become rounded and occupies most of the nucleus. The nuclear envelope is about to be dissolved.

FIGURES 7 *a* TO *e*

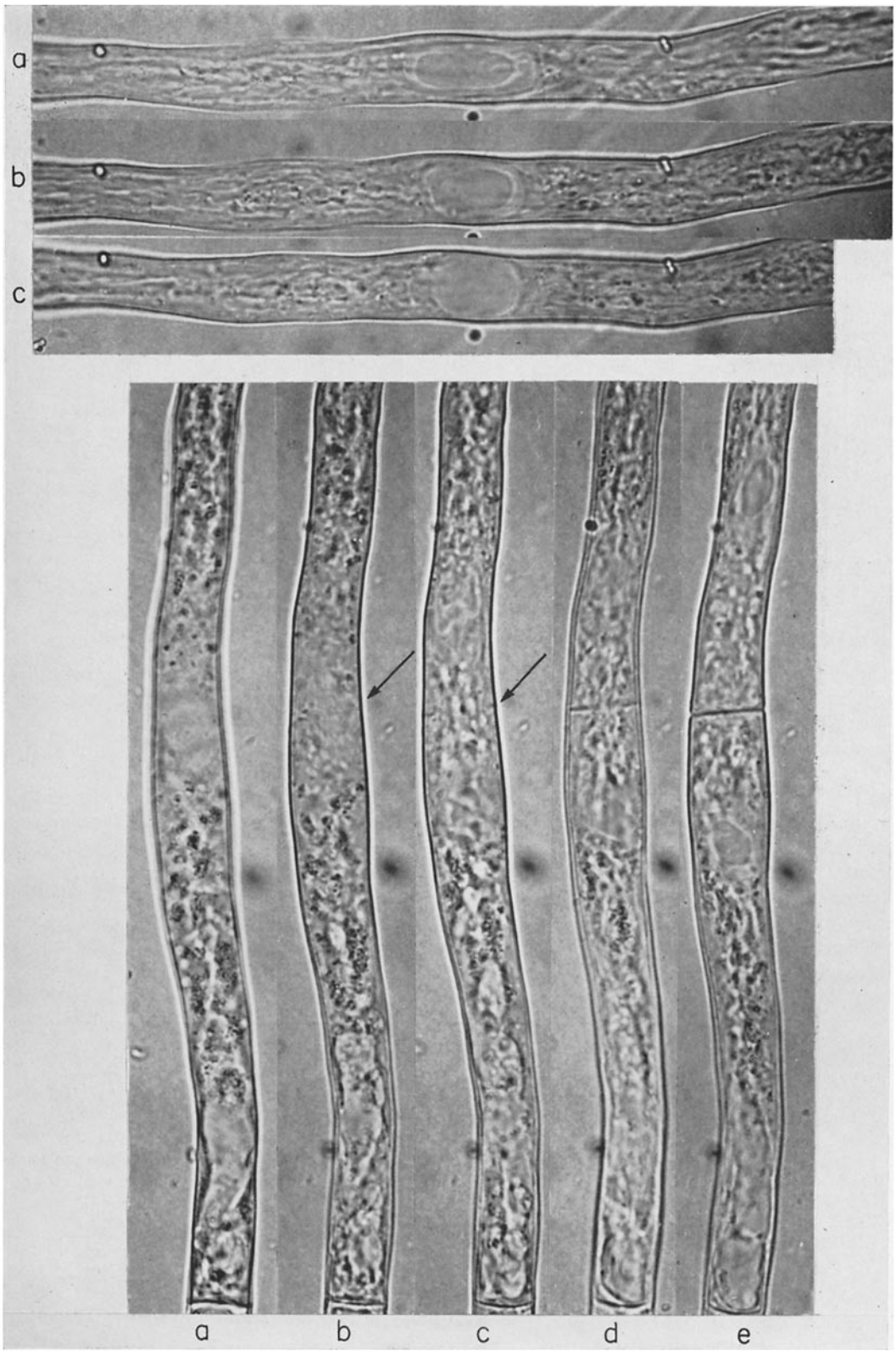
Successive photographs of a living cell illustrating some phases of its mitosis and division.

a. *Metaphase-anaphase*. Daughter plates (central pair of dark grey bands) separated by the barrel-shaped spindle and capped by "end-plates" of nucleolar material (light bands).

b. *Telophase*. The arrow points toward the half-spindles.

c to *e*. Three steps in the growth of the transverse cell wall, an event which in *Basidiobolus* invariably follows the division of the nucleus. The beginnings of the new wall are just becoming visible (in optical section) at the level indicated by the arrow. Note that the daughter nuclei are gradually becoming more distinct. The interval between *a* and *e* is 20 minutes.

Figs. 6 and 7 were made from slide cultures and are magnified 870 times.



FIGURES 6 and 7

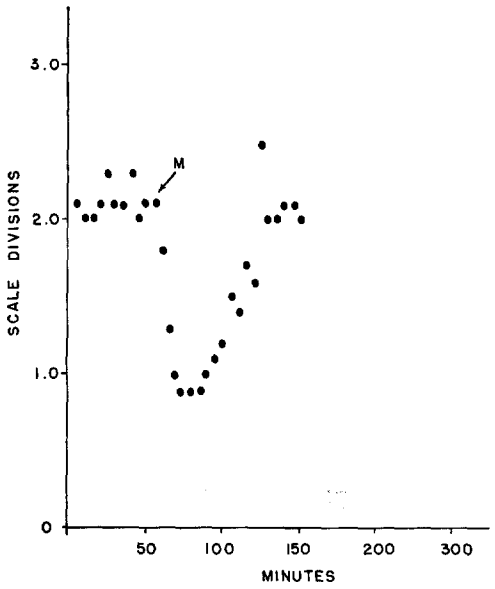


FIGURE 8

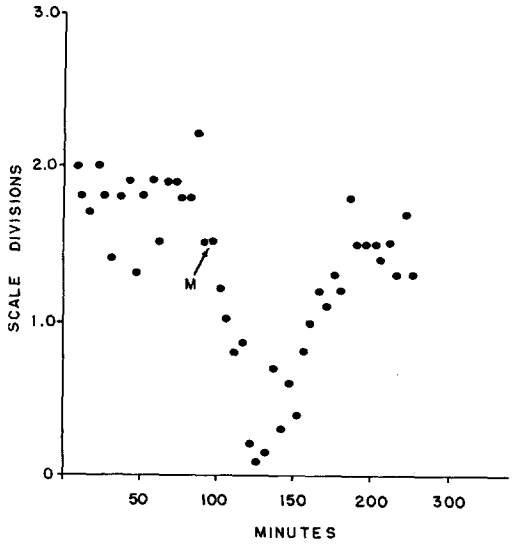


FIGURE 9

FIGURES 8 AND 9

Increment in length of two hyphae recorded at 5-minute intervals to show decline of rate of growth of the cell wall tube during mitosis. *M*, start of mitosis. One scale division equals 11 microns.

but they were not a constant feature of the nucleoli of growing cells.

There is evidence that the nucleolus contains both protein and ribonucleic acid. It is brightly and tenaciously stained by mercuric bromphenol blue (Fig. 12). Soudek and Stranska (27) found that it is rapidly dissolved by trypsin at a concentration of 1 mg per ml of a weakly alkaline phosphate buffer. They also noticed that the affinity of the nucleolus for pyronin and toluidine blue was lost in the course of 30 minutes' incubation at 40°C in ribonuclease (0.1 mg/ml). The present writer's experience has been similar. It is illustrated in Figs. 11 *a* to *d*. These findings are reinforced by Soudek and Stranska's (27) further observation that the nucleolus strongly absorbs ultraviolet light at 2537 A.

2. Mitosis

The nucleolus plays an important part in mitosis. Prophase begins with the gradual disappearance of the nuclear membrane, a softening of the contours of the nucleolus, and the loss of some of its affinity for haematoxylin (Figs. 13 *b*, *c*). The disintegration of the nucleolus is arrested by the transformation of some of its substance into a mass of fibres arranged in parallel along either its short or its long axis (Figs. 13 *f*, 14 *d*, and 18 *a*

FIGURE 10

Successive photographs of mitosis and division of a living cell of *Basidiobolus*, growing in 15 per cent gelatin with yeast extract and glucose. The direction of growth is towards the right. Phase contrast microscopy. Magnification, about 900.

a, *b*. Two views of the nucleus at rest, taken 40 minutes apart. Mitosis began soon after the second picture had been taken, as can be inferred from the position of the nucleus relative to the marker (an impurity in the gelatin) which from now on is opposite the nucleus at the lower edge of each panel.

c. The nucleolus is beginning to fade.

d to *f*. Stages of metaphase. The cytoplasm is becoming coarsely granular.

g to *i*. Daughter plates (dark grey bands) being separated from each other by the expanding spindle (light central band). The cytoplasm filled with a coarse granulation.

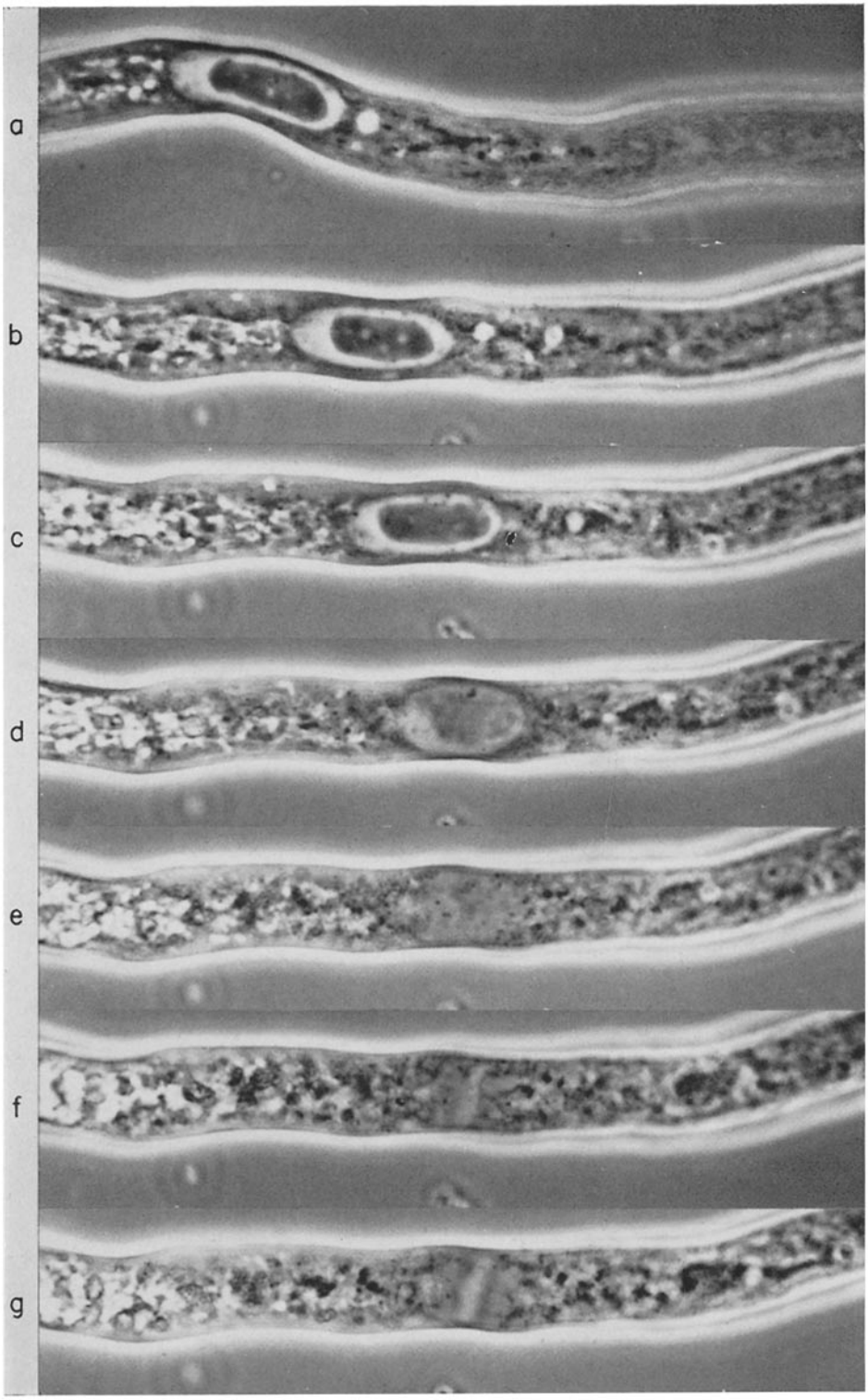


FIGURE 10

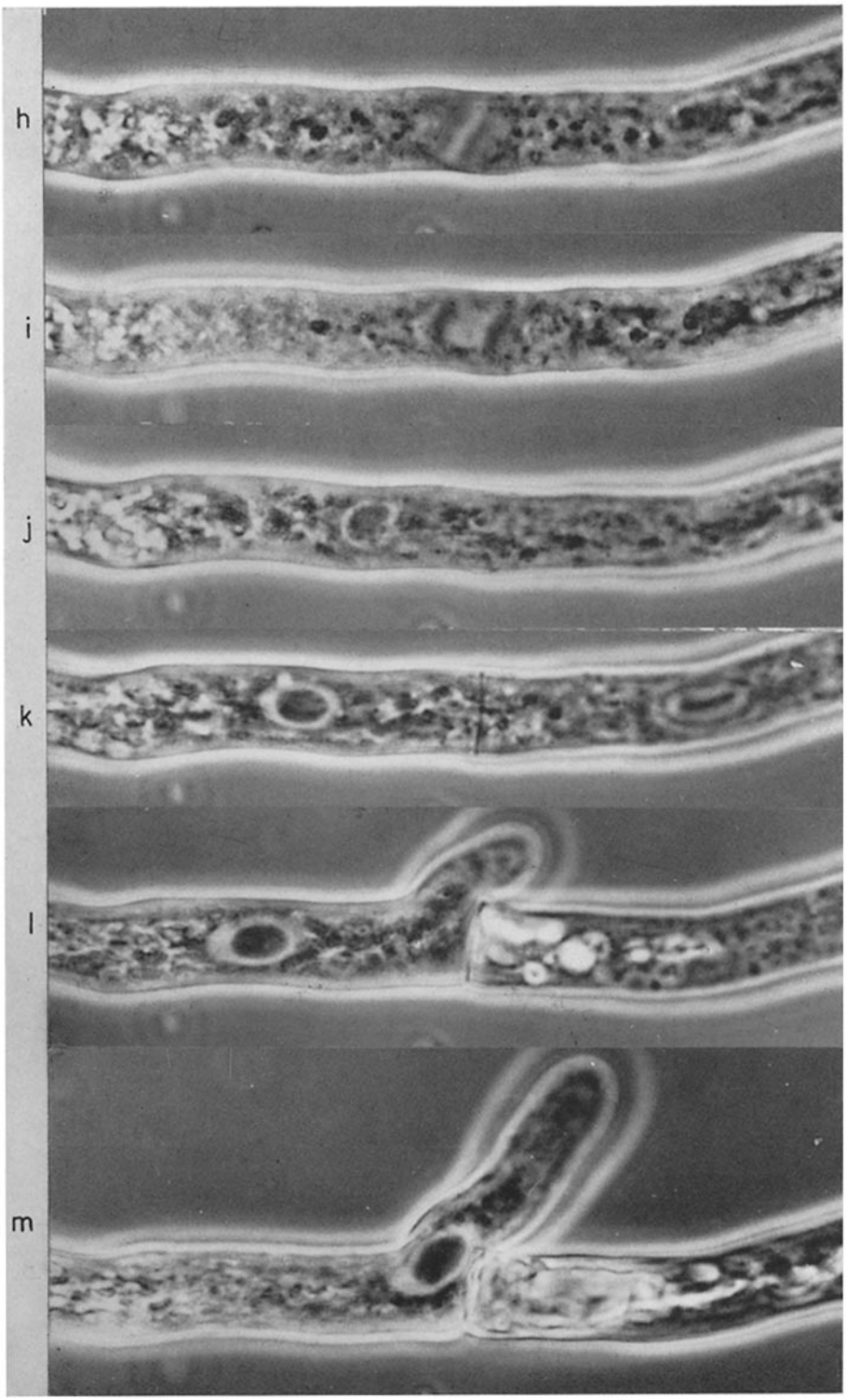


FIGURE 10

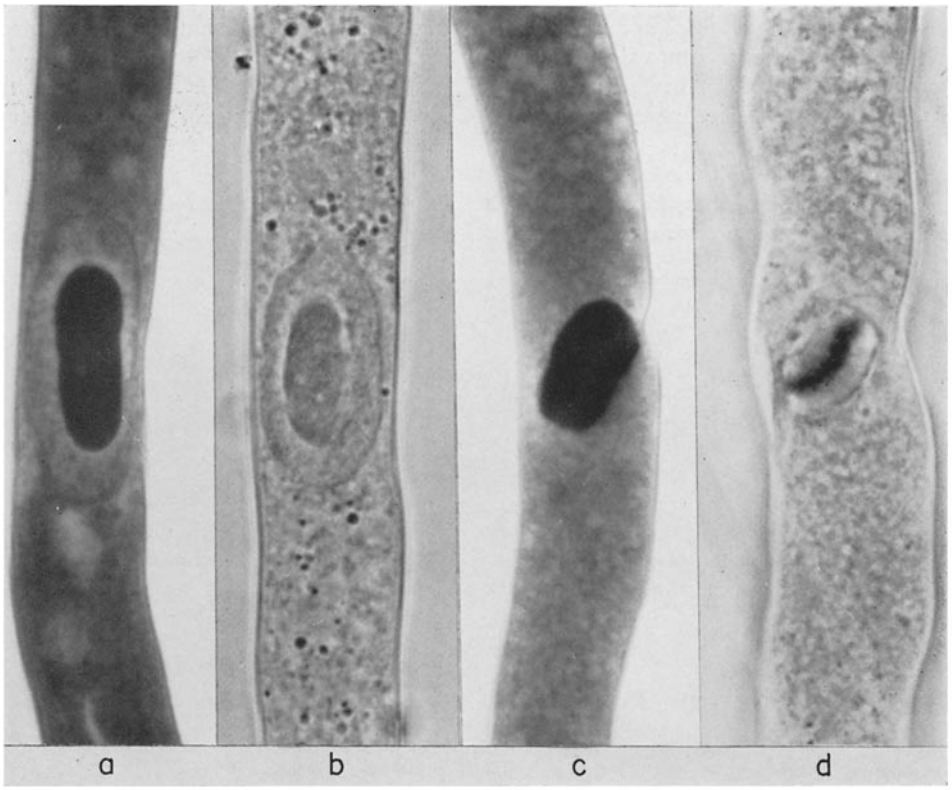


FIGURE 11

Effect of ribonuclease on the stainability of the nucleolus and the "end-plates" of the metaphase figure. Demonstrated in four different cells from cultures fixed in 5 per cent formalin plus 3 per cent mercuric chloride in water.

a. After 5 minutes in 0.01 per cent toluidine blue; rinsed with and photographed in water.

b. Nucleus of another cell, stained in the same way after 3½ hours digestion with 0.5 mg/ml of ribonuclease (Freehold) in phosphate buffer at pH 6.9. Buffer controls stained like *a*.

c. Metaphase plate imbedded in brick-shaped remains of nucleolus. Toluidine blue 0.01 per cent, 5 minutes. Compare with living cell of Fig. 5 *a* and with haematoxylin-stained metaphase of Fig. 13 *e*.

d. Another cell with a nucleus in a similar state of metaphase. Digested and stained as under *b*. The chromosomes are stained but the end-plates are not. Magnification, all figures, 1750.

FIGURE 10 *continued*

g to *i*. Daughter plates (dark grey bands) being separated from each other by the expanding spindle (light central band). The cytoplasm filled with a coarse granulation.

j. Proximal daughter nucleus visible. The other is out of focus.

k. Nuclei growing. Cell divided by a transverse septum.

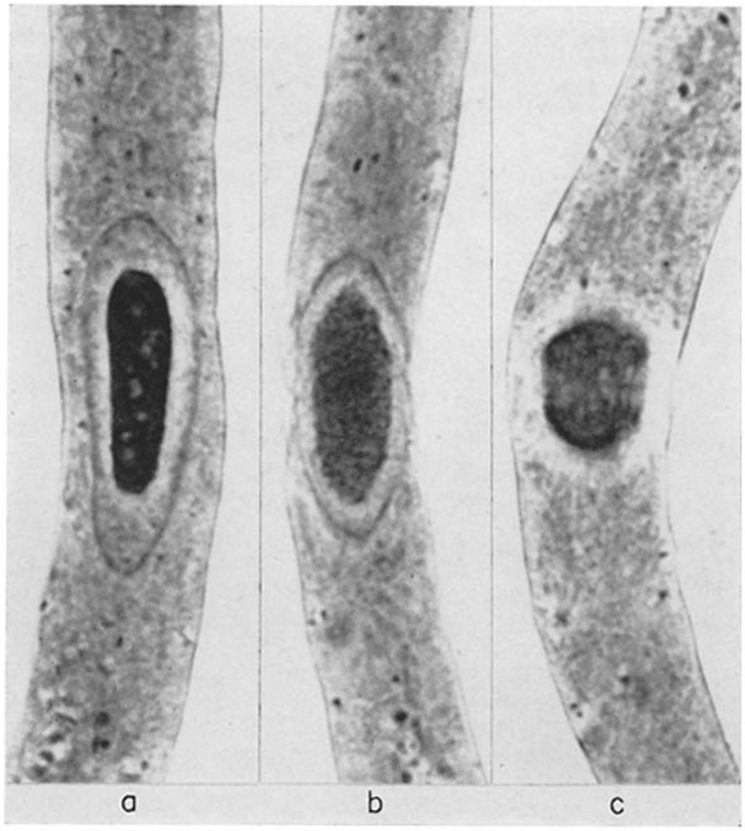
l, m. Migration of proximal cell into branch. Withdrawal of distal cell from chamber to the right of the septum.

The interval between the fading of the nucleolus in *d* and the completion of the wall in *k* was 25 minutes. The bud (*l*) was formed 55 minutes later. The last picture *m* was made after another 30 minutes had passed.

to *c*). Within and at right angles to the bundles of fibres is a disc, or rather a Saturn ring, of closely packed chromosomes. The Feulgen procedure shows this more clearly than haematoxylin (Figs. 14 *e* and *e'*, Fig. 16 *g'* and Figs. 17 *a* to *d*). The fibres thus appear to be those of the *metaphase spindle*, and this is confirmed by later events. Be it noted that the chromosomes are at the equator of the spindle, not necessarily at the equator of the transformed nucleolus. The spindle may arise in a peripheral segment of the nucleolus and may be inclined at odd angles to its long axis (Fig. 18 *c*). The only fixed relationship is that between the spindle fibres and the chromosomes. Furthermore, the shape of early metaphase spindles is often irregular and resembles the type of shapes assumed by nucleoli at prophase. All this suggests that the formation of the spindle is initiated not from outside the nucleolus but by the chromosomes within.

The remainder of the nucleolar substance, a variable amount, forms a pair of caps or "end-plates" over the ends of the spindle cylinder (Fig. 13 *g*). These end-pieces are once again deeply stainable with haematoxylin and may also be recognised in living cells by a degree of refractility which is much higher than that of the fading nucleolus at the start of prophase. Perhaps the two processes of making a fibrous spindle out of some of the nucleolar matter and increasing the density of the remainder are chemically connected.

That the polar caps or end-plates are, in fact, derived from the nucleolus is made probable by the fact that they have the same affinity for toluidine blue as the intact nucleolus and lose it to the same extent in the course of digestion with ribonuclease (Figs. 11 *c*, *d*).



FIGURES 12 *a* TO *c*

An interphase nucleus (*a*), a nucleus at prophase (*b*), and one at anaphase (*c*) of cells of the newly isolated London strain of *Basidiobolus* stained by the mercuric bromphenol blue technique for the detection of protein. Helly fixation. Magnification, 2340. Explained in the text.

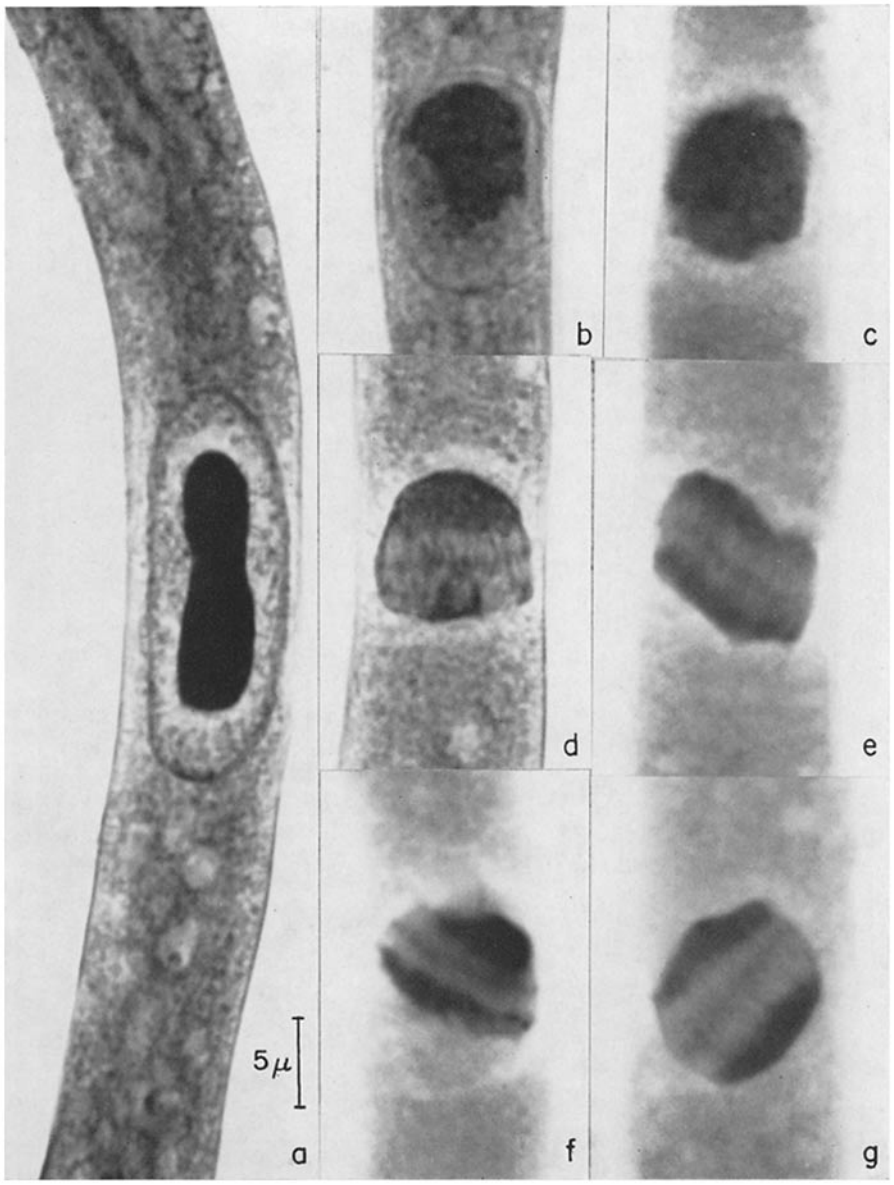


FIGURE 13

Basidiobolus fixed with Helly (*a*, *b*, and *d*) or *Flemming-Benda* (*c*, *e*, *f*, and *g*), stained with iron alum haematoxylin and photographed mounted in water, (*a*, *b*, *d*) or D.P.X. polystyrene mountant (*c*, *e*, *f*, *g*). Magnification, 2340.

a. Resting nucleus with distinct envelope, granular and rod-like chromosomes, and a dense nucleolus.

b to *g*. Successive stages of the partial dissolution of the nucleolus and of the appearance of the spindle within its remains. The dark band at the equator of the spindle in *e*, *f*, and *g* represents chromosomes on the metaphase plate. Compare with Figs. 6 *a*, 11 *d*, and 14 *e*.

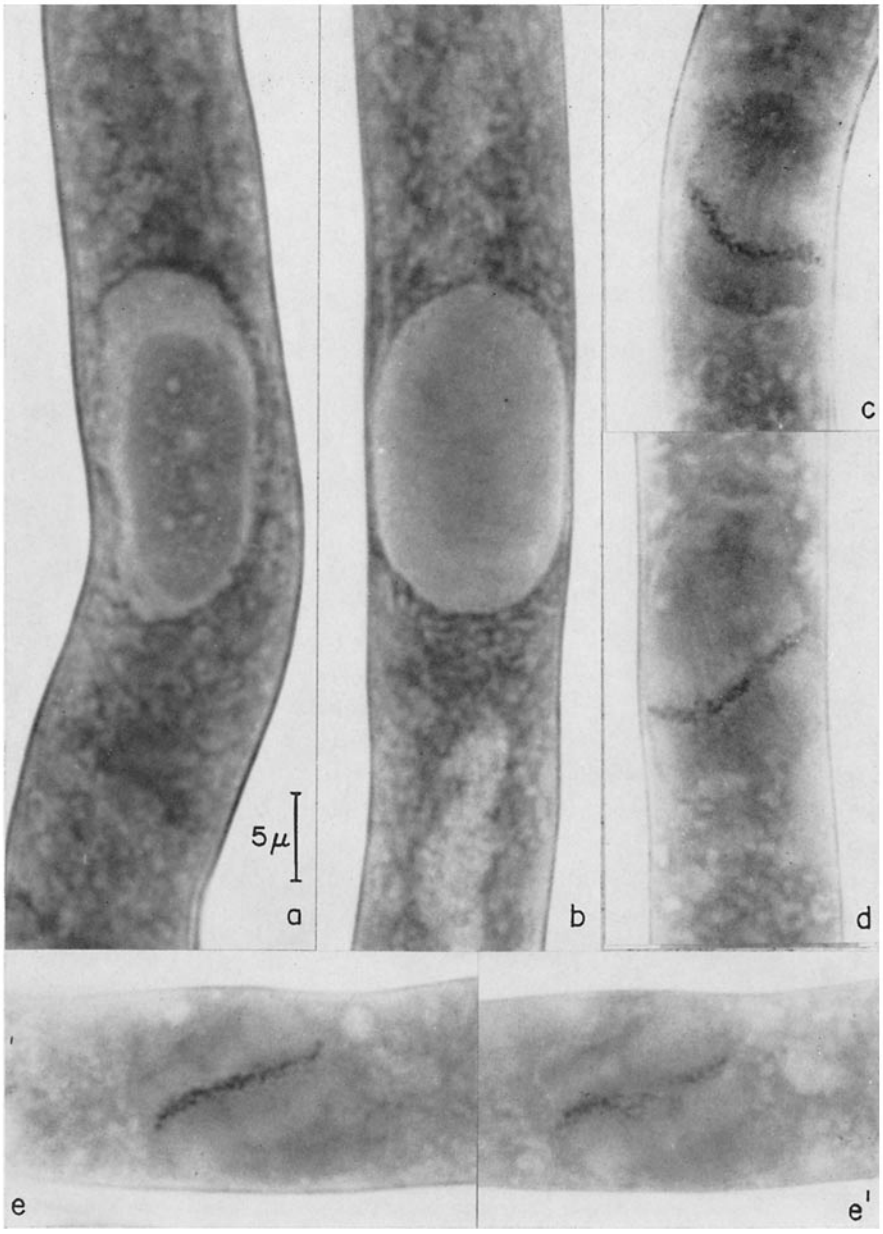


FIGURE 14

Helly-fixed nuclei examined in aceto carmine to show the nucleolus and the relationship of the chromosomes to the nucleolar substance at metaphase.

a. Resting nucleus.

b. Early prophase. Nucleolus diluted and swollen. It fills the nuclear cavity. Compare with Fig. 6 *b*.

c to *e'*. Chromosomes at metaphase inside the remains of the nucleolus. In *c* and *d* the radiations of the spindle are faintly visible. *e* and *e'* are photographs of the same nucleus taken at two different levels of focus. All magnifications, 2340.

Further evidence that the chromosomes are at metaphase embedded in material derived from the nucleolus comes from Helly aceto carmine preparations. The nuclei in Figs. 14 *c* to *e'* require little explanation. The position of the chromosomes inside the enlarged nucleolus is plain. Nothing has been seen that would suggest the presence of centrioles inside or outside the end-plates.

It is apparent from a comparison of living cells in mitosis and haematoxylin-stained and Feulgen preparations that the chromosomes of the daughter plates travel but a short distance on the spindle, still a flat disc at this time, before they become so submerged in the substance of the end-plates that in haematoxylin preparations they cannot be distinguished from it (Fig. 15 *a*). As the end-plates are pushed farther apart by the growing spindle they become rounded, and the chromosomes inside now occupy a smaller area than they did at metaphase. This causes the spindle fibres to diverge towards the middle of the cell and produces the two separate conical half spindles so clearly illustrated in the works of earlier students of our fungus (Figs. 15 *b*, *c*). The spindle fibres are gradually dissolved at anaphase, and at telophase the reconstruction of the daughter nuclei is well under way. The end-plates now break down into granules and droplets, some of which are probably utilised in the construction of the new nucleoli (Figs. 15 *d*, *e*). Telophase in *Basidiobolus* recalls telophase in the green alga *Spirogyra*, where there is a similar close association of chromosomes and nucleolar substance. In the words of Godward (10), "There is the 'chromatid plate' whose chromatids are frequently already so attenuated as to be invisible to the eye, embedded in a dense mass of nucleolar substance of variable shape, sometimes a uniform plate, sometimes irregular and perforated or separated into blobs. Very soon this substance begins to dissolve, and if blobs were not present before, they now appear, usually spherical, large and small. . . . As the nucleoli increase in size they may fuse. Meanwhile the nucleolar substance which has been dissolving may be used up completely or one, two, or more small spheres of it may remain to be incorporated as they are in the resting nucleus. . . .the nucleolar substance with which telophase begins, . . . is used up as the nucleolus proper is organised, presumably in the formation of the nucleolus." Further remarks concerning *Spirogyra* are in the Discussion.

3. The Nucleus at Rest and in Mitosis as Seen in Feulgen Preparations of the CBS Strain of Basidiobolus

The chromosomes in Helly-fixed whole mounts of *Basidiobolus* were only faintly and indistinctly stained by iron haematoxylin. Aceto carmine, applied directly after Helly fixation, showed the chromosomes much more clearly but it did so only at metaphase. Only the Feulgen technique provided transparent, selectively stained preparations suitable for the study of the entire chromosome cycle. However, only a general description can be given because the chromosomes of the fungus are minute, numerous, and closely packed at metaphase. The positive Feulgen reaction of the chromosomes in resting nuclei is weak (Figs. 16 *a*, *c*). It becomes much stronger at prophase, which finds the chromosomes sinking into the surface of the swollen nucleolus (Fig. 15 *b*). In some nucleoli the chromosomes remain at the surface, spread out in variously curved, sometimes saddle-shaped, flat sheets. Typical examples of this frequently encountered phase are shown in Figs. 16 *d* to *f'*. Next, the chromosomes gather along one edge of the sheet, endowing it with a thick (rolled-up) rim. This is later somehow transformed into the closed circle or narrow ellipse of metaphase (Figs. 14 *e*, *e'* and 16 *g*, *g'*) which careful focusing reveals to be a pierced disc or Saturn ring. Other nucleoli of prophase are penetrated by the chromosomes which are irregularly arranged all through their interior. It seems that this arrangement, too, leads eventually to the ring plate of metaphase. The difference between the two prophase patterns may merely be one of different degrees of softening of the nucleoli. But the possibility cannot at present be excluded that the sheet arrangement, which is the more frequently encountered one of the two, is obligatory and *follows* on the clustering of chromosomes within the nucleolus. A time scale is provided by the nuclear envelope, which is always still just visible around intranucleolar chromosomes clusters (Fig. 16 *c*), but is no longer present at the time when the nucleolus is covered with a curved sheet of prophase chromosomes.

Metaphase plates, invariably presented in side views and bordered by faint indications of spindle fibres, resemble a wreath of thorns (Figs. 17 *a* to

d). They are strongly Feulgen-positive and the most frequent and most easily recognised of all phases of mitosis. Separating daughter plates are less common. They sometimes provide a better view of separate chromosomes than early metaphase plates and suggest that the number of 60 proposed by E. W. Olive (18) is of the right order of magnitude. The nucleolar substance and the substance of the polar caps, because they react negatively, are hard to see in Feulgen preparations, but their limits can usually be inferred from slight differences in refractility and affinity for carmine that exist between them and the cytoplasm. Events after metaphsae follow a conventional course and require no special explanation. They are illustrated in Figs. 17 *e* to *h*. The clarity with which mitotic figures stand out against resting nuclei and seemingly empty hyphae in Feulgen preparations makes it easy to count them. This has made it possible to demonstrate quantitatively the effect of certain procedures, which for a time increase the incidence of mitosis in colonies of *Basidiobolus*. Two such effects will be described.

D. *Stimulation of Mitosis*

1st Experiment

It was noticed that removal of a small segment from the edge of a growing colony to a dish with fresh medium was soon followed by the simultaneous occurrence of many more mitoses among the cells of the explant than would have occurred among them in the same interval of time had they been left where they came from. This observation, often repeated, suggested that an inhibitor of mitosis might have accumulated between the hyphae in the original colony. Its removal, through diffusion, in the new environment might be the cause of the observed burst of mitoses in the explant. The fact that mitoses follow each other rapidly among the first few cells of the small mycelia arising from germinating conidia points in the same direction. To test this hypothesis, cultures were prepared on sheets of cellulose overlying yeast extract glucose agar. 18 hours later some of the sheets were transferred to fresh dishes of the same medium kept at the same temperature. Some were fixed 40 minutes later, others at the end of the 1st hour. Another set was transferred to agar without food (water agar) and was left there for 1 hour. Controls were provided by overnight cultures fixed at the beginning of the experiment. Another lot of overnight cultures were left in place and fixed at the same time as the second lot of the experimental cultures. This was done to safeguard against the possible occurrence of large fluctuations of the incidence of mitosis among the controls. The results of this trial are set out in Table II. As will be seen, the transfer to fresh medium was indeed followed by an increase in the number of cells in mitosis. It would be tempting, but erroneous, to dispose of this effect as the mere consequence of a fresh supply of food. The cultures used in these experiments were very small, 7 to 9 mm in diameter, actively growing and surrounded by a vast excess of available food. The removal of a diffusible inhibitor active at very low concentrations seems a more plausible explanation. But it is also clear from the low number of mitoses among the "C" set of cultures that removal of an inhibitor is not enough to get mitosis going. A source of energy is also required. This is emphasised by the

2nd Experiment

As set out in Table III, the results of this trial showed that after 1½ hours on water agar the number of mitoses had dropped to a low value. Return to yeast extract glucose agar was followed, 40 minutes later, by a dubious slight increase in the number of cells in mitosis in one culture and after another 20 minutes by a definite large increase. Remarkable, in view of the small numbers involved, is the similarity of the numbers of mitoses in the eight control cultures recorded in Table II. Limited as they are, these observations indicate, perhaps, that *Basidiobolus* has possibilities as a subject for experiments on the physiology of mitosis.

DISCUSSION

The structure of the cytoplasm and the transformation it suffers during mitosis, the nuclear envelope, the organisation of the spindle, its relationship to the chromosomes, and the probable absence of centrioles have been touched upon only lightly in the text of the paper because the author feels that their discussion had better be deferred until *Basidiobolus* has been studied in electron micrographs. The slow growth of *Basidiobolus* and the regularity with which mitosis is followed by cell division will perhaps make this

TABLE II

Experiment 1

Set of cultures	Nature of medium	Length of incubation	Number of mitoses in Helly-Feulgen preparations counted at 600 times magnification. Each value from a separate culture.				
A	0.1% yeast extract-0.4% glucose agar	18 hours	23	36	34	37	Control
B	Transferred to fresh dish of same medium as before	40 min.	78	99			Experimental cultures
B′	" "	60 min.	166	136			
C	Water agar	60 min.	7	6	6	4	
A′	As in A	19 hours	23	35	35	35	Control

TABLE III

Experiment 2

Set of cultures	Nature of medium	Length of incubation	Number of mitoses counted as in Exp. 1			
A	0.1% yeast extract-0.4% glucose agar	18 hours	65	68		
B	Water agar	1½ hours, changed to fresh dish of water agar at end of first hour.	6	0		
C	Returned from water agar to yeast extract glucose agar	40 min.	1	12		
D	As under C	60 min.	100	152	84	92

organism useful to students of the "cell-growth duplication cycle" (19).

The feature of *Basidiobolus* that will appear most commonplace to cytologists, namely, that its nucleus divides by an ordinary form of mitosis, is in reality the most remarkable and least suspected of its properties, doubly so because, in growing mycelia, cells with only one nucleus are not so common as cells with several to many nuclei and because there are indications that vegetative nuclei of fungi 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. For a recent discussion of some of the problems involved and prospects of their solution, see Girbardt, 1962 (8). Some of the best, but by no means the only, evidence for this view of fungal mitosis is to be found in *Mucor* and *Phycomyces* (23, 24), *Endogone* (2), *Saprolegnia* (3, 25), *Blastocladiella* (28), and *Empusa* (18). These genera are all of them members of the Phycomycetes, the very class to which *Basidiobolus* itself belongs. A few preparations of *Conidiobolus villosus* and a species of *Empusa* have been freshly examined for comparison with *Basidiobolus*, their close relative in the accepted classification. These species, as is well known, are coenocytic and contain large numbers of very small nuclei. It was found that these nuclei too divide in the seemingly direct manner of *Mucor* nuclei.

There is no reason to assume that the chromosomes of unconventionally dividing phycomycete nuclei are not equitably distributed at mitosis, but it is equally certain that they go through their orderly movements without having first been marshalled on a metaphase plate, and it is prob-

able that they do it without the help of an ordinary spindle apparatus.

One can think of one slender link between the atypical divisions of vegetative mucorine and zygomycete nuclei and the mitosis of *Basidiobolus*. In the former, it has been firmly established that the nucleolus divides together with the chromatin (*i.e.*, the clustered chromosomes) (24), and it looks as if it performed a mechanical function. In *Basidiobolus* the nucleolus also plays an important part in the separation of sister sets of chromosomes: it gives rise to the spindle. In both kinds of fungi there is close contact between the chromosomes and the nucleolus during the whole of division. However, one difference wants emphasising: in the phycomycete nuclei listed above the chromosomes remain at the periphery of the nucleus all through its division and do not enter the nucleolar material, there to align themselves on a metaphase plate, as in *Basidiobolus*.

The intranuclear body that, in accordance with the usage of fungal cytology, has been, and will be, referred to in this paper as "nucleolus" is similar in composition to the nucleoli of higher organisms and shares some of their staining properties. It must be admitted that there is at present no evidence that, in the manner of proper nucleoli, the intranuclear body of *Basidiobolus* is regularly associated with one or more chromosomes.

A similarity between mitosis in *Basidiobolus* and in *Spirogyra*, where the spindle is also barrel-shaped and terminates in end-plates of nucleolar substance, was noted from the first (6, 18). It has already been mentioned in the description of the events of telophase. In *Spirogyra* the nucleoli of the resting nucleus contain ". . . coiled structures through which the greatly enlarged nucleolar organising regions of the chromosomes pass" (9). This has not, so far, been noticed in *Basidiobolus* and although the nucleolus is not homogeneous, it does not, during interphase, contain Feulgen-positive structures. A longer and closer look may yet reveal them. The completeness with which the chromosomes are at prophase transferred from their position near the nuclear envelope to the surface of the nucleolus almost suggests that the chromosomes have, in fact, a foothold in the nucleolus even during interphase. Another difference between mitotic nuclei in the two organisms concerns the spindle. In *Spirogyra* two half spindles are formed outside the nucleus and later penetrate it. In *Basidiobolus* a single spindle is formed inside the nucleolus. Both Raciborski (20) and Olive (17) found prophase a difficult stage to describe and to understand. They thought that the nucleolus is completely dissolved at the beginning of it and were not aware that the chromosomes sink into the substance of the nucleolus, and that much of it survives prophase in the form of the end-plates of meta- and anaphase. Fairchild (6) did consider the possibility that the material of the nucleolus might somehow be utilised in the formation of the spindle. In the present study, too, the events of prophase have proved difficult to unravel and have perhaps still not been correctly explained.

A helpful critic has urged the writer not to overlook the possibility that "the spindle may first form outside the nucleolus and then, as this becomes dispersed, invade the nucleolar space." The idea is plausible, but the view, described above, that the spindle arises *within* the nucleolus under the influence of the chromosomes accords better with the available evidence. The material of the nucleolus is diluted at the beginning of prophase, but it is not dispersed, a point once more emphasised by Figs. 18 *a* to *c*. A spindle has never been seen outside the nucleolus, not even at advanced stages of the softening that overtakes that body at prophase. The spindle first becomes

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FIGURE 15

Helly. Iron alum haematoxylin. Photographed in water. *a* to *c*, magnifications, 2340, *d* and *e*, 1750.

Anaphase to telophase. Spindle between endplates (*a*) and half-spindles between young daughter nuclei (*b* and *c*). Note that the mitochondria in *c* fail to penetrate into the space occupied by the half spindles. In the cytoplasm of both *d* and *e* faint traces of the spindle fibres may still be recognised. The nuclear envelopes in *d* are still in several separate pieces. The envelope around the left nucleus in *e* seems complete.

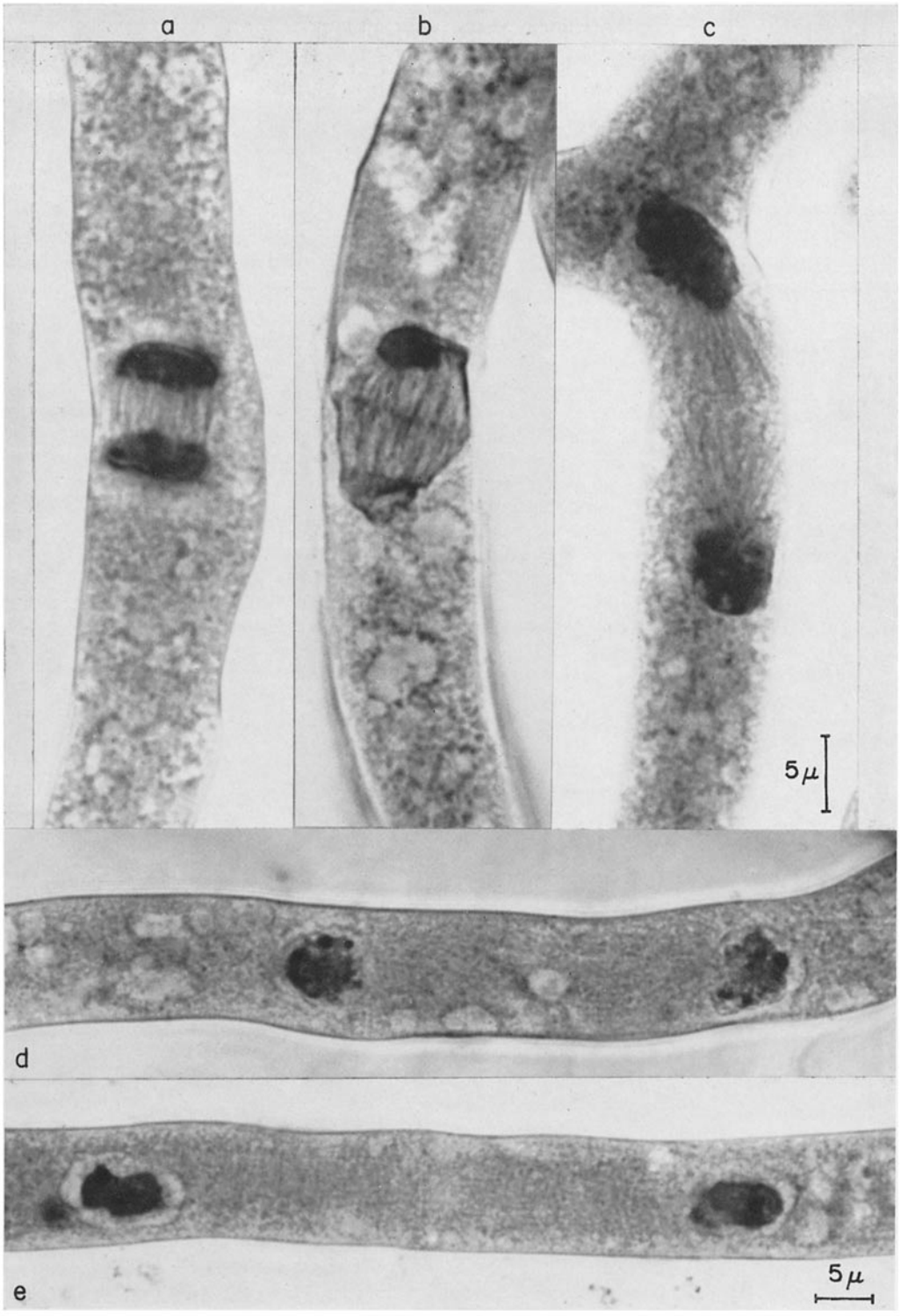

FIGURE 15

visible in association with metaphase chromosomes at a time when the latter are embedded in nucleolar material. The true origin of the spindle will only be revealed in electron micrographs of sections. Meanwhile, there is no doubt that the nucleolus makes a major contribution to the spindle and that its substance never entirely disappears from the dividing nucleus.[2]

The cell structure of *Basidiobolus* isolates it from its fellow phycomycetes and leaves its origins and present affinities a matter for speculation. Meanwhile, its large cells with their large single nuclei provide a promising addition to the range of types of living cells available to the experimental biologist. That was the opinion of Raciborski, writing in 1896. It is still true today.

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[2] Dr. M. Girbardt, Jena, has independently arrived at the same conclusion. See abstract in *Ber. Deutsche Bot. Ges.*, 1962, **74**, 424.

FIGURE 16

Helly. Feulgen. Aceto carmine. Magnification, 2340.

a. Resting nucleus. A shell of loosely arranged chromosomes, thick at the poles, thin along the sides of the nucleus, surrounds the Feulgen-negative nucleolus. A similar nucleus may be seen, slightly out of focus, to the right of the prophase stage in *c*.

b. Prophase. Chromosomes at the surface of the swollen nucleolus. The chromosomes have retracted from all contact with the nuclear envelope, (*n*, *e*).

c. On the left a nucleus in early prophase. Chromosomes at the surface of as well as inside the nucleolus. Nuclear envelope still faintly visible.

d to *f'*. Prophase. Chromosomes spread out in a sheet which covers one side of the nucleolus and is rolled up into thick rim, the future metaphase plate, at one end. *e'* and *f'* are pictures of *e* and *f* at a different level of focus.

g and *g'*. Side views, at two different levels of focus, of a ring-shaped metaphase plate inside the transformed nucleolus whose limits are faintly visible in *g'*.

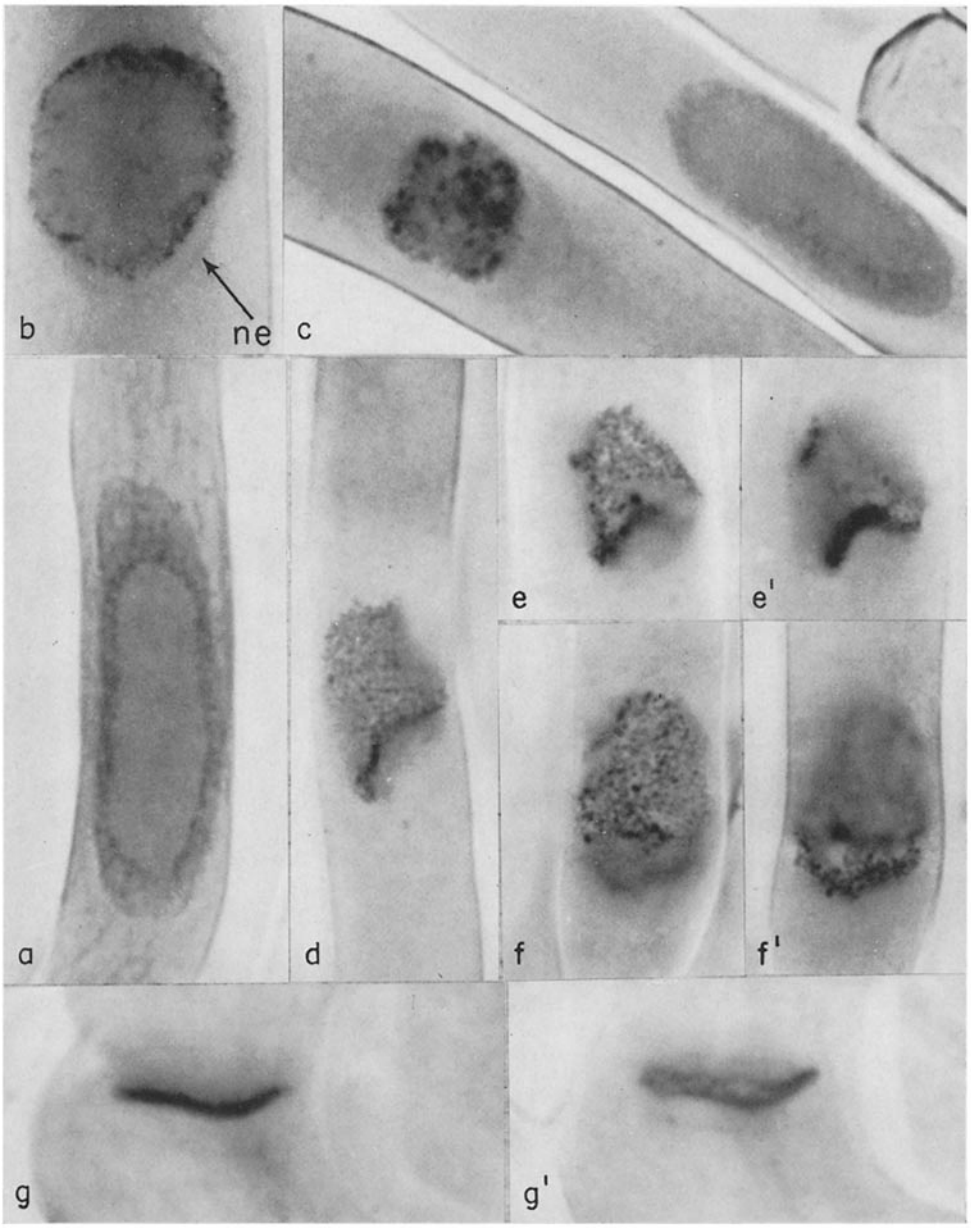

FIGURE 16

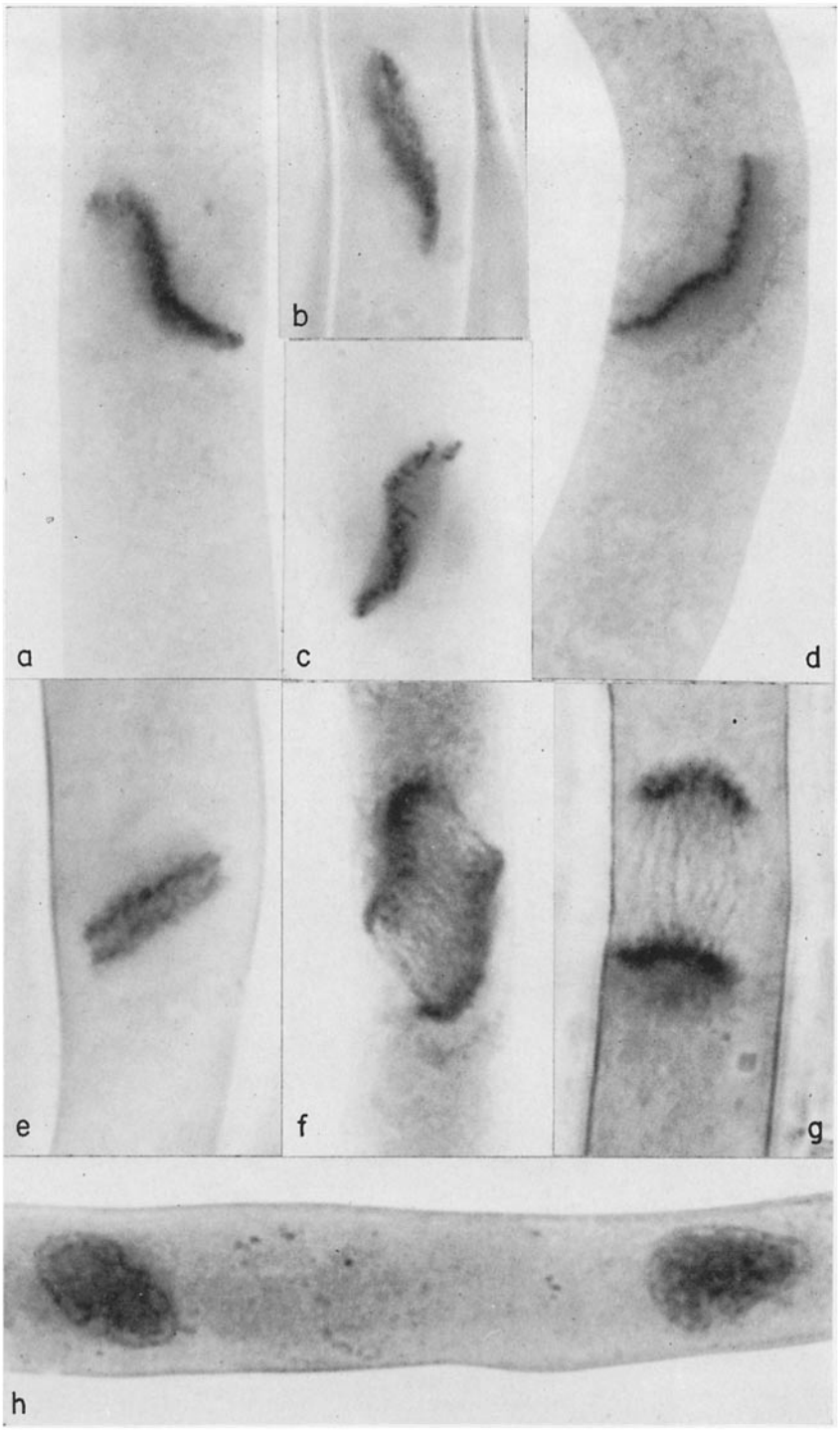

FIGURE 17

Helly. Feulgen, Aceto carmine.

a to *d*. Profiles of metaphase plates.

e to *g*. Anaphase-Telophase. Spindle fibres outlined in faintly Feulgen-positive matter.

h. Young daughter nuclei still lobed and with chromosomes and nucleolar matter not quite sorted out. All magnifications, 2340.

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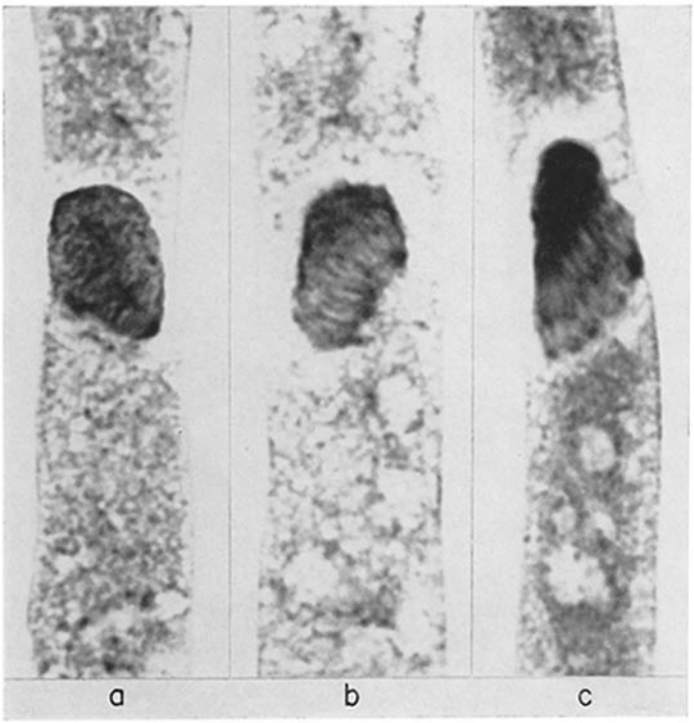

FIGURES 18 *a* TO *c*

Spindle fibres arising in nucleolar material early in the metaphase of mitosis. Note that the fibrous bodies still have the shapes of prophase nucleoli. Note also a large piece of little changed nucleolar material above, and a small one below and to the right of the incipient spindle in *c*. Helly, acid fuchsin. Magnification, 2340.

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