A FIBER APPARATUS IN THE NUCLEUS OF THE YEAST CELL

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ABSTRACT

The structure and mode of division of the nucleus of budding yeast cells have been studied by phase-contrast microscopy during life and by ordinary microscopy after Helly fixation. The components of the nucleus were differentially stained by the Feulgen procedure, with Giemsa solution after hydrolysis, and with iron alum haematoxylin. New information was obtained in cells fixed in Helly's by directly staining them with 0.005% acid fuchsin in 1% acetic acid in water. Electron micrographs have been made of sections of cells that were first fixed with 3% glutaraldehyde, then divested of their walls with snail juice, and postfixed with osmium tetroxide. Light and electron microscopy have given concordant information about the organization of the yeast nucleus. A peripheral segment of the nucleus is occupied by relatively dense matter (the "peripheral cluster" of Mundkur) which is Feulgen negative. The greater part of the nucleus is filled with fine-grained Feulgen-positive matter of low density in which chromosomes could not be identified. Chromosomes become visible in this region under the light microscope at meiosis. In the chromatin lies a short fiber with strong affinity for acid fuchsin. The nucleus divides by elongation and constriction, and during this process the fiber becomes long and thin. Electron microscopy has resolved it into a bundle of dark-edged 150 to 180 A filaments which extends between "centriolar plaques" that are attached to the nuclear envelope.

INTRODUCTION

The structure and reproduction of the nucleus of the yeast of baking and brewing, *Saccharomyces cerevisiae*, was the subject of bitter polemics at the turn of the century (8, 14) and a mycology text of our time informs us that "the controversy over the structure of the nucleus . . . continues undiminished" (1). Some progress there has been. Many would now agree that the yeast nucleus owes part of its elusive character to the presence of many small, closely packed chromosomes which are in much the same condition at rest and during mitosis (18, 21, 27, 30, 35, 38, 44). It has also beeen known for a long time (8, 13, 35, 37, 38, 44) that the nucleus divides by lengthening accompanied by the gradual shifting of its contents to its extremities until the attenuated middle portion gives way. Electron microscopy, reviewed by Hawker (11), has established that the nuclear envelope remains intact during this process. The mechanism of this form of division has remained obscure. The literature contains no adequately documented evidence of the occurrence of a mitotic apparatus in baker's yeast and many firm claims of its absence. However, it is only a short while ago that fibrous devices were first seen (or rediscovered) in dividing vegetative nuclei of several fungi including *Polystictus* (5, 6), *Albugo* (3), *Allomyces* (44, 45) and a fission yeast, *S. versatilis* (44). To search

for a fiber apparatus in the nucleus of *Saccharomyces* seemed therefore not unreasonable. The increase in morphological information that has attended the general adoption of glutaraldehyde as a fixative for electron microscopy (17, 48) also suggested that it might be worthwhile now to take a fresh look at the yeast nucleus.

MATERIALS

Three baking yeasts, Fleischmann's, L'Allemand's, and an Australian one, have been examined, as well as a brewing yeast used to make lager beer, and a few preparations of *S. carlsbergensis* obtained from the Centraalbureau voor Schimmelkultures at Baarn, The Netherlands. Most of the electron microscopy and much light microscopy was done on a tetraploid yeast, strain 1376, from the collection of Dr. Hawthorne at the University of Washington at Seattle. The nuclear features of all the yeasts were much alike and resembled also those depicted in many earlier papers on *Saccharomyces*. The yeasts were grown on 1.5% agar containing 0.5% "Difco" yeast extract and 2.0% glucose. Meiosis was induced on a medium described by McClary et al. (28) and consisting of, in grams per liter: glucose, 1.0; KCl, 1.8; yeast extract, 2.5; sodium acetate, 8.2; and agar, 15.0.

METHODS

Phase-Contrast Microscopy of Living, Growing Cells

Following the example of Mason and Powelson (23) and Mueller (32) we have examined growing yeasts in a medium of high refractility which reduces the brightness of the luminous haloes which surround bacteria and yeasts in the phase-contrast microscope when they are examined in watery media. 21% Difco gelatin containing 0.5 to 1% yeast extract and 2.0% glucose was satisfactory. The haloes did not entirely disappear but higher concentrations of gelatine caused a flattening of the contrast between cytoplasm and nuclei. A loopful of the melted gelatin was solidified by placing it in the center of a slide which was resting on the lid of a Petri dish filled to the brim with water that had been chilled in the refrigerator at 4 to 6°C. After it had set, the gelatin was inoculated with growing cells from an overnight culture with the help of a fine, flexible glass fiber. A cover slip was placed on the gelatin and the slide pressed against the lid of a second dish filled with hot water (around 70°C). As soon as the gelatin began to spread, the slide was returned to the cold dish and promptly sealed with wax from a small birthday candle. In such cultures the yeasts soon began to bud, and 4 to 6 hr later had sufficiently freed themselves from bright granular inclusions to afford clear views of the nuclei. They were examined with Zeiss phase-contrast optics comprising the VZ condenser (NA 1.4), an achromatic objective × 100, and a × 10 eyepiece. The microscope was illuminated with light from a Bausch and Lomb tungsten ribbon lamp passing through an interference filter transmitting maximally around 5460 A. The light was adjusted to provide Koehler illumination. Photographs were taken on Kodak Royal and RS-Pan cut film. They were enlarged twofold in printing.

Examination of Living Yeast Cells in 0.5% Acetic Acid

Low concentrations of acetic acid, as Henneberg (13) showed fifty years ago, change the optical properties of the nucleus in the unstained yeast cell so that it becomes visible in the ordinary microscope. Instructive preparations were obtained by examining cells from growing cultures between a cover slip and a thin film of water agar containing 0.5% acetic acid (pH 3.5). Films of agar were prepared by dipping an object slide twice into molten acetic acid–agar and allowing it to set between the depositions of the first and second coats. The set films were inoculated with the help of a glass fiber. All but an area measuring 4 to 6 mm^2 was then cut away. This area was covered with a glass cover slip and sealed with wax from a candle.

Preparation of Stained Specimens for Examination in the Light Microscope

A high density of cells in the exponential phase of growth and occupying a single layer on yeast extract–glucose agar was obtained in the following way: 1 ml of a heavy suspension of cells from an overnight slant culture was spread evenly, by repeated tilting, all over the agar in a standard Petri dish. Excess inoculum was removed with the pipette that delivered it and with pieces of blotting paper. The dish was then dried for 10 min while it stood upright and open in a 37°C incubator. An inoculum in which the cells lie, on the average, 20 to 30 μ apart is satisfactory.

FIXATION

After 6 to 8 hr of incubation at 30°C, slabs of agar measuring 10 × 15 mm were cut from the culture and placed, cells up, on a glass slide. A No. 1 cover slip was thinly coated with fresh egg white and placed gently over the cells. A flattened wire or "agar knife" was next slipped underneath the slab of agar and, while the fingers of one hand held the cover slip, the agar was several times pressed gently against the cover slip with the flattened wire. The agar slab was then lifted off the cover slip, in a single rapid movement, and the moist film of yeasts remaining on the

glass was immersed in Helly's fixative (minus sodium sulfate) consisting of 5 g of mercuric chloride and 3 g of potassium dichromate in 100 ml of distilled water. To 10 ml of this reagent, 0.6 ml of formalin was added immediately, before use, from a store kept over powdered chalk or magnesium carbonate. After 10 min in the fixative the films were rinsed several times with, and finally stored in, 70% alcohol.

Preparations to be stained with iron alum haematoxylin were fixed for 10 min in the reagent of Schaudinn: two parts of saturated aqueous solution of mercuric chloride plus one part of absolute alcohol and 2% v/v of glacial acetic acid. These films too were rinsed with and stored in 70% alcohol.

STAINING

FEULGEN TEST: Films fixed in Helly's or Schaudinn's were hydrolyzed in 1 N HCl at 60°C for 8½ to 9½ min, rinsed with tap water and distilled water, and left for 3 hr in a "Columbia" staining jar of the Feulgen reagent. After seven quick rinses with standard SO_2-water they were washed for 20 min in running tap water and mounted over a drop of acetocarmine (26, 43). Some preparations were examined without having been counterstained.

HCL-GIEMSA'S: The procedure followed was the same as that described earlier in a study of the yeast *Lipomyces* (42). Hydrolyzed, stained cells were flattened by strong thumb pressure on the cover slip. Unless this was done, the nuclei in HCl-Giemsa preparations of budding yeasts appeared opaque and uninformative.

HEIDENHAIN'S IRON ALUM HAEMATOXYLIN: Cells fixed in Schaudinn's were mordanted overnight in 2% iron alum, stained for at least 6 hr with haematoxylin prepared according to Baker and Jordan (2), and destained in the mordant. The progress of differentiation was followed with a × 40 water-immersion lens. After ½ hr in running water the stained films were mounted over a drop of tap water and sealed with wax from a candle.

ACID FUCHSIN: We tried this dye because we knew that it has a strong affinity for the fibers of the mitotic spindle of at least one fungus, *Basidiobolus ranarum* (43). Films fixed in Helly's were removed from the 70% alcohol in which they had been stored, rinsed with distilled water and 1% acetic acid in water, and immersed for 2½ to 3½ min in 0.005% acid fuchsin in 1% acetic acid. They were examined with a water immersion lens for the right degree of contrast and rinsed with and mounted over a drop of 1% acetic acid. No useful amount of stain is retained by the cells if acetic acid is omitted from the dye solution and/or the washing water. The optical equipment, illumination, and photographic technique were the same as that described in an earlier paper (42). Methods that were used only for special purposes are explained in the text.

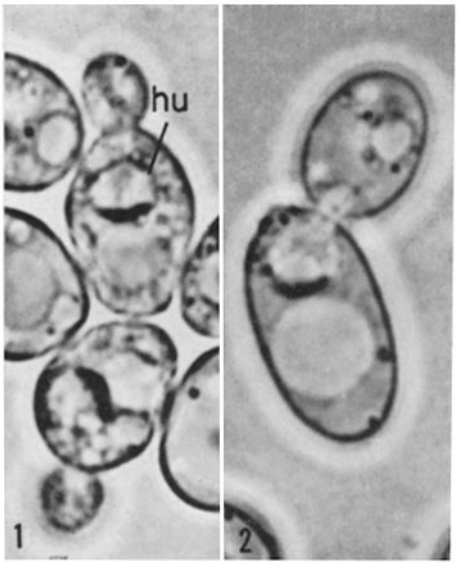
Electron Microscopy

One ml of a dense suspension of cells from an 18 hr slant culture of the tetraploid yeast was added to each of two 500 ml conical flasks containing 11 ml of liquid medium (as described above, without agar). The flasks were placed in a 30°C incubator room and given a shake every half hour. After 5 to 6 hr the cells were spun down and taken up in 3% glutaraldehyde in 1/15 M sodium–potassium phosphate buffer at a final pH of 6.8. The cells were washed four times by centrifugation in 9 ml of the same buffer 8 hr later, and resuspended in 4 ml of Ryter-Kellenberger buffer (47), pH 6.1, plus 1 ml of snail juice (Industries Biologiques Francaises S. A., Gennevilliers, France). Digestion was allowed to proceed for 1 to 1½ hr at 25°C. When they were examined under a cover slip at the end of this time, most of the cells had only a few shreds of wall adhering to them. The digested yeasts were once more spun down and postfixed in Ryter and Kellenberger's osmium tetroxide–calcium fixative (47) overnight. They were then washed with Kellenberger buffer, soaked in uranyl nitrate, dehydrated, and embedded in Vestopal in the usual way. Sections were cut on a Porter-Blum microtome, stained with lead by the method of Reynolds (41), and examined in a Philips-100 electron microscope. This procedure has given us the information we were seeking, but we are aware that it wants improving in many respects.

RESULTS

Observations on Living Cells

Under the ordinary microscope the nucleus of the living yeast cell is not readily distinguished from the cytoplasm. It becomes visible in the presence of 0.5% acetic acid (pH 3.4) (Figs. 1 to 2). The nucleus then appears clear and watery except for a peripheral cap or crescent of high refractility, the *Kernkopf* of Henneberg (13). Townsend and Lindegren (49) state that the acid-treated cells remain viable, and we have confirmed this. Yeasts were kept in 0.5% acetic acid in water for 18 min. A sample of them was examined for crescents, which were prominent. Another sample was streaked out on a film of ordinary yeast extract–glucose agar and observed continuously under the microscope. Of 13 numbered cells, all but one soon resumed growth, budded and divided in normal fashion. So did the great majority of the numerous cells surrounding them. The main body of



FIGURES 1 and 2 Unfixed yeast cells on water agar containing 0.5% acetic acid. Ordinary microscopy. The nucleus (*nu*) stands out clearly. It contains a crescent of refractile matter which appears black. Fig. 1, from a culture of L'Allemand baking yeast. Fig. 5, from a culture of tetraploid No. 1376. × 2700.

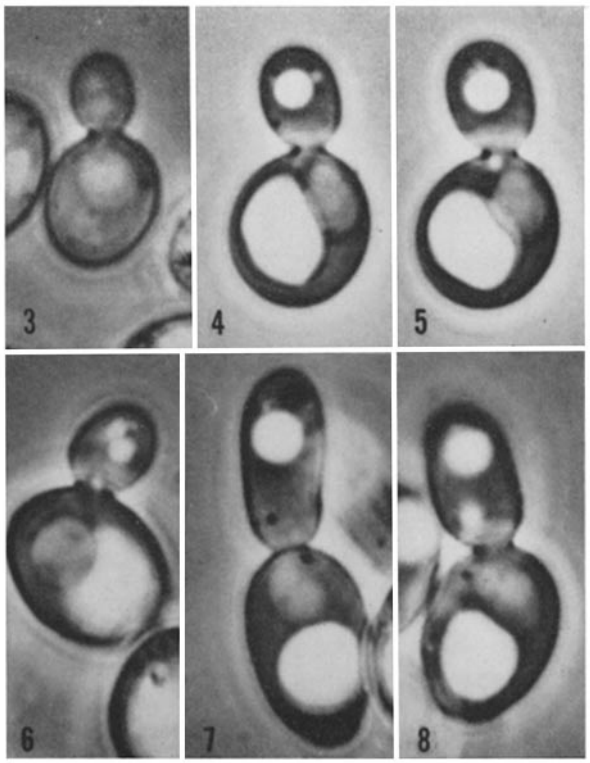
the nucleus of acid-treated cells is of a watery transparency, but when such cells were fixed with 70% alcohol and stained, their nuclei were found to contain the usual amount of chromatin at its usual site. There is therefore no reason for suspecting that the dense crescent of the nuclei of living cells suspended in dilute acid represents the displaced former content of the main body of the nucleus.

Phase-contrast microscopy (Figs. 3 to 8) reveals the same two components of differing density in the nucleus of yeasts growing in yeast extract–glucose gelatin. Under these conditions the dense crescent occupies a larger fraction of the nucleus than it does in the presence of acid. It is also far less refractile. The crescent is most frequently found at that pole of the nucleus which is closest to the vacuole. A small, round, phase-gray bead makes a dent in the contour of many of the smaller nuclei at a point opposite the crescent (Figs. 3, 9, at 0). In larger nuclei it is common to find the bead replaced by a sharp tooth of relatively dense matter cutting into the nucleus from the side. Two such narrow intrusions may arise from opposing points of the nuclear margin and give the impression of cutting the nucleus in half (Figs. 7 and 8). The crescent and intrusions, either shallow or deep, are visible in most nuclei whose images are not blurred by the presence of too many lipid droplets in their vicinity. They are seen so often and, as will be shown later, their equivalents appear so regularly in preparations preserved in several different ways that we believe that the dense crescent and the intrusions into the nucleus are normal and constant features. Optical differences between the parts of the nucleus fade from sight during division but are soon reestablished in the daughter nuclei. Division follows the course previously described by Mueller (32) and by Nečas et al. (37) and outlined in the Introduction. A representative sequence is illustrated in Fig. 9.

Observations with the Light Microscope on Fixed and Stained Cells

FEULGEN TEST: (Figs. 10 to 14) The main, least dense portion of the nucleus gives a positive Feulgen reaction. The crescent does not. The absence of this bulky component from the image of the nucleus in Feulgen preparations accounts for the oddly angular shapes and relatively small size of the chromatinic moiety. At the margin of the Feulgen-positive portion a small, round, seemingly punched out patch that is Feulgen negative is commonly seen. Equally frequent are deep indentations or grooves which appear to divide the nucleus into two halves (Figs. 10 and 11). These peculiarities of shape and contour line, also recorded by Nagel (35), correspond closely to the intrusions into the lighter portion of living nuclei which are visible under the phase-contrast microscope (Figs. 3 to 8). Nuclei in division and young daughter nuclei are invariably less strongly Feulgen positive than "resting" nuclei in cells with small buds.

HCL-GIEMSA'S: In hydrolyzed preparations stained with Giemsa's, the chromatin occupies the same portion of the nucleus as in Feulgen-acetocarmine preparations. We have confirmed the earlier observations of others and ourselves (29, 38, 44) that this portion of the nucleus becomes transformed into a cluster of chromosomes at the beginning of meiosis. Many nuclei in acid-Giemsa preparations are traversed by the same clear, narrow fissures which we have already encountered in the nuclei of Feulgen specimens. A good example of this is provided by Fig. 6 of Lindegren et al. (21). Usually, however, unless the nuclei are flattened, the greater density of the Giemsa stain makes the



FIGURES 3 to 8 Living cells growing in 21% gelatin with yeast extract and glucose. Phase-contrast microscopy. All nuclei contain a gray crescent. From preparations of baking yeasts, except Figs. 4 to 6 which are from cultures of tetraploid No. 1376. × 2700.

FIGURE 3 Near the bud the bright portion of the nucleus is indented by a gray bead or dimple. The lower third of the nucleus is occupied by a gray crescent.

FIGURES 4 and 5 Photographs of the same cell taken a few minutes apart. There is a crescent at the lower right. The upper part of the nucleus appears bisected by a gray line.

FIGURE 6 Nearly one half of the nucleus is occupied by a dark crescent, and a small gray patch seems to be floating in the brighter portion of the nucleus.

FIGURES 7 and 8 Examples of nuclei with indented contours. The dark crescent is distinct in Fig. 8.

fissures and intrusions less conspicuous than they are in Feulgen preparations. In sufficiently transparent nuclei the chromatin on either side along the middle of the central groove or fissure is regularly found more deeply stained with Giemsa's than the rest of the chromatin (Figs. 30 to 33). A similar effect is noted in Feulgen preparations. It will be discussed in a later section. The material of the *crescent* can be stained directly with Giemsa's but loses most of its affinity for this stain in the course of hydrolysis, in striking contrast to the *chromatin* whose normally weak affinity for the Giemsa stain is vastly increased by hydrolysis. The unstained or lightly tinted portion of the nucleus occupied by the crescent in acid-Giemsa preparations has often been overlooked.

HEIDENHAIN'S IRON HAEMATOXYLIN: The dense peripheral crescent of the nucleus retains the haematoxylin lake longer than anything else in the cell. That degree of differentiation which leaves the crescent sharply defined and solidly stained also reveals, close to, but not always at the opposite pole, a lightly colored granule or a pair of them united in the form of a slender rodlet, slightly thicker at the ends than in the middle (Figs. 15, 16). These small but distinct objects, which are found regularly, seem to lie inside the substance of the main body of the nucleus which in this kind of preparation is almost colorless.

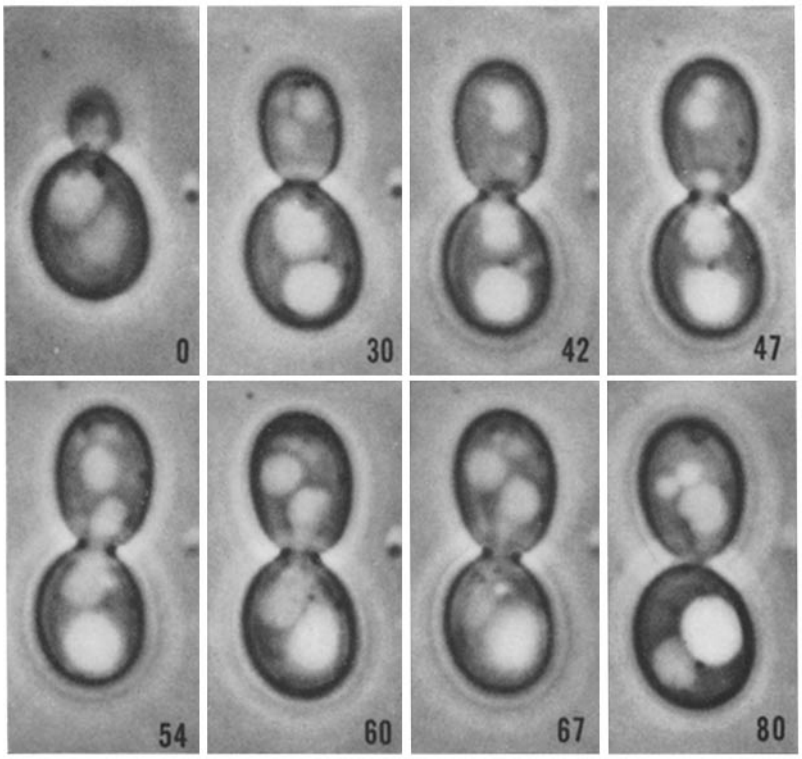
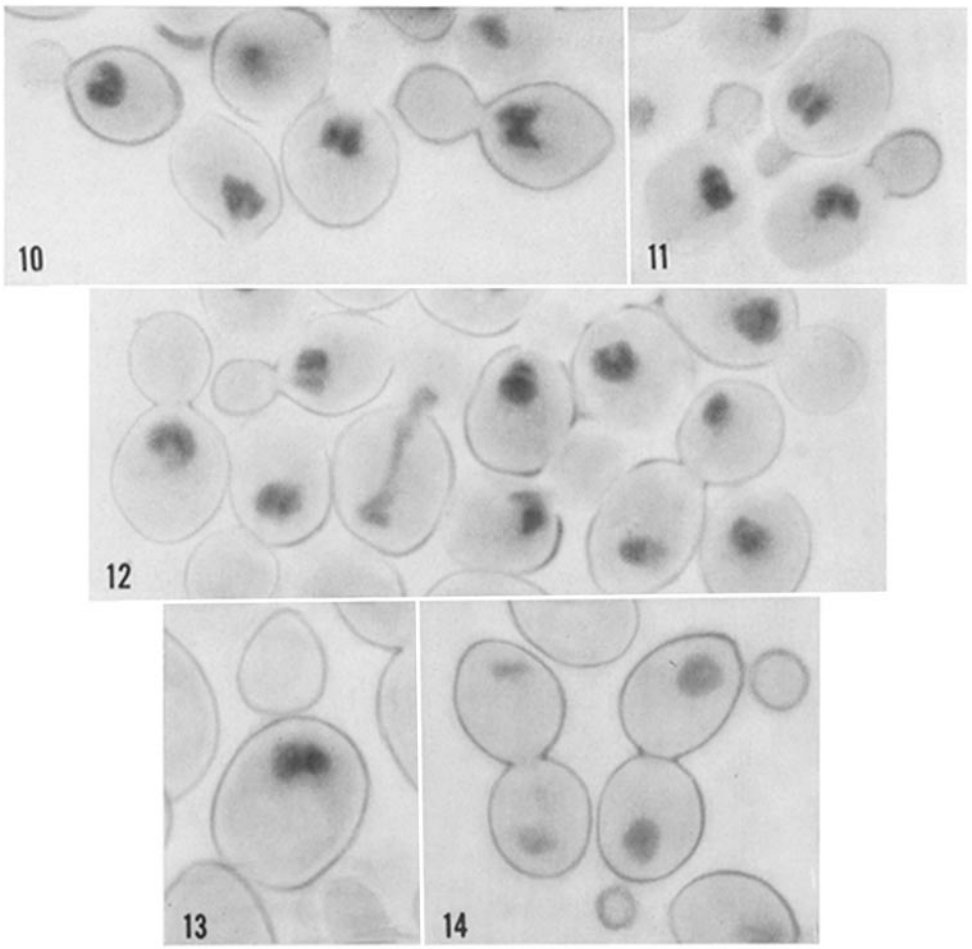


FIGURE 9 Time-lapse phase-contrast photomicrographs of the division of the nucleus in a budding cell of the L'Allemand strain. Numbers indicate minutes elapsed since the first photograph was taken. Note the gray bead indenting the upper pole of the nucleus in the first picture of the sequence. The gray crescent is faintly visible at the lower edge of that nucleus. × 2750.

Comparison with the images provided by the phase-contrast microscope suggests that the granules and the rodlet are somehow related to the nick or dimple and the fine toothlike intrusions which cut into the contours of living yeast nuclei (Figs. 3 to 8). In dividing nuclei the crescent assumes irregular shapes and often breaks into several small pieces which spread over the length of the nucleus, frequently along one side of it (Figs. 17 and 18). Daughter nuclei receive roughly equal shares of this material and probably add to it by synthesis. Illustrations of nuclei with crescents stained deeply with haematoxylin, some of them showing also the granule at the opposite pole, are found in many papers on yeast cytology. They will be referred to in the Discussion.

Iron haematoxylin is an excellent stain for mitotic chromosomes of higher organisms and protozoa and was much used by cytologists until the introduction of rapid nuclear staining with acetocarmine. It is unfortunate that in *Saccharomyces* iron haematoxylin is avidly retained only by that component of the nucleus which is Feulgen negative and that the dye is most readily extracted from the Feulgen-positive portion which represents the chromosomes.

ACID FUCHSIN: In our search for a fiber apparatus in dividing yeast nuclei, we tried the effect of acid fuchsin because we knew that in the fungus *Basidiobolus* acid solutions of low concentrations of this dye stain the fibers of the mitotic spindle very clearly (reference 43, and further work since). The response of *Saccharomyces* was surprisingly informative. The result of a few minutes' staining in a 1:20,000 dilution of acid fuchsin in 1% acetic acid told us as much and more about the yeast nucleus than preparations stained by the procedure of Heidenhain. The crescent as well as the bead or rodlet at the opposite pole of the nucleus takes up the dye faster than the chro-



FIGURES 10 to 14 Helly fixation. Feulgen reaction, acetocarmine. Fleischmann's yeast, except Fig. 13 which is of the tetraploid strain. Several nuclei contain a small peripheral patch of Feulgen-negative matter. Many others appear bisected by a narrow Feulgen-negative groove. The crescents are invisible because they are Feulgen negative and have lost their affinity for acetocarmine during acid hydrolysis. Note relatively pale dividing nucleus fourth from left in Fig. 12 and how in Fig. 14 the equally pale pair of telophase nuclei in newly formed sister cells on the left contrasts with the strongly Feulgen-positive nuclei in budding cells on the right. × 2700.

matin and stands out in strong contrast against it (Figs. 19 to 25). The relationship of the rodlet to the chromatin is illustrated in Figs. 24 *a*, *b*, and 25 *a*, *b*. In each pair of figures the (*a*) cell is stained with acid fuchsin. The (*b*) picture shows the same cell stained with Giemsa's after hydrolysis. It is seen that the hydrolyzed crescent has no affinity for Giemsa's and that the rodlet is embedded in chromatin. The term rodlet may be extended to the polar intranuclear dot or bead, e.g. the beads in Fig. 19, because most of the dots or beads, by their apparent change of position with change of focus, reveal that they are in reality short cylinders. In dividing nuclei the rodlet expands into a long, thin, straight fiber with a small knob at each end (Figs. 26 to 29). This fiber, which is also visible in dividing nuclei of living cells exposed to 0.5% acetic acid (Fig. 2) recalls the *Zentralstrang* or central cord in mitotic nuclei of the basidiomycete *Polystictus* which Girbardt (5, 6) has demonstrated in living hyphae and more recently in electron micrographs. The length, uniform thickness, and

crystal-like straightness of the fully expanded fiber are remarkable but, except in the terminal stages, there is no correlation between either the orientation or the length of the intranuclear fiber and the direction and degree of elongation achieved by a dividing nucleus. It is not unusual to see a short fiber lying crosswise in a ribbon-shaped nucleus which has already penetrated for some length into a bud. It seems possible that the fiber expands to its full length and aligns itself parallel to the long axis of the nucleus suddenly and rapidly at a late stage in the division of the nucleus. It vanishes from sight after the stage of its longest extension but in stained preparations a minute rodlet is already visible in each daughter nucleus before the severance of the thin strand by which daughter nuclei remain connected until late telophase.

It will now have become apparent that the groove that seems to bisect many nuclei stained with Feulgen's or Giemsa's (Figs. 10 to 12, 33, 34) is the negative image of a fiber of varying length buried inside the chromatinic portion of the nucleus. The nature of the dents in nuclear contours seen under the phase-contrast microscope (Figs. 7 and 8) is not immediately obvious in stained preparations, but it has been explained by electron microscopy. To anticipate: the dents correspond to invaginations of the nuclear envelope terminating in a point of origin of the intranuclear fiber.

Observations Concerning the Composition of the Dense Crescent

The selective staining of crescents and intranuclear fibers with acid fuchsin in acid solution made this a useful procedure with which to look for possible effects of protoeolytic enzymes on these structures. Cells of the L'Allemand strain fixed in Helly's on eggwhite-coated cover slips were kept in 70% alcohol overnight, washed ten times with distilled water, and digested for 2 hr with 0.3% pepsin (twice crystallized, Worthington, Freehold, New Jersey) in 0.3 N HCl. Controls were kept for an equal length of time in the same concentration of acid. Digested cells had lost their affinity for acid fuchsin and showed no trace of either crescent or fibers, although in normal fixed cells the crescent at least is visible, even without staining, on account of its relatively high refractility. Controls stained deeply, and the crescents of their nuclei were intact. Trypsin (twice crystallized, salt-free, Worthington) applied as 0.1% solution in 0.1 M potassium phosphate buffer at pH 7.5 gave essentially the same result though its action was slower and less uniform. Controls in buffer were normal. The crescents and intranuclear fibers also stain distinctly with the bromphenol blue–mercuric chloride reagent of Mazia et al. (25) but stand out less clearly from the stained cytoplasm than they do in acid fuchsin prepara-

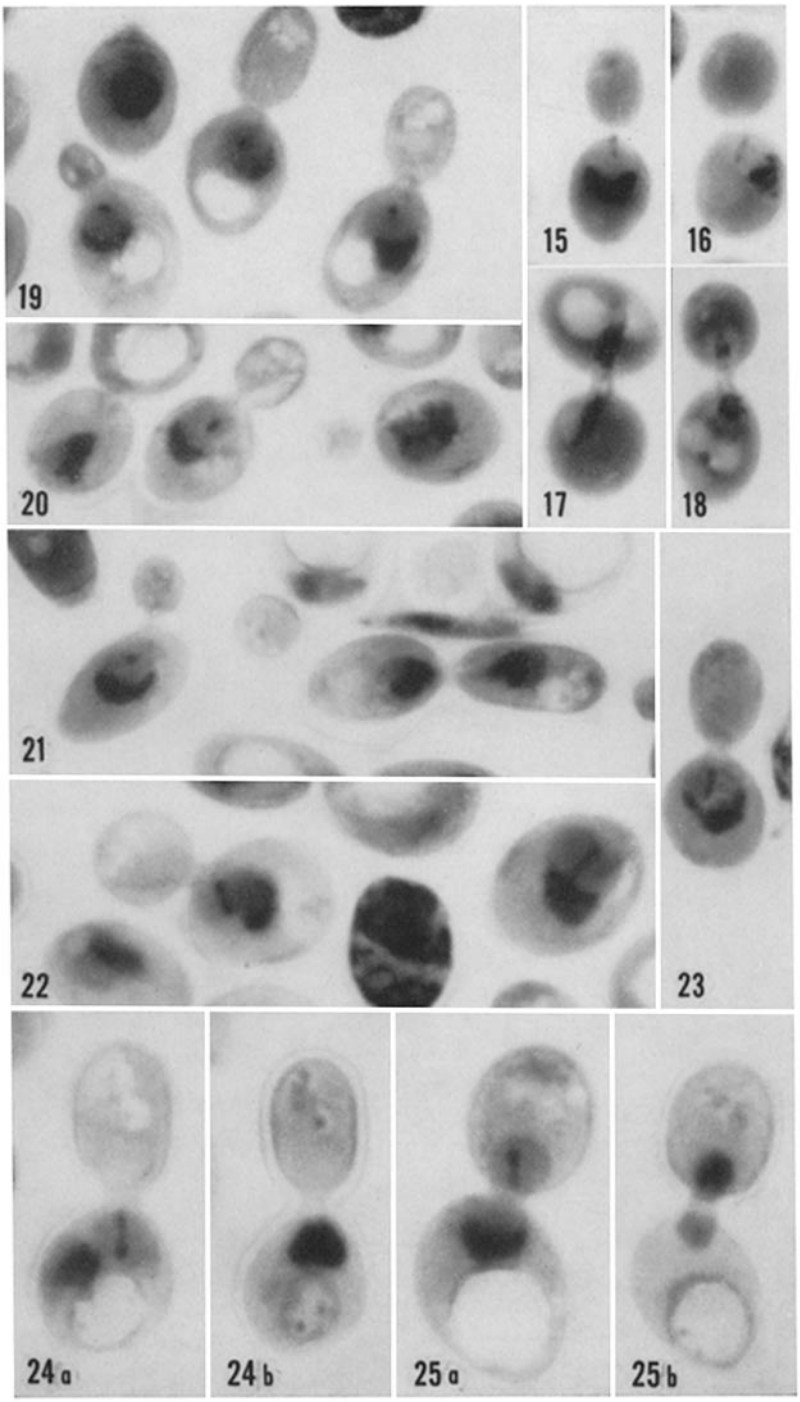
FIGURES 15 to 18 Schaudinn fixation. Iron alum haematoxylin.

FIGURES 15 and 16 Resting nuclei, each containing a densely stained crescent and a short dumbbell-shaped intranuclear fiber. The fiber appears suspended in the unstained chromatin. × 2700.

FIGURES 17 and 18 Crescent material spread out along the main axis of dividing nuclei. This material is basiphilic but Feulgen negative.

FIGURES 19 to 23 Helly fixation. Acid fuchsin staining. A short fiber in the shape of a bead or rodlet lies in the lightly stained chromatinic half of most of the nuclei. The crescent-shaped nonchromatinic moiety of the nucleus is deeply stained with acid fuchsin. Note anaphase of division in Fig. 21. Daughter nuclei of this size contain a short new fiber of their own. It is here obscured by crescent material but just visible in the triangular nucleus, product of a recent division, at the left in Fig. 20. Figs. 19 to 21, × 2700; Fig. 22, × 3600; Fig. 23, × 2700.

FIGURES 24 and 25 The relationship of the fiber to the chromatin is illustrated in Figs. 24 *a*, *b*, and 25 *a*, *b*. In each pair of figures the (*a*) cell is stained with acid fuchsin. The (*b*) picture shows the same cell stained with Giemsa's following hydrolysis. × 3600.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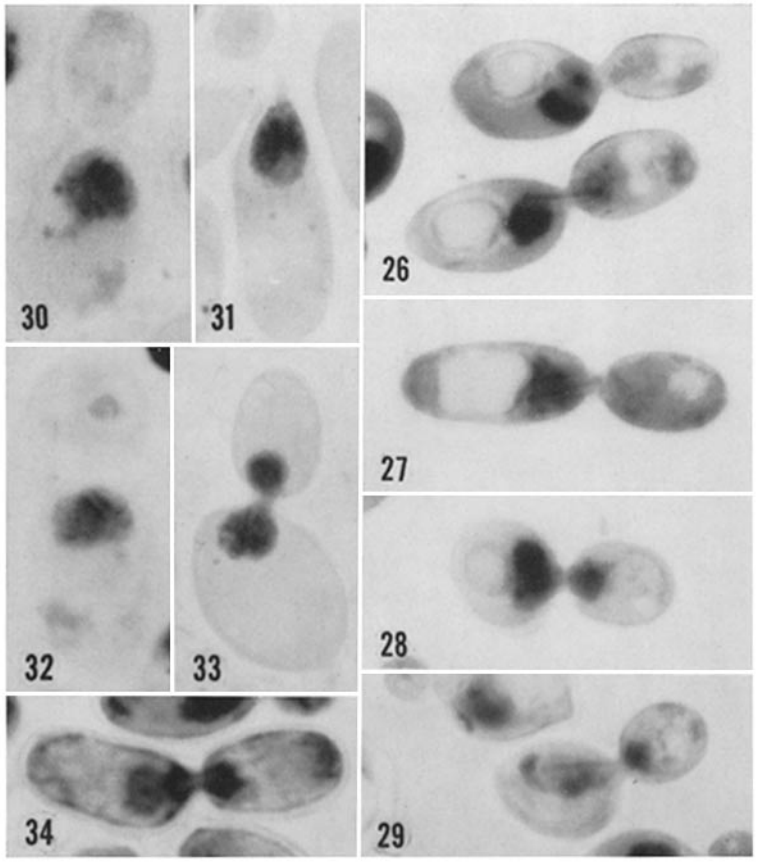


FIGURES 26 to 29 Helly fixation. Acid fuchsin stain. Examples of fully expanded fibers inside dividing nuclei. Chromatin faintly stained, crescents deeply stained. In Figs. 26 and 28 the crescent material is lagging behind the chromatin. In Figs. 27 and 29 it is mixed with the chromatin. The fibers are tipped at each end by a small knob. × 3600.

FIGURES 30 to 34 Nuclei from much flattened HCl-Giemsa preparations. The nuclei are bisected by a seemingly empty groove which is lined along part of its length by deeply stained chromatin. The groove is the site of the fiber shown in the adjoining Figs. 26 to 29. The crescents of the nuclei of Figs. 30 to 33 have not accepted the Giemsa stain. Fig. 34 is of a heavily stained preparation photographed in red light from a Kodak Wratten filter A 25. The crescent is at the left. The nucleus has begun to divide. It contains a short fiber which, being unstained, appears as a white rodlet embedded in chromatin. Figs. 26 and 27, tetraploid No. 1376. Fig. 28, L'Allemand yeast. Fig. 29 and the rest, Fleischmann's yeast. × 2700.

tions. These experiences permit the conclusion that the crescent contains a basic (i.e. strongly acidophil) protein. Since it is part of the nucleus, the crescent possibly contains a nucleoprotein. Since the crescent is Feulgen negative and loses its basiphilia in the course of acid hydrolysis, one suspects it of consisting of or containing a protein combined with ribonucleic acid. It has not yet been possible to verify this suspicion through experiments with ribonuclease. Action of this enzyme renders fixed cells strongly acidophilic all over (through absorption?) and also greatly increases their affinity for iron haematoxylin. It was hard, under these circumstances, to distinguish the crescents from the cytoplasm, and a decision on the action of ribonuclease on the crescents could not be made. It is our impression that crescents were still present after 1½ to 3 hr of digestion

at 37°C with enzyme concentrations of 300 to 400 μg/ml. Observations bearing on this point have been made by Mundkur (34) and will be referred to later. A systematic cytochemical study of the crescent material, using other dyes as well as extractants, is desirable but was not part of our plan of work. In the nuclei of many other fungi, including three yeasts, *Sporobolomyces*, *Lipomyces*, and *Schizosaccharomyces* (45), haematoxylin and acid fuchsin are avidly taken up by the nucleolus and, pending further information, we tentatively agree with Yoneda (55) in regarding the dense crescent as the equivalent of a nucleolus.

Summary of the Evidence from Light Microscopy

The yeast nucleus consists of two materials differing in optical density and composition. The main body of the nucleus is less dense than the cytoplasm, is filled with fine-grained Feulgen-positive matter, and acquires increased affinity for the Giemsa stain in the course of acid hydrolysis. At meiosis, chromosomes appear in this portion of the nucleus. Another portion of the nucleus is almost as dense as the cytoplasm. It occupies a peripheral segment of the nucleus, commonly on the side nearest to the vacuole. This segment is often crescent-shaped in optical section, but bulkier, more irregular shapes are also seen, especially at the start of division and immediately after it. The material of the crescent is Feulgen negative but has marked affinity for iron haematoxylin and for acid fuchsin in acid solution. It retains little affinity for Giemsa's stain after hydrolysis. Both Feulgen-positive matter (i.e the chromosomes, in whatever form they may be present) and crescent material are divided between daughter nuclei. Opposite the crescent a small organelle is regularly found which appears at first sight as a bead but which closer study reveals to be a small rodlet. During division the rodlet expands into a long straight fiber, tipped at either end by a small knob. The intranuclear fiber has marked affinity for acid fuchsin, more so when it is short and compact than when it is expanded. In living nuclei the smallest fibers appear as a dark patch or line in the low density portion of the nucleus (Figs. 3 to 5). In Feulgen preparations their presence reveals itself by indentations of the margins or by a furrow (or tunnel) which seems to cut the nucleus in two.

Electron Microscopy

The electron micrographs about to be described were obtained from two embeddings of the tetraploid yeast No. 1376. Under the light microscope the nucleus of this yeast looks like the nuclei of the other yeasts we have examined and shares with them features described in the literature. We think it probable therefore that the results of our electron microscopy are valid for *Saccharomyces cerevisiae* in general. We have obtained satisfactory preservation of the plasma membrane, the ribosomes of the cytoplasm, and the envelope and contents of the nuclei, but not of the mitochondria.

Chromatin (Figs. 34 to 37)

The main body of the nucleus is fine-grained and lacks recurrent, definite and interpretable features. The uniform granulation of the main portion of the nucleus was to be expected from the evenness of the positive Feulgen reaction and accords well with the view of Moor and Mühlethaler (31) that the yeast nucleus contains "no component larger than about 100 A . . . except in one direction." In electron micrographs nothing was seen that could be related to the sleeve of deeply stained chromatin which in Feulgen and Giemsa preparations is found on either side of the intranuclear fiber (Figs. 30 to 33). We never encountered the broad interlaced cords of matter of very low density which have several times been described in nuclei fixed with potassium permanganate and which some regard as profiles of chromosomes (50, 56).

Material of the Dense Crescent (Figs. 35, 36, and 39)

The sites occupied by this material and the amount of it per nucleus agree with expectations from acid fuchsin preparations. In small nuclei the crescent is compact. In dividing ones the dense matter is more loosely arranged and no longer confined to a peripheral segment of the nucleus. Despite their strong acidophilia the crescents obviously correspond to the "peripheral clusters" of basiphil granules which Mundkur (33, 34) discovered in electron micrographs of yeast nuclei. Crescent material is also visible in some of the micrographs published by Hagedorn (10). Mundkur (33, 34) has carried out cytochemical tests on his specimens, and records a striking loss of contrast in the main body of the nucleus after treatment

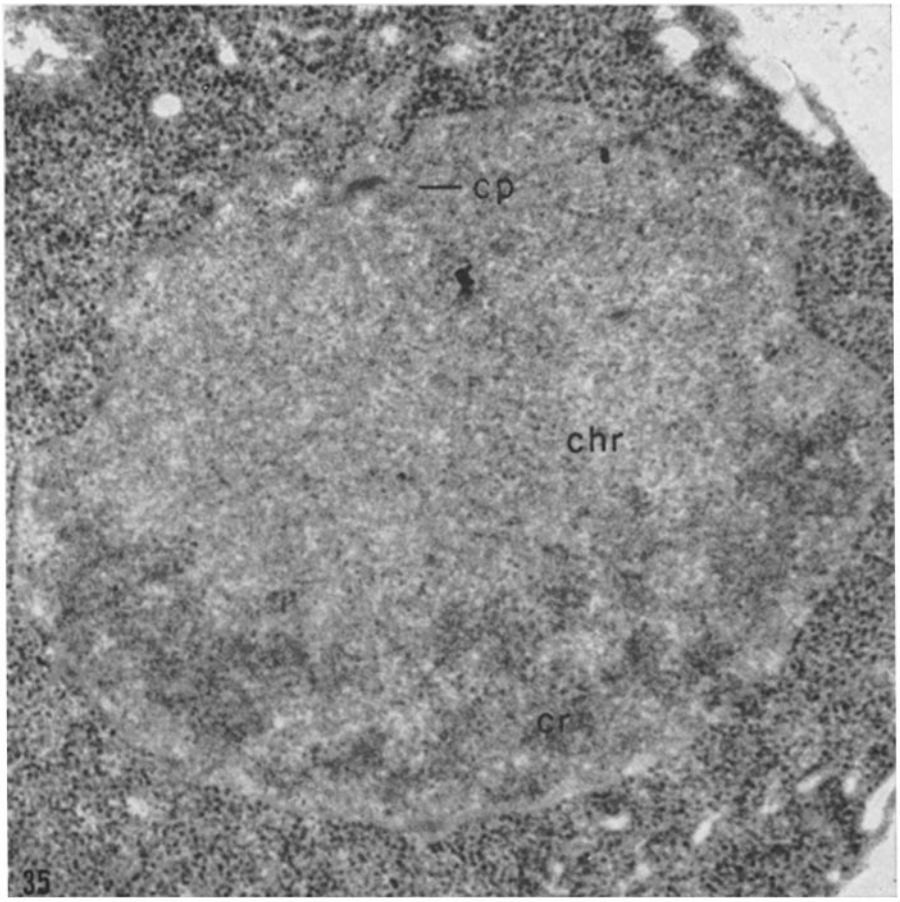


FIGURE 35 Low-power view of a nucleus. This and all remaining illustrations are electron micrographs of sections of the tetraploid yeast No. 1376. A crescent (*cr*) of dense matter contrasts with fine-grained chromatin (*chr*). The dark bar at the edge of the nucleus opposite the crescent is a profile of a centriolar plaque (*cP*). × 50,000.

with deoxyribonuclease. There was loss of some material from the peripheral clusters after digestion with ribonuclease, a fact which indicates that their composition is complex. This accords with our impression that the crescents remain stainable after long exposure to ribonuclease.

THE NUCLEAR ENVELOPE (FIG. 36)

Profiles of the nuclear envelope in our sections resemble those previously published by Vitols et al. (50) and Moor and Mühlethaler (31). The envelope is pierced by numerous pores which in our material are usually filled with a cluster of closely packed minute granules, less dense and much smaller than the ribosomes which fill the cytoplasm. This material is not seen, at least not in this concentration, elsewhere in the nucleus. In some instances the pores contained larger clumps of dense matter, of the order of size of those composing the dense crescent.

THE FIBER APPARATUS

The electron microscope resolves the intranuclear rodlets and fibers of acid fuchsin preparations into bundles of optically hollow 150 to 180 A filaments or tubules which run between two points on the nuclear envelope (Fig. 37). The filaments end in the vicinity of paired, flat, broad, dense structures about 1500 A long and several hundred Angstroms wide which are set into large pores of

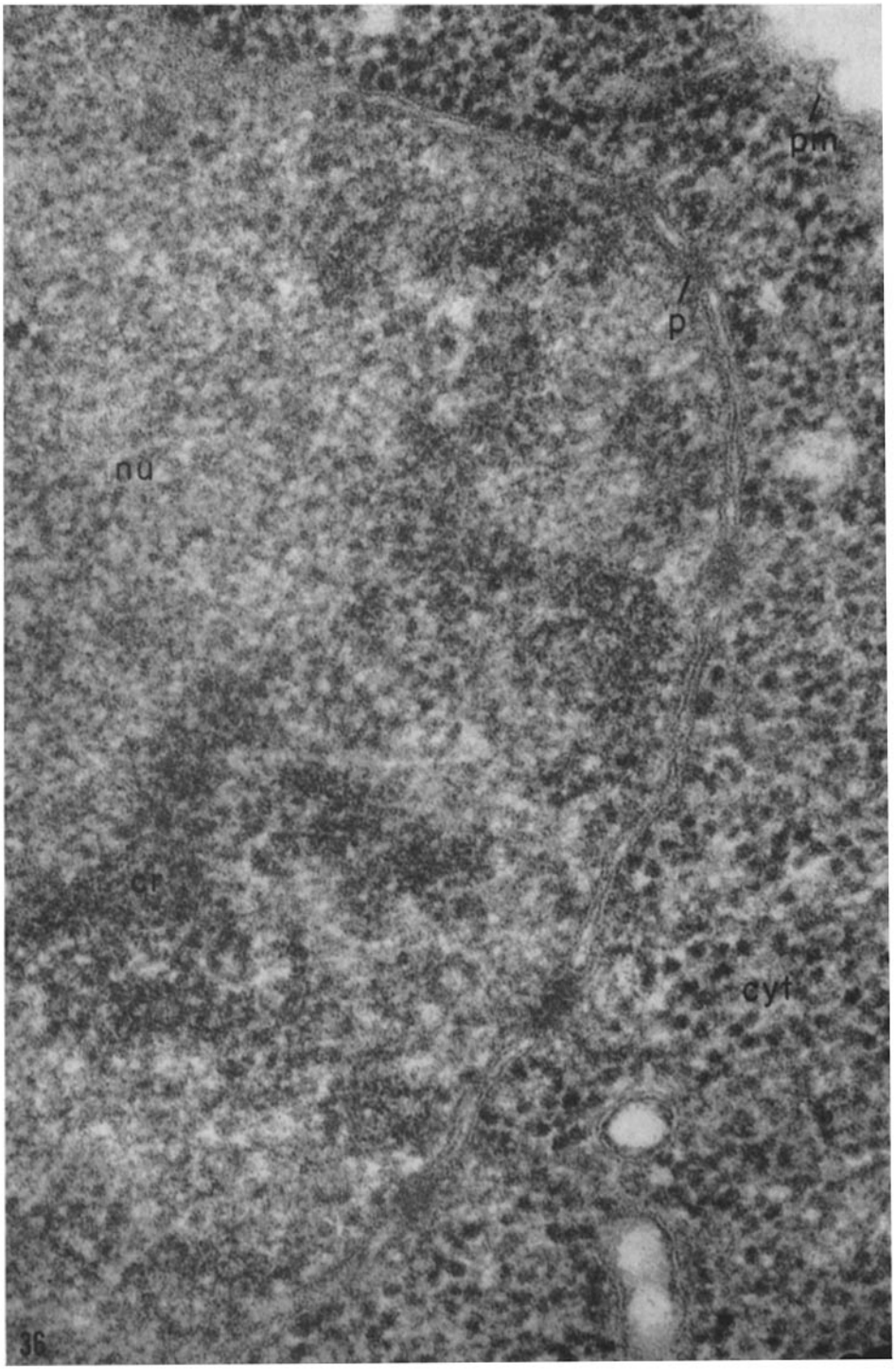


FIGURE 36 Most of the illustration is taken up by a corner of a nucleus (*nu*) separated by the two leaves of its envelope from the cytoplasm (*cyt*) which is crammed with ribosomes and bounded, at top right, by the plasma membrane (*pm*). There is much dense crescent material (*cr*). The pores (*p*) of the envelope are filled with fine-grained matter of high density. × 118,000.

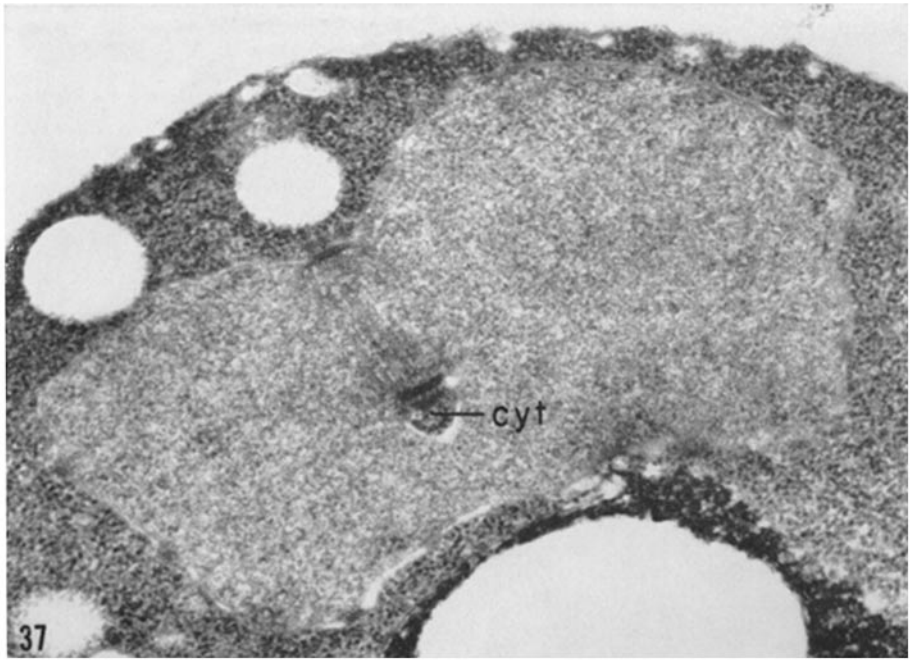
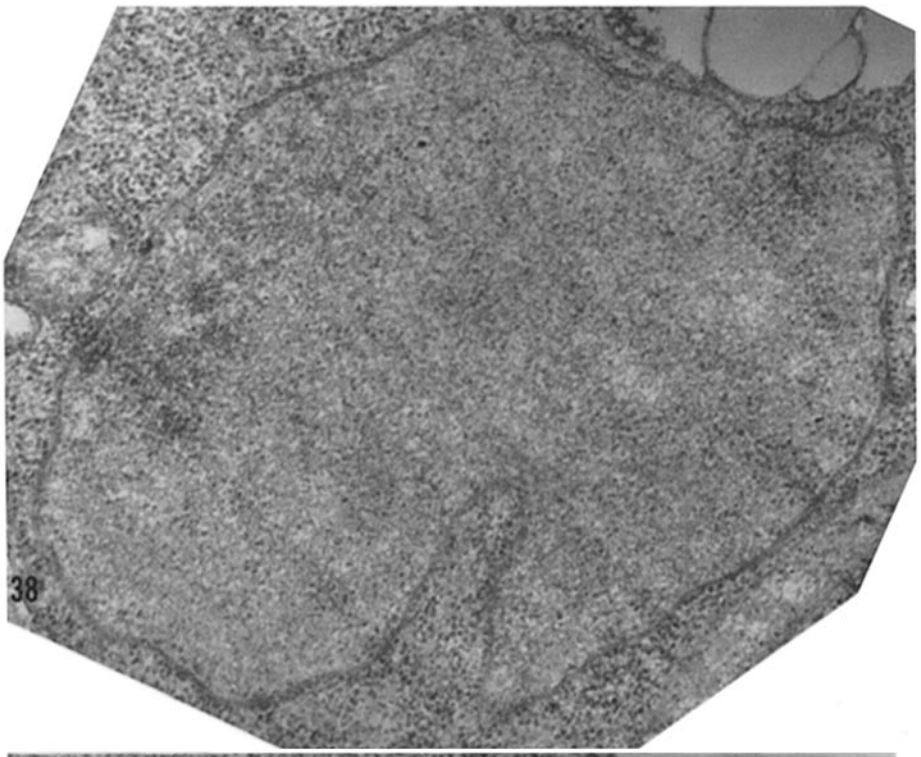


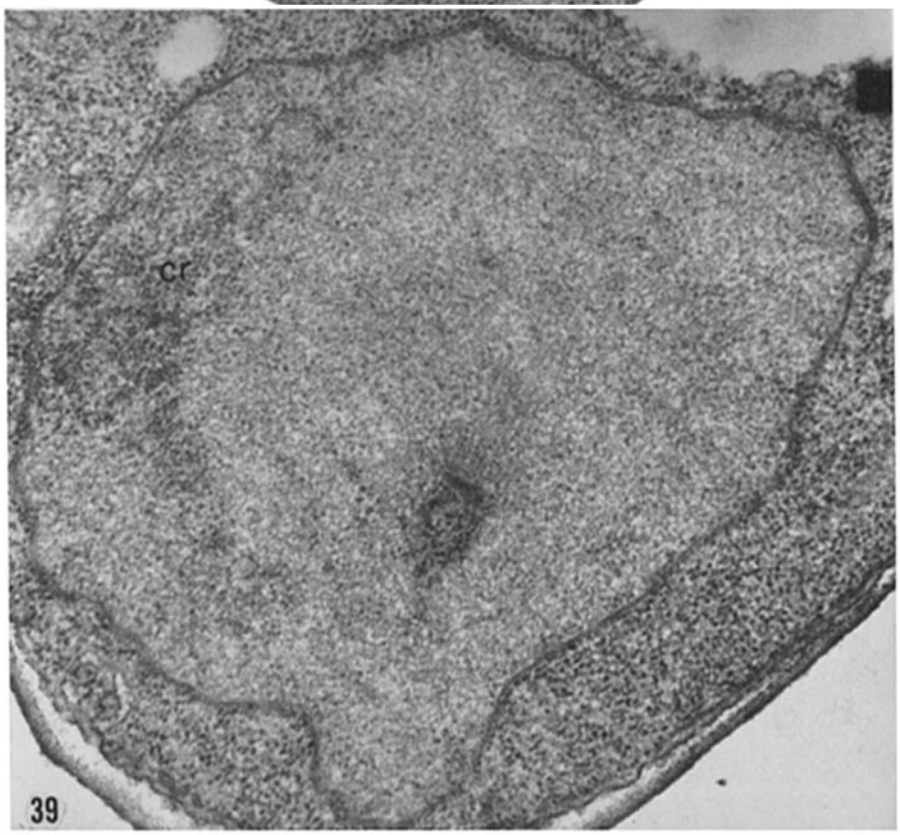
FIGURE 37 Illustrates a typical small nucleus with a short fiber. Centriolar plaques are at either end of a fiber which is composed of a small number of filaments. Each plaque consists of two bars that are parallel to each other. The lower plaque lies at the bottom of a deep well of cytoplasm (*cyt*) that intrudes into the chromatinic portion of the nucleus. × 50,000.

the nuclear envelope and will be referred to as "centriolar plaques," a term suggested to us by Dr. Hewson Swift. The plaque on the side of the nucleus nearest to the bud lies usually in a shallow depression of the nuclear envelope (Figs. 37 and 43). The opposing plaque, which may be anywhere on the side of the nucleus or at a point close to the vacuole, usually lies at the bottom of a much deeper, tunnel-like indentation of the envelope (Figs. 37, 39, and 40).

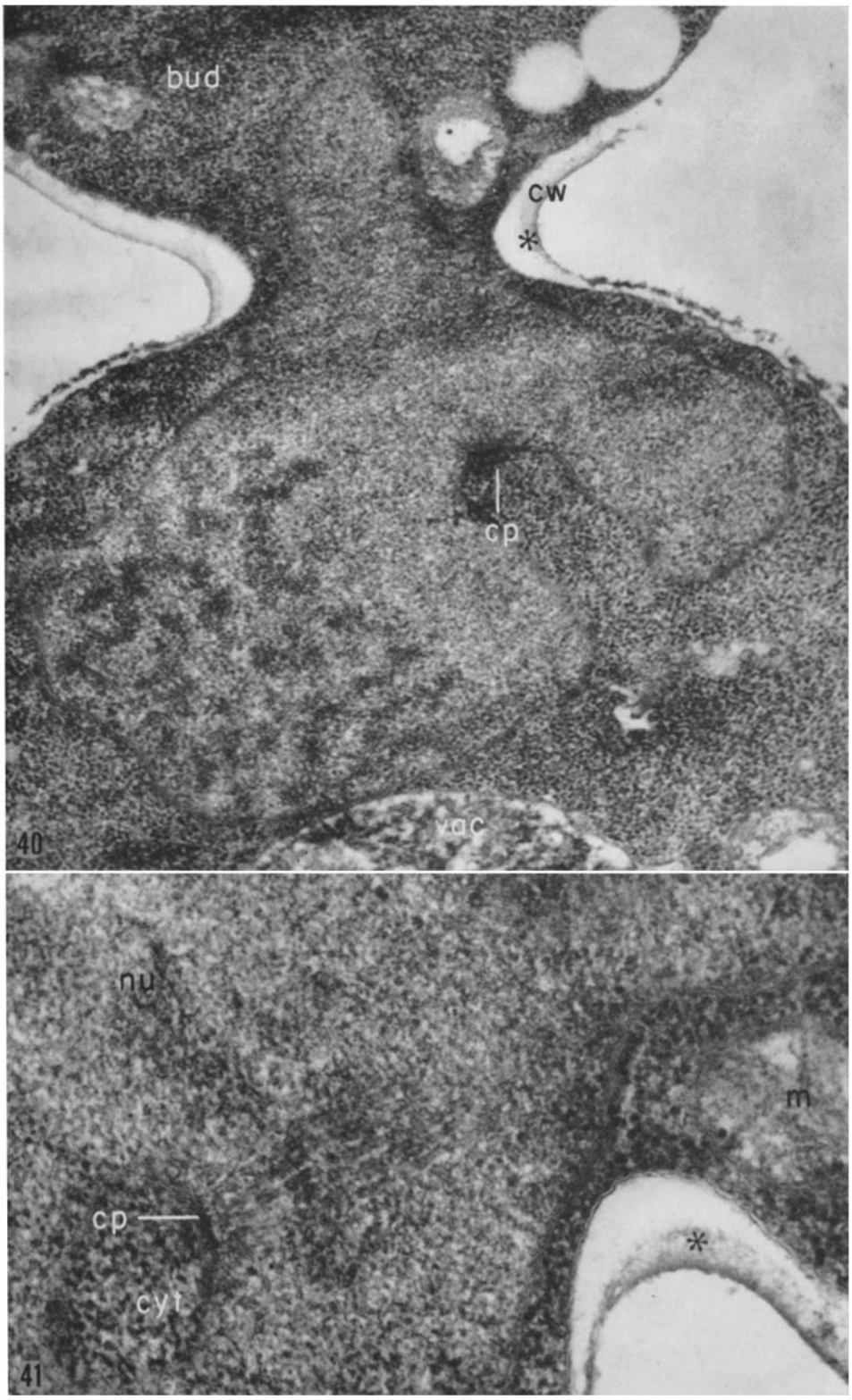
THE FILAMENTS OF THE INTRANUCLEAR FIBER: The component filaments of short fibers flare out from their points of origin and may have wavy contours; those of longer fibers are straight and run parallel to each other (Fig. 43). This difference between short and long fibers corresponds to the difference between the slender lozenge shape of the intranuclear rodlets (e.g. Figs. 23 and 24 *a*) and the wirelike straightness of fully expanded fibers in acid fuchsin preparations. The edges of the component filaments of fibers in our material are not so evenly stained as the contours of spindle fibers described by other authors (16, 17, 22). The same holds for cross-sections and oblique sections, though clusters of these (Figs. 50 and 52) were sufficiently different from the rest of the nuclear content to be recognizable. The number of filaments visible at the same time in one section is always small. In thirteen out of sixteen different favorable profiles it was between 5 and 8; the remainder had 4, 9, and 11 countable filaments. This is compatible with a tentative count of 15 filaments in a patch of cross-sections and is in keeping with the thinness of the fibers in the light microscope. Pairs of centriolar plaques are invariably so placed on the nuclear envelope that the fiber connecting them does not pass through the crescent of dense material.

THE CENTRIOLAR PLAQUE: (Figs. 41 to 51) The plaques associated with the intranuclear fiber appear to consist of an inner dense component accompanied on its outer cytoplasmic aspect by a less dense bar of the same length but lesser width. Short filaments are sometimes seen crossing the gap between the dense component and its less dense companion. In favorable sections one such





FIGURES 38 and 39 Two sections of the same nucleus. Crescent at upper left. The finger of cytoplasm which indents the lower margin of the nucleus in Fig. 38 is cut across near its tip in Fig. 39. At this point is a dense centriolar plaque from which a bundle of filaments (i.e. a fiber) extends into the nucleus. Compare with the living nuclei of Figs. 7 and 8. × 36,000.



FIGURES 40 and 41 Two sections of the same nucleus differently oriented and not at the same magnification. Asterisks in both figures are at corresponding spots. In Fig. 40, the nucleus is at an early stage of its division and has entered the bud (top). Loosely arranged cords of crescent material at lower left. The right half of the nucleus is indented by an intrusion of cytoplasm which leads to a centriolar plaque (*cp*). The region surrounding the plaque is shown more highly enlarged in another section in Fig. 41. A bundle of filaments (a fiber) extends from the plaque into the nucleus. *cw*, a shred of undigested cell wall; *cyt*, cytoplasm; *m*, mitochondrion; *nu*, nucleus; *vac*, vacuole. Fig. 40, × 28,000; Fig. 41, × 83,000.

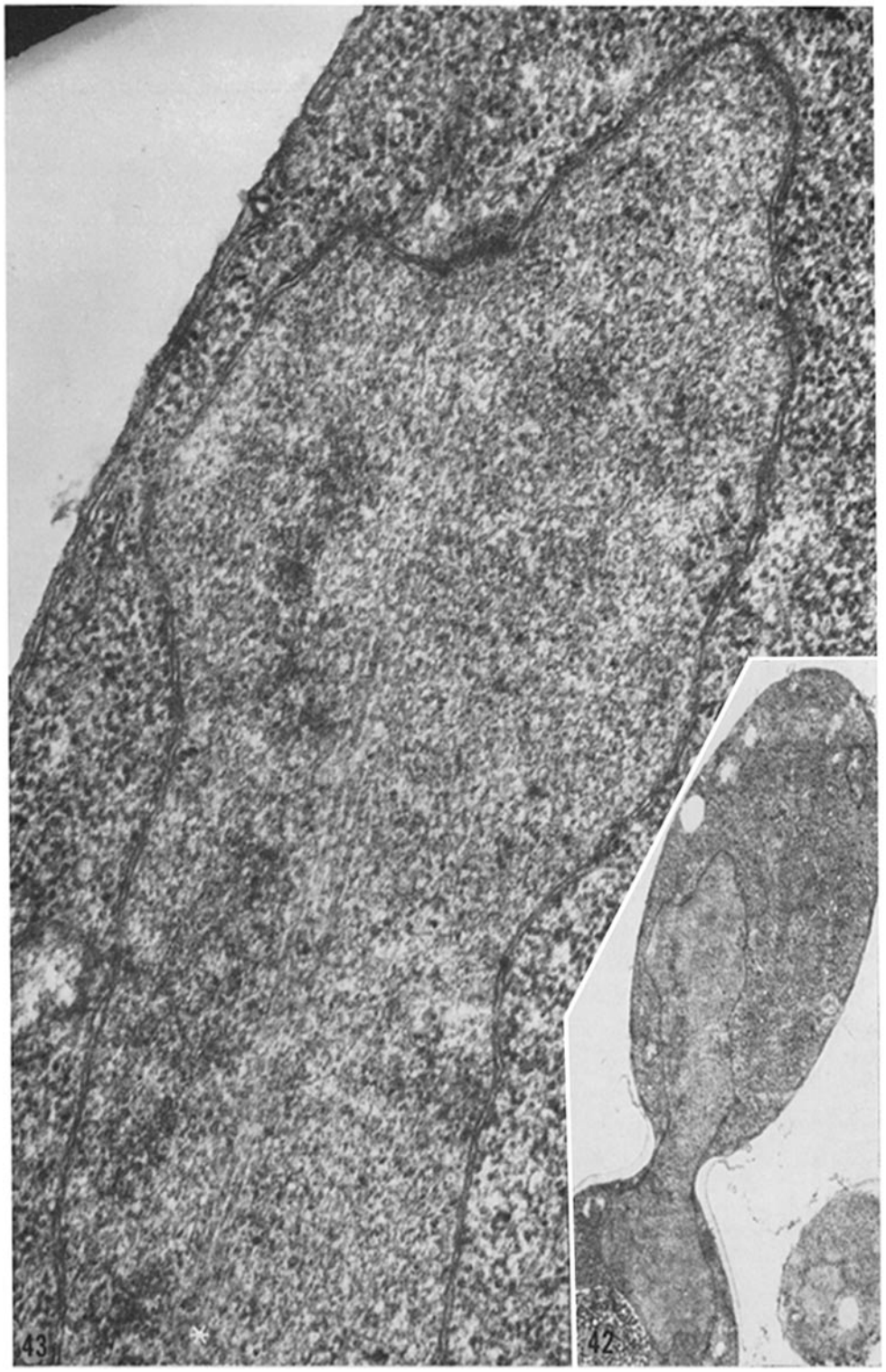


FIGURE 42 Low-power view of nucleus at an advanced stage of division. Bud is at the top. Vacuole of the mother cell is at lower left.

FIGURE 43 Tip of the nucleus shown in Fig. 42. Note centriolar plaque (at top) in a shallow depression of the nuclear envelope. A straight bundle of filaments is discernible in the nucleus along a line connecting the plaque with the asterisk at the bottom of the page. The filaments become more clearly visible if one looks along this line while the page is held flat in front of the eyes. Immediately to the left of the filaments are scattered clumps of crescent material. Compare this illustration with Figs. 26 to 29. × 83,000.

assembly is connected, by a bundle of 150 to 180 A tubules or filaments, with another one on the opposite side of the nucleus (Fig. 37).

Many of the plaques in our material have an amorphous structure, but several have been seen which appear barred at right angles to their long axis. Again, others seem to consist of vesicular elements which perhaps represent coiled up ends of tubules (a suggestion made by Dr. M. Girbardt). We have several times seen indications of a short cone of rather coarse filaments diverging outward from the centriolar plaque into the cytoplasm (Figs. 46–48). We know the plaques only in straight and oblique profiles. Full views of them were not encountered and not enough profiles of any one plaque have been obtained to permit a three-dimensional reconstruction. A profile that appears as two rounded clusters of vesicular elements (Fig. 44) has been seen several times and at present cannot be related to the most frequently seen type of plaque. The possibility that the centriolar plaque has the shape of a much flattened fibrous cylinder is not excluded by our evidence, but at present this seems unlikely.

DISCUSSION

The literature on yeast nuclei is large and full of contradictions. It is therefore encouraging to find good agreement between the few observations that have been made on the nucleus of *living* yeast cells. Some of Henneberg's illustrations (13), e.g. Fig. 13 of his plate 1 and Fig. 15 of his plate 8, could scarcely be bettered, and Townsend's and Lindegren's sketches of structures seen in unfixed viable cells mounted in weak acetic acid (49) anticipate a certain amount of what we have been able to discern in living cells and in fixed cells stained directly with acid fuchsin. That this agreement does not cover the terminology used by these authors to explain their drawings is another matter. In living nuclei of yeast *protoplasts* the dark crescent has been clearly seen and illustrated by Nečas (36).

As long as only the geometrical relations of the parts of the nucleus are considered, there is again a large measure of detailed correspondence between our view of the yeast nucleus, as seen in fixed specimens, and many earlier accounts of the nuclear organization, especially in papers based on preparations stained with iron haematoxylin. Examples of the "dot and crescent" are among drawings of yeast nuclei by Guilliermond, 1903 (8); we recognize the crescent in the "chromatin patch" on one side of the "nucleolus" as seen by Wager and Peniston, 1910 (51) and again in the "companion body" of Nagel, 1946 (35). The latter

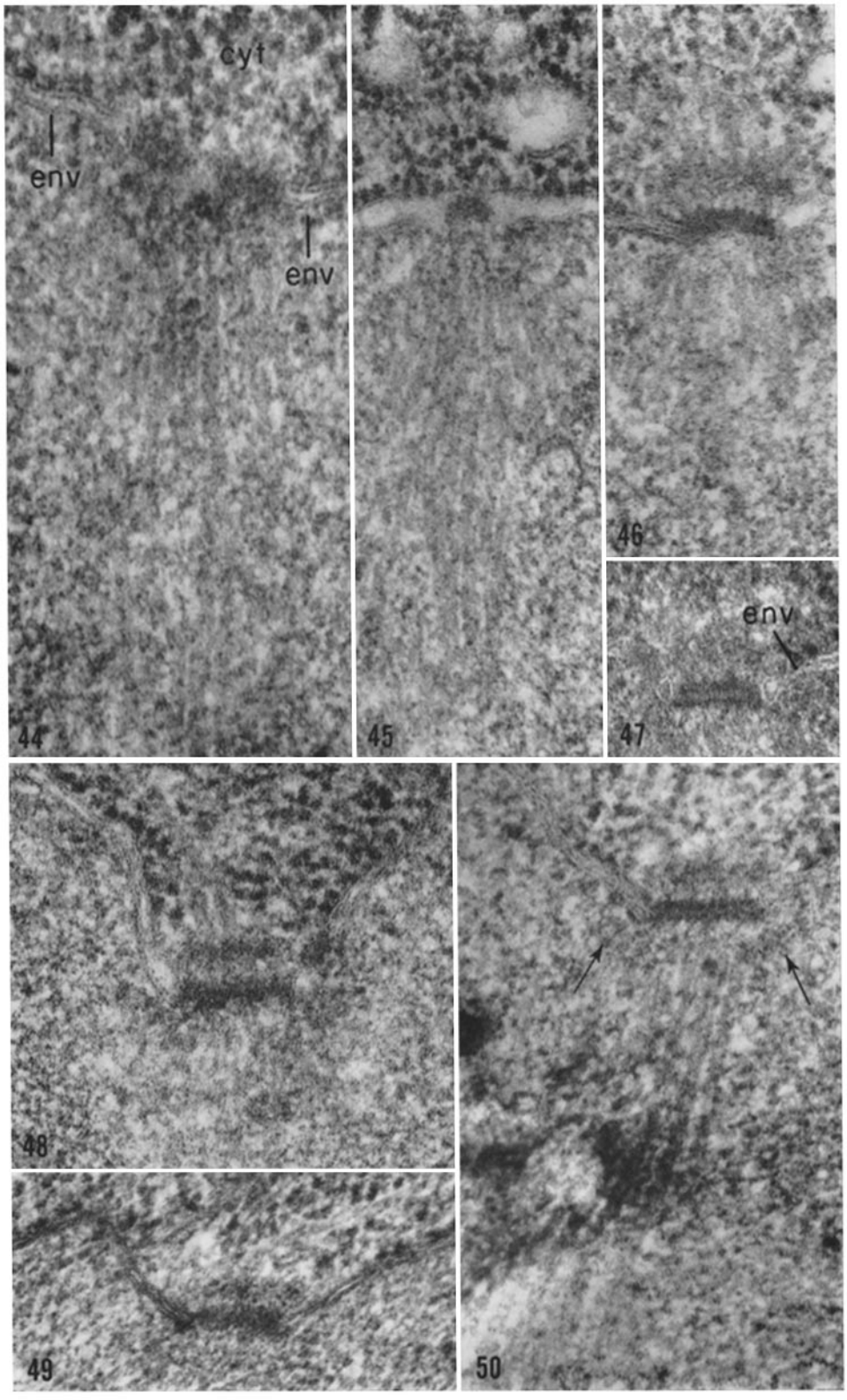
FIGURES 44 to 50 Examples of centriolar plaques. All figures are oriented in the same way with the cytoplasm at the top and the nucleus filling the remaining space. × 118,000.

FIGURE 44 Two clusters of small dense elements with circular profiles set in a wide pore of the nuclear envelope (*env*) are associated with a bundle of filaments. This kind of plaque profile was seen only rarely. *Cyt*, cytoplasm.

FIGURES 45 and 46 Parts of two sections of the same plaque. In Fig. 45 a divergent bundle of filaments extends from the plaque into the nucleus. In Fig. 46 the plaque is much larger and is accompanied on its cytoplasmic aspect by a band of small circular profiles which is perhaps a daughter plaque. A few indistinct filaments seem to be extending from this region into the cytoplasm. The filaments of the intranuclear fiber beneath the plaque are much shorter and do not approach it as closely as those in Fig. 45.

FIGURE 47 A two-layered centriolar plaque filled neatly into a pore of the nuclear envelope (*env*). There are indications that filaments are radiating from it into the cytoplasm.

FIGURES 48 to 50 Typical centriolar plaques set in shallow depressions of the nuclear envelope. Two filaments, wider than the intranuclear ones, extend outward from the presumed daughter plaque in Fig. 48. Fig. 49 is the plaque of the nucleus of Fig. 43. The course of the filaments in Fig. 50 is not straight because of a fold in the section. At some distance from the plaque the contours of the filaments appear wavy. Arrows point to circular profiles presumed to represent cross-sections of filaments.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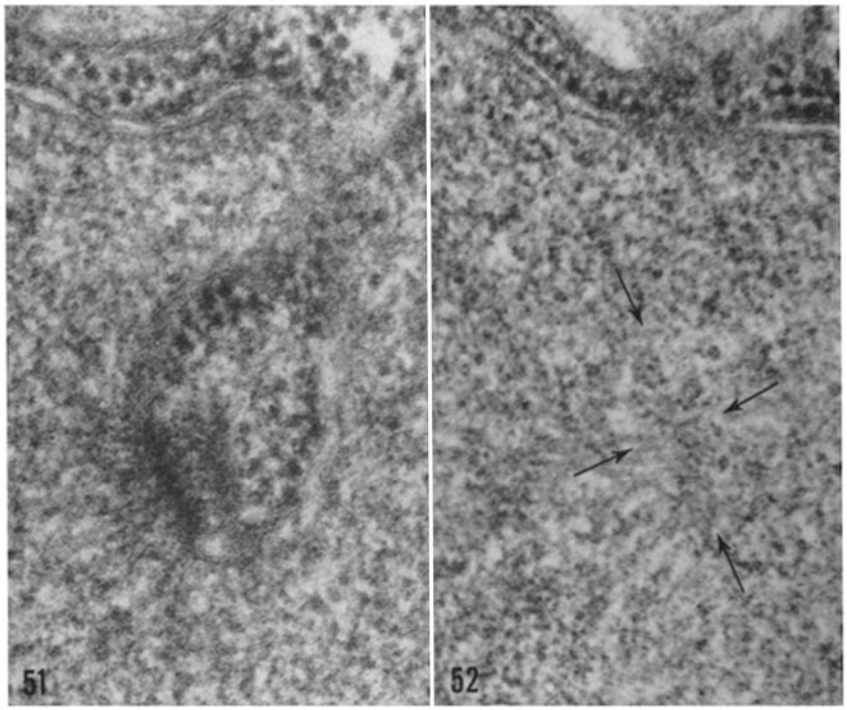

FIGURES 51 and 52 Corresponding regions of adjoining sections of the same nucleus. At the top in both figures can be seen part of the same mitochondrion, ribosomes of the cytoplasm, and the membranes of the nuclear envelope. In Fig. 51 there is a centriolar plaque set into a pore of the nuclear envelope which is deeply indented at this point. The plaque has the usual appearance and resembles that of Fig. 48. In Fig. 52 arrows delimit a region in which cross-sections of filaments can be more or less definitely discerned. × 118,000.

author has shown, what some of her successors, notably Lietz (19) have chosen to disregard, namely that the companion body, the crescent that avidly retains iron haematoxylin, is Feulgen negative and that the Feulgen-positive matter is confined to the rest of the nucleus. Lindegren's stylized series of drawings from iron haematoxylin preparations (20) accurately portrays the changing configurations of the crescent material and of the main body of the nucleus during division. Only the intranuclear fiber is missing from these illustrations. Renaud (40) obviously did see something of that fiber though in most of his drawings it is invisible and lies buried inside the lightly stained chromatinic portion of the nucleus. Renaud mistook the fragmented, haematoxylin-loving but Feulgen-negative crescent for a mass of chromosomes. That the crescent and the Feulgen-positive material are two different simultaneously present parts of the yeast nucleus is well understood by Yoneda (55). The "intranuclear crystal" of Nečas (36) is probably identical with the fiber of the present paper.

There are no serious disagreements among those who have studied the chromatin of the yeast nucleus in Feulgen or acid-Giemsa preparations. Feulgen preparations are to be preferred because their greater transparency directs attention to the grooves in the nuclei which are the site of the intranuclear fiber. It wants to be remembered that neither of these methods stains the whole nucleus and that their results overemphasize the chromatinic portion and lead to neglect of the Feulgen-negative crescent.

The nuclei of *Saccharomyces*, unlike those of higher organisms, are least strongly Feulgen positive at the time of division. They are most strongly Feulgen positive at the time when the DNA content of the cells abruptly doubles (52, 53, 54), that is, in the compact "resting" nuclei of cells with small buds. There is no further increase in DNA content until the emergence of the next generation

of buds. The pale Feulgen reaction of dividing nuclei (Figs. 12 and 14) is thus an expression of the dilution of the DNA of the resting nucleus in the larger volume of the dividing one. The physical state of the chromosomes is not known. Mazia (24) has drawn attention to quantitative data which suggest that one chromosome of *Saccharomyces* (which has many; see reference 12) probably contains considerably less DNA than a single chromosome of the *E. coli* type. A yeast chromosome, to be accomodated in its nucleus, therefore need not be so tightly coiled as the single, but very long, bacterial "genophore." Chromatin patterns of the bacterial kind (which do occur outside bacteria) have in fact not been seen in *Saccharomyces*. We do not know whether the chromosomes are attached to the fiber (the hyperchromatic "manchette" around its middle portion, mentioned above, would be a likely site) and whether the fiber plays a part in the sorting out of duplicated chromosomes. At present it seems improbable that the fiber does so because it is shortest in the nuclei of cells with small buds in which DNA is known to be in a process of duplication and the separation of chromosomes is believed to be taking place (52, 53). The behavior of *Saccharomyces* chromosomes at mitosis thus remains obscure. On the other hand, it is difficult to imagine meiosis being performed without an attachment of chromosomes to spindle fibers. There is, in fact, already strong evidence, from the analysis of meiotic recombinations, that yeast chromosomes have centromeres (12).

It would be reasonable to assume that the elongation of the fiber provides the force for the elongation of the nucleus, but there are reasons for doubting this. As mentioned above, it is not unusual to find short fibers lying crosswise in elongated nuclei which have advanced part of the way into the bud. The fiber appears to attain its greatest length suddenly and rather late in the process of division, and we think it possible that the fiber's main contribution to the division of the nucleus may be its form-giving *rigidity* and that elongation of the nucleus is achieved principally by the expansion (i.e. growth in area) of the nuclear envelope. On this view the filaments of the intranuclear fiber would have something in common with the filaments of the skeleton of the axopods in *Actinophrys sol* which appear to provide rigidity rather than movement and which, interestingly enough, grow out from the nuclear envelope (15). It speaks in support of our opinion that the ends of dividing *Saccharomyces* nuclei are more or less flat (Figs. 9, 12, and 43) whereas in *Schizosaccharomyces versatilis*, where there is no elongation of the nucleus without elongation of the fiber, they are drawn (or pushed out) into sharp points (reference 44, and unpublished observations).

Saccharomyces is not the first yeast in which an intranuclear fiber has been seen. Guilliermond (9) described an early example of it in meiotic nuclei of *Schizosaccharomyces octosporus*, and we have regularly seen the same kind of fiber in mitotic nuclei of another fission yeast, *S. versatilis* (44).

It is interesting that fibers have recently also been demonstrated for the first time in problem nuclei of a different sort, the macronuclei of ciliate protozoa (7, 46). But here, as in *Saccharomyces*, the nature and behavior of the chromosomes remains to be elucidated.

The plaques which are associated with the intranuclear fiber differ considerably from ordinary centrioles. Their shape is not yet known but it is apparently not that of a fibrous cylinder. This is scarcely surprising. Ordinary centrioles have close affinities to and are in some instances directly derived from the basal bodies of flagella, e.g. in *Allomyces* (39), but there are no motile, flagellated forms in the life cycle of *Saccharomyces*. We are ignorant of the details of duplication of the centriolar plaques and the setting up, at telophase, of a new fiber apparatus inside a newly formed daughter nucleus. Our experience makes it seem probable that the two components of the plaque received by each daughter nucleus separate immediately while remaining attached to the nuclear envelope. A short bundle of filaments develops between the components, perhaps from precursors formed during the preceding interphase, and each of them generates a daughter plaque on the side which faces the cytoplasm. On this view the behavior of the plaques would resemble that of the curious centriole of the hypermastigote flagellate *Joenia* as described by Cleveland (4). The fiber stays short and the plaques with which it is associated remain close to each other until the beginning of nuclear division when the fiber begins to grow longer, slowly at first but finally much faster. A polar bead in a nucleus of an acid fuchsin preparation represents, we believe, the earliest member of this series, namely a very short fiber between two centriolar plaques.

Terminology

A centriolar plaque is too small to be recognizable in the light microscope as something separate from the fiber that extends from it into the nucleus. A fiber together with its two plaques constitutes a "fiber apparatus." That is something that can be seen in the light microscope. The stain of choice is acid fuchsin. Only negative images of the fiber apparatus are visible in acid-Giemsa preparations. Because the relationship of the intranuclear fiber to the chromosomes is not known and the nature of its contribution to the division movements of the nucleus is uncertain, we have avoided calling it a mitotic spindle.

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Note Added in Proof: Since this paper was written, we have found in electron micrographs of an ordinary baking yeast (L'Allemand) both longitudinal and well defined cross-sections of the same kind of intranuclear fiber apparatus that is shown in the electron micrographs of the tetraploid yeast No. 1376 in our paper. In both yeasts the fiber apparatus, as the text makes clear, is visible, if not resolved, in the light microscope in preparations stained with acid fuchsin.

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