THE DIMORPHISM PHENOMENON IN YEASTS

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I. INTRODUCTION AND HISTORICAL REVIEW

The pathogenic fungi may be divided into two groups. Those in the first group can invade only the superficial skin and are referred to as "dermatophytes". The yeastlike fungi in the second group can infect not only the skin but also the deeper organs. There are a number of yeastlike pathogenic fungi which occur in a yeast (Y) phase *in vivo* and under special conditions *in vitro*, but usually grow in a mycelial (M) phase *in vitro* upon being incubated at room temperature. This characteristic has been termed "dimorphism". The dermatophytes do not show this dimorphism. Many nonpathogenic fungi show a similar or identical phenomenon, existing in M (or F) phases and Y phases. The term dimorphism will be used here to refer to the potentiality of yeasts and yeastlike organisms to give rise to elongated, filamentous, or mycelial cells under certain conditions and will apply to pathogens and nonpathogens alike. The purpose of this review is to discuss the conditions affecting dimorphism in yeasts and yeastlike pathogenic fungi and the significance of this phenomenon in the pathogenesis by certain yeastlike fungi.

The term "yeast" will be used here in accord with the definition given by Henrici (Skinner *et al.*, 149), that "yeasts are true fungi whose usual and dominant growth form is unicellular".

The line of demarcation between fungi in which the budding or yeastlike form is a usual mode of development and those which exist predominantly in the mycelial (M) phase is by no means sharp. Some fungi which develop a true mycelium, such as Exoascaceae (Taphrinaceae) and Ustilaginales, may have bud-

ding stages in their life histories, and budding may appear under abnormal conditions in many forms (de Bary, 5).

In some of the Hyphomycetes (Moniliales), as Anderson (2) points out, a condition of conidial formation exists which simulates true budding. The end of the elongated sporophore (conidiophore) develops a sinus which squeezes off the spore by gradually narrowing, and the conidia, on germination, send out true germ tubes by the further growth of which a mycelium is formed.

Geotrichum species can be shown to go through a number of phases of a M → Y transition. As Henrici's (149, p. 298) description is most concise, the reader is referred to the original article.

In the budding process of yeasts a protuberance forms on some part of the parent cell, and at the same time that the protoplasm connecting the parent and daughter cells is constricted, a wall is formed, and the new bud may become disassociated from the cell from which it was formed, to exist as an individual entity. In the process of septation, however, the parent cell elongates and a cross wall is laid down without the occurrence of a constriction between the parent and daughter cells. Details and some of the controversial aspects of budding in yeasts have been discussed by Wager (168), Guilliermond (57, 59, 60), Swellengrebel (157), Wager and Peniston (169), Knaysi (75), Nagel (101), Lindegren (85, 86, p. 6–7), Richards (129), and Beams *et al.* (11). If cell elongation and cell constriction may be viewed as two processes whose rates of occurrence may be independent, then in the process of bud formation constriction may occur concomitantly with elongation (and growth), but in hyphal formation constriction is lacking and the laying down of a cell wall isolates the daughter from the parent cell. If this relatively crude assessment of one phase of the cell division mechanism in yeasts has some validity, then it should be possible to demonstrate a form of growth intermediate between true mycelial formation and budding. This, in fact, can readily be demonstrated with certain fungi exhibiting dimorphism, *e.g.*, *Histoplasma capsulatum* and *Candida albicans*. Such an intermediate form of hyphal growth is commonly referred to as a pseudomycelium.

It has been known for some time that numerous species of yeasts and yeastlike fungi will, under certain conditions, grow in the form of filaments or mycelia, whereas under other conditions they will grow in yeast phases. Even before the researches of Hansen, as Levan (82) points out, brewers had observed so-called involution forms in aging yeast cultures. Five years before Pasteur completed his work for the doctorate, a yeastlike fungus, *Sporotrichum* sp. (*Candida albicans*), had been described by Gruby (56) as the etiological agent of what is now referred to as thrush.

The work of Reess (127) has recorded the formation of filament (F) forms[1] (faden) by *Saccharomyces cerevisiae* and other yeasts under varying conditions

[1] In general, the term filament or F form has been used and will be used here to denote a general lengthening of cells but not to a degree or of a nature which could properly be termed pseudomycelial or mycelial. The term is more properly used, however, with reference to elongation of bacterial cells.

of culture. Hansen's (64) detailed investigations on the physiology and morphology of yeasts demonstrated the presence of M and F forms in old cultures and in those incubated at temperatures below the optimum for growth.

It is common to find occasional short strings of yeast phase cells in young actively growing yeast cultures, but the designation of "filamentous" should not be made unless such growth becomes the usual morphology and is accompanied by cell elongation. There are thus two basic morphological types to be considered, the ellipsoidal or ovoid yeastlike cell and the elongated cell (elongated beyond that which is the usual morphology).

However, depending upon whether the daughter cells become disassociated[2] from the parent cell or not, the following morphological entities should be possible:

(a) Normal appearing budding cells.

(b) Budding cells in which there has been interference with the cell division mechanism, giving rise to clusters of cells.

(c) Single occurring cells which have elongated beyond their usual morphology.

(d) Filaments of cells in which cell division has been impaired while elongation has occurred concomitantly.

Hansen's work revealed a number of characteristics of the dimorphism phenomenon:

(a) The potentiality of Y → F formation varied widely with different yeast strains.

(b) The Y → F effect was not complete in that some Y forms were invariably found in cultures no matter how marked the Y → F transition.

(c) Though certain cultures showed little impairment of the cell-division mechanism when they were incubated at a reduced temperature, a general lengthening of the cells was evident.

Reess (127) described in some detail the morphology of yeasts in various stages of the brewing operation and under various conditions of incubation. On substrates which usually offer conditions favorable for sporulation of *Saccharomyces apiculatus* he found long forms which never resembled mold filaments (niemals Pilzfaden hervorgehen), probably the first observation of what we now call a pseudomycelium. Reess' description of *Saccharomyces cerevisiae* Meyen also brings out a concept on growth rate to explain the occurrence of filaments, a concept which still has many adherents today:

"Sprossungzellen meist rundlich oder oval, . . . bei langsamer Vegetation alsbald isoliert, bei rasher Vegetation verzweigte kurze Zellreihen zusammensetzend."

The significant point here is that the strings of cells were interpreted as the

[2] In this review "cell division" will refer to the condition in which the daughter cell or bud becomes disassociated from the parent cell; cell multiplication will merely indicate the formation of a new cell and will be used, therefore, synonymously with reproduction. The term "growth" usually encompasses cell division and cell multiplication, but in the work discussed here a distinction is made among these three phenomena, since they can be separately assessed cytologically; *e.g.*, the filamentous forms undergo growth and cell multiplication but are damaged in their cell division mechanism.

result of an increased rate of growth. Presumably the rate of cell division could not keep pace with an increased rate of cell multiplication, thus resulting in filaments of cells. It is also conceivable that the impairment of the cell division mechanism without a change in the rate of cell multiplication would also give rise to the same cytological entity of filament formation.

Aderhold (1) outlined five stages of development in a freshly implanted yeast culture. During the latter stages he noted that the yeast cells formed clusters of budding cells which settled to the bottom of the vessel. Many of the cells also occurred in the form of filaments. Such clusters and filamentous forms were also observed by Lepeschkin (80) in cultures of *Schizosaccharomyces pombe* and *S. mellacei*.

Anderson's (2) study of yeastlike fungi isolated from the human intestinal tract also confirmed the Y $\rightarrow$ F enhancement present in old cultures of numerous species and genera of yeasts. In these the cells had elongated while remaining attached to each other, forming hyphal threads. He also reported the presence in old cultures of giant cells that were frequently ten times the diameter of the normal cells found in young cultures. Anderson noted the common occurrence in older cultures of buds surrounding a central enlarged globular cell. Anderson also noted that in old cultures grown on an agar medium the F forms or pseudomycelium developed predominantly beneath the surface of the medium, and that cells taken from the surface of old colonies were essentially yeastlike in morphology although they may have undergone other changes. He recognized that the formation of a series of elongated cells does not necessarily imply that the yeast has given up the budding habit in favor of hyphal formation and septation. In the majority of cases such series of cells are formed by the apical budding of each cell in turn and the subsequent elongation of the members of this series of cells.

The morphological difference between yeasts which never produce new cells by septation and those which may show septation under proper conditions was recognized by Will (175, 176, 177), and Geiger (51). Hansen (65) was most vigorous in his contention that "it is not . . . the form and size of the cells *per se*, in which the distinguishing characters lie, but in the form and size contingent upon particular conditions of cultivation." It was also realized that morphological characters alone were not sufficient for differentiating species of yeasts. Thus, biochemical and cultural schemes of classification were recommended by Will (175, 176, 177, 178, 179, 180) and Geiger (51).

Anderson (2) classified budding fungi as follows:

(a) Those in which budding is a secondary phase, usually occurring under unusual or abnormal conditions, or during only a short period of the life cycle of the organism.

(b) Those in which budding is the primary phase, occurring under all the usual conditions of growth, but always with mycelial formation, more or less rudimentary, as a secondary phase.

(c) Those in which budding is the only known method of vegetative multiplication.

Intermediate morphologic types may be found as supplementary to the three main groups originally postulated by Anderson (2). The occurrence of chains of round or oval yeast cells would necessitate a distinct group between his groups (a) and (b). Actually, the transition from Y $\rightleftharpoons$ M growth may exhibit a gamut of gradually changing morphological types as difficult to segregate as shades of gray between white and black.

The possibility that yeasts have descended from more complex fungi through loss of mycelial formation has much in its favor (Guilliermond, 58; Stelling-Dekker, 151). See also the review of Mrak and Phaff (96). It might be possible to utilize the relative ease of F or M formation by yeasts under a certain set of conditions as one criterion for their phylogenetic position.

II. THE ENHANCEMENT OF FILAMENT FORMATION IN YEASTS

A. Effect of temperature

Temperature appears to be one of the most important environmental factors affecting dimorphism. In general, optimum temperatures favor growth in the Y phase and temperatures somewhat below the optimum favor growth in the M phase. The effect of a lowered temperature of incubation in enhancing Y $\rightarrow$ F formation was perhaps first revealed by the studies of Reess (127). A culture of *S. cerevisiae* incubated at 16–18 C showed multiple buds and longer cells than one grown at a higher temperature (note figure 1 from plate I). This same phenomenon is also evident in figures 5, 7, 8, plate I.

Hansen's (64) work revealed this effect of temperature most vividly. The usual morphology of cells of *S. cerevisiae* I[3] and *S. pastorianus* I is recorded in figures 1 and 2 of plate II. The cells are, in general, elliptoid in appearance; occasionally there are elongated cells occurring singly or in short chains. This lengthening is more or less pronounced depending on the strain. Hansen's figures clearly show that a consistent effect of a lowered temperature of incubation with these yeasts was an interference with what we refer to as the cell-division mechanism and a lengthening of the cells. This effect is less pronounced with *S. cerevisiae* I (figure 3, plate II), but quite marked with *S. pastorianus* I (figure 4, plate II). This lengthening of yeast cells at lowered temperatures of incubation was also evident in numerous other cultures studied by Hansen. It is also evident from Hansen's figures that regardless of the degree of Y $\rightarrow$ F formation, normal appearing cells were always present in the culture.

Although comparative temperature studies were not made by Aderhold (1), it may readily be inferred from his results that there was a tendency to filament formation at lowered temperatures of incubation. This was consistently true for five different yeast strains studied. In all cases the morphology of the cells grown at 25–27 C was filamentous; frequently they were longer than cells examined from bottom (presumably warmer) fermented cultures. This is evident in plate III (compare figures 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10).

Zikes' (186, 187) studies on the effect of temperature on yeast morphology

[3] The name and number designations for the strains are those given by Hansen (64).

indicated that lower temperatures gave rise to elongated cells in different yeast genera and that also at lower temperatures the cells tended to hang together in colonies. The normal appearance of the yeast cells recurred after incubation at an optimum temperature. Zikes termed these aberrant forms "fluchtige Varietaten", or unstable variants.

Richards (128) observed that cultures of *S. cerevisiae* maintained within the narrow temperature limits of 29.5–30.5 C exhibited the maximum deviation from normal cell shape (enhancement of Y → M).

Blastomyces dermatitidis from lesions appears as budding yeast cells having a thick refractile wall; no mycelium is found. On blood agar or beef infusion agar incubated at 37 C, yeastlike cells similar to those found *in vivo* may be seen (Stober, 153; Benham, 12; Baker, 4). However, many of these yeast cells may show short mycelial fragments, suggesting that there is a tendency to mycelial growth. Yeastlike growth occurs on blood agar media at 37 C even if the inoculum consists of mycelial cells (26). On Sabouraud's glucose agar at room temperature the mycelial phase predominates.

By studying yeast phase formation of *B. dermatitidis* in a chemically defined semifluid medium, Salvin (142) showed that temperature was the most important environmental factor controlling dimorphism in this organism. No amino acid, carbohydrate, nor growth-substance tested was found to be an essential accessory factor for the growth of the yeast phase at 37 C. This evidence was in accord with the findings of previous workers (36, 62, 84, 93, 108, 111, 130). Nickerson and Edwards (111) proposed the term "thermal dimorphism" to characterize this phenomenon.

PLATE I

FIG. 1. Left—*Saccharomyces cerevisiae*, a bottom yeast from a secondary fermentation. 400 ×.

Right—*S. cerevisiae*, a bottom yeast after 30 hr incubation in culture at 16–18 C. 400 ×.

FIG. 2. Left—*S. cerevisiae* from top fermenting ale yeast after 4 days on cooked carrot slices. 400 ×.

Right—From the same culture, showing characteristic filaments. 400 ×.

FIG. 3. *S. cerevisiae* (showing spore formation) after 4–5 days on a carrot slice. 750 ×.

FIG. 4. From L to R—A germinating spore triad; germinating spore tetrad; spore germination of triad: (a) 24, (b) 54, (c) 56, (d) 74 hr after inoculation into wort. All of *S. cerevisiae*. 750 ×.

FIG. 5. R—Organisms found in alcoholic fermentation: (a) *S. cerevisiae*; (b) milk souring organisms. 400 ×.

L—*S. cerevisiae* taken from this fermentation and cultured in grape-sugar at 15 C. 400 ×.

FIG. 6. R—(a) *S. cerevisiae* sporulating; (b) *S. ellipsoideus* sporulating. 400 ×.

L—Same culture of *S. cerevisiae* cultivated in beer ferment. 400 ×.

FIG. 7. From L to R—*S. ellipsoideus* from early wine fermentation (600 ×); same from bottom fermentation of wine (300 ×); same from top fermentation of wine (300 ×).

FIG. 8. *S. ellipsoideus* (same strain as in figure 7) grown in grape juice at 15–17 C. 400 ×.

FIG. 9. R—Vegetative form of *S. apiculatus* at the beginning of a top fermentation of grape juice. 600 ×.

L—Same at end of fermentation. 600 ×.

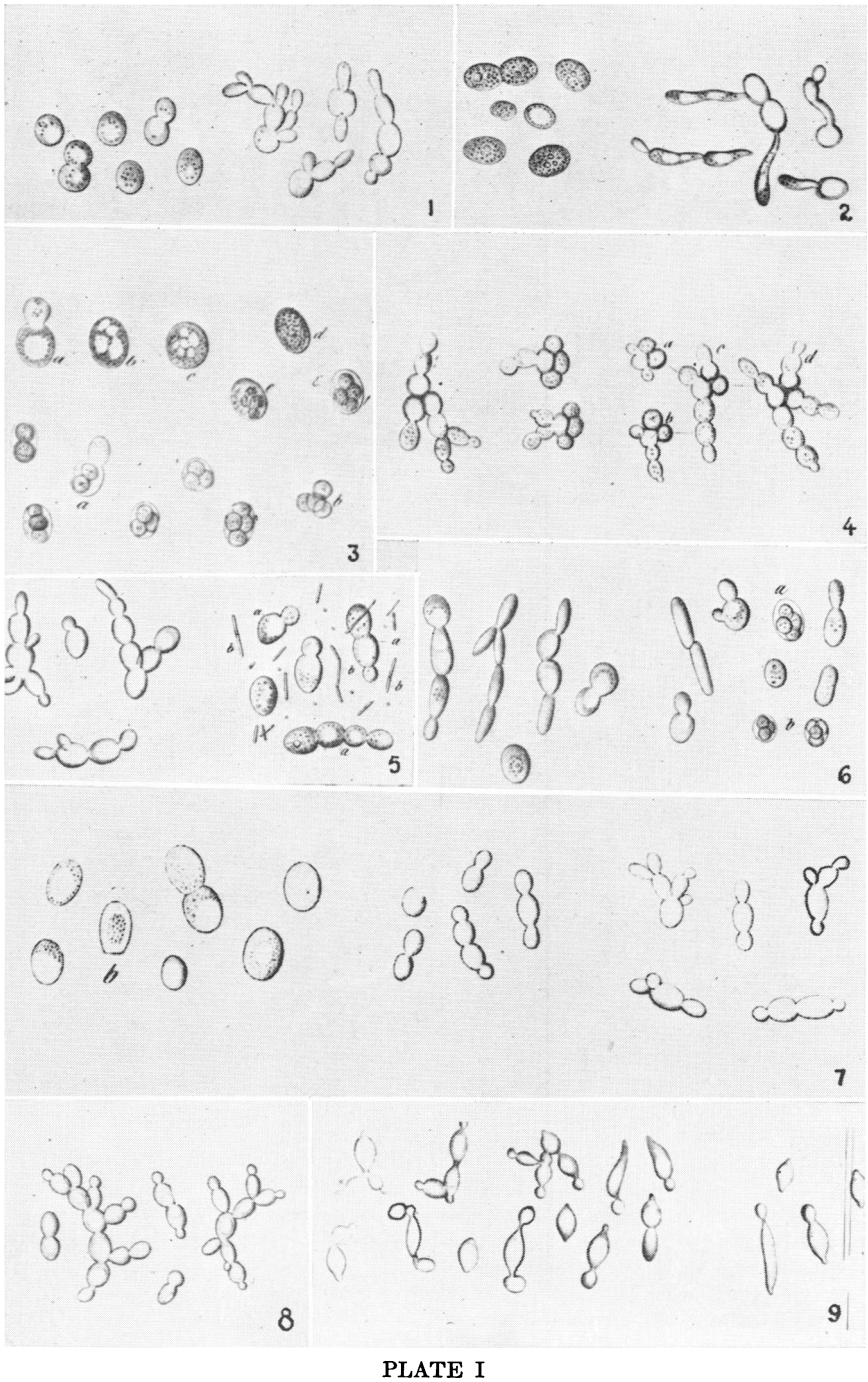

PLATE I

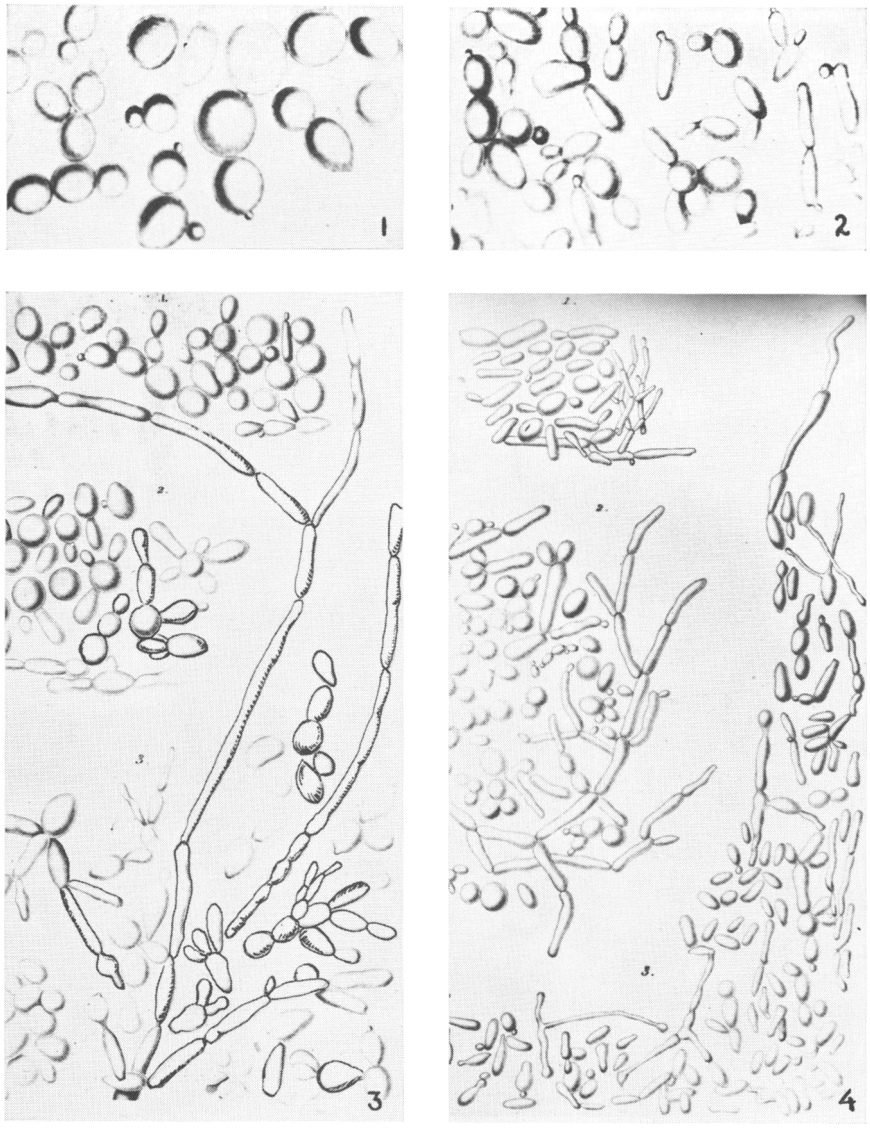

PLATE II

FIG. 1. *Saccharomyces cerevisiae* I.
FIG. 2. *Saccharomyces pastorianus* I.
FIG. 3. *S. cerevisiae* I from film of beer culture; from top to bottom: incubated at 20–30 C, incubated at 6–15 C, from film of old culture.
FIG. 4. *S. pastorianus* I from film of beer culture; from top to bottom: incubated at 20–28 C, incubated at 3–15 C, from film of old culture.

There is some evidence that the optimum temperature of growth of a microorganism is identical with the temperature at which deviation from linearity, in an Arrhenius plot of its metabolic activity, becomes evident. For thermophilic

bacteria, this is 45–49 C (49); for *B. dermatitidis*, 30 C (111). Nickerson and Edwards have suggested that the departure from linearity above 30 C for the mycelial form of *B. dermatitidis* may result from a change in reversible processes which govern Y $\rightleftharpoons$ M conversion.

Blastomyces brasiliensis occurs in infected tissues as multiple budding yeastlike cells. The yeast phase grows on blood agar, beef infusion glucose agar, or Sabouraud's glucose agar, if incubated at 37 C. At room temperature incubation the organism grows in the mycelial phase, forming round to pyriform conidia (Moore, 95; Conant and Howell, 28). This organism, as *B. dermatitidis*, also exhibits thermal dimorphism (111, 142).

B. Effect of nutritional factors

For a number of the yeastlike fungi, factors other than temperature, usually of a nutritional nature, are necessary for the maintenance of the yeast phase. These requirements may be supplemented by or be independent of the temperature of incubation.

Coccidioides immitis appears in infected tissues as a nonbudding, spherical, thick-walled cell which contains numerous endospores. These large thick-walled cells are called "spherules". On glucose agar the organism develops a cottony aerial mycelium which is composed of branching septate hyphae (Dickson, 38; Baker, Mrak, and Smith, 3). Lack (78) was able to reproduce the parasitic cycle of this organism *in vitro* under conditions of partial anaerobiosis and in the presence of partially coagulated egg albumen.

Histoplasma capsulatum occurs in infected tissues as yeastlike cells resembling Leishman-Donovan bodies, but on blood agar and on Sabouraud's glucose agar at room temperature only the mycelial phase of the fungus develops (27, 35). DeMonbreun (35) believed that the mycelial form was fixed and that conversion to the yeast phase was impossible by cultural methods but could be accomplished by animal passage. Cifferi and Redaelli (25) brought about the M $\rightarrow$ Y transformation *in vitro* by growing the cells in blood agar at 37 C.

A detailed study was made by Salvin (140) to ascertain the conditions necessary for yeast phase growth of *Histoplasma capsulatum*. Using the YP medium composed of proteose peptone, neopeptone, tryptone, glucose, sodium chloride, disodium phosphate, and distilled water, he found that the best growth occurred at pH values between 6.3 and 8.1 and at incubation temperatures of 37 C or higher. At 43 C the yeastlike phase grew less abundantly than at 37 C, but at neither temperature were hyphae found. At 37 C and pH 7.3 no growth occurred unless a small percentage of agar, silica gel, oil, or some other such substance that increased the viscosity of the medium was added. The reason for this was not clear.

Salvin also grew other yeastlike pathogens on the YP medium. Five strains of *B. dermatitidis* inoculated onto the YP medium in the Y phase developed primarily as mycelial growth. Six strains of *Coccidioides immitis*, consisting of a suspension of spherules from infected mouse testis, showed no development of the Y phase. Of six strains of *Sporotrichum schenckii*, two developed entirely in

the Y phase, the others showing both Y and M growth. Incubation was at 37 C with all strains. The yeastlike phase does not always occur at 37 C but depends upon the presence of certain other factors (Salvin, 141). Salvin showed that biotin and a reduced sulfur group such as a sulfhydryl group must be included in the medium. The requirement for a reduced sulfur group was best fulfilled when the S occurred in an organic molecule of small size. The tripeptides, glutathione and diglycylcystine, permitted some mycelial formation among the yeast cells. The position of the S in the molecule seemed to have little significance since cysteine and methionine were found to have equal activity. Salvin converted the yeastlike phase to the mycelial phase at 37 C by changing the amino acid constitution of the medium, thus demonstrating that dimorphism in *H. capsulatum* was not solely governed by temperature.

Nickerson and Van Rij (112) shifted the Y $\rightleftharpoons$ M equilibrium toward the Y phase, using organisms which very readily formed a mycelium. The characteristic effect of cysteine on *Candida albicans* was to produce short hyphal cells with very close spacing of blastospore clusters (figure 3 in plate VI). A 10^{-2} molar concentration of cysteine reduced mycelial formation in cultures of *Candida albicans*, *C. tropicalis*, *Trichosporon capitatum*, and *Hansenula anomala*. The authors believed that this phenomenon could not be explained solely by redox potential differences since exposing these cultures to a 10^{-2} molar concentration of ascorbic acid did not result in an inhibition of the Y $\rightarrow$ M transformation. It also appeared that some specificity was required of the —SH donor since sodium thioglycolate was not as effective as glutathione, which in turn was less effective than cysteine.

Nickerson (109) demonstrated that the maintenance of *C. albicans* in the yeast phase on a medium composed of glucose, $(NH_4)_2SO_4$, inorganic salts, and biotin correlated with the presence of the reducing sugar glucose. When he replaced the glucose with commercial starch washed relatively free of reducing sugar, an abundant filamentous growth with chlamydospore formation resulted. Nickerson's explanation was that the metabolism of glucose establishes an intracellular redox potential which maintains a concentration (possibly critical) of —SH

PLATE III

FIG. 1. Cells of strain Johannesberg I from the sediment of a young must culture. 800 ×.

FIG. 2. Forms in film from culture of strain Johannesberg I incubated at 25–27 C. 500 ×.

FIG. 3. Bottom yeast forms of yeast Johannesberg II. 800 ×.

FIG. 4. Film forming cells from same culture, at 25–27 C. 500 ×.

FIG. 5. Bottom yeast forms of the yeast Piesport. 800 ×.

FIG. 6. Film forming cells of same yeast, 25–27 C incubation. 500 ×.

FIG. 7. Bottom yeast forms of the yeast "Crimea" I. 800 ×.

FIG. 8. Film forming cells of same yeast, 25–27 C incubation. 500 ×.

FIG. 9. Walporzheim yeast, bottom fermentation. 800 ×.

FIG. 10. Cells of Walporzheim culture taken from film after 6 days' incubation at 25–27 C.

FIG. 11. Filaments of cells from an old film of same culture. 800 ×.

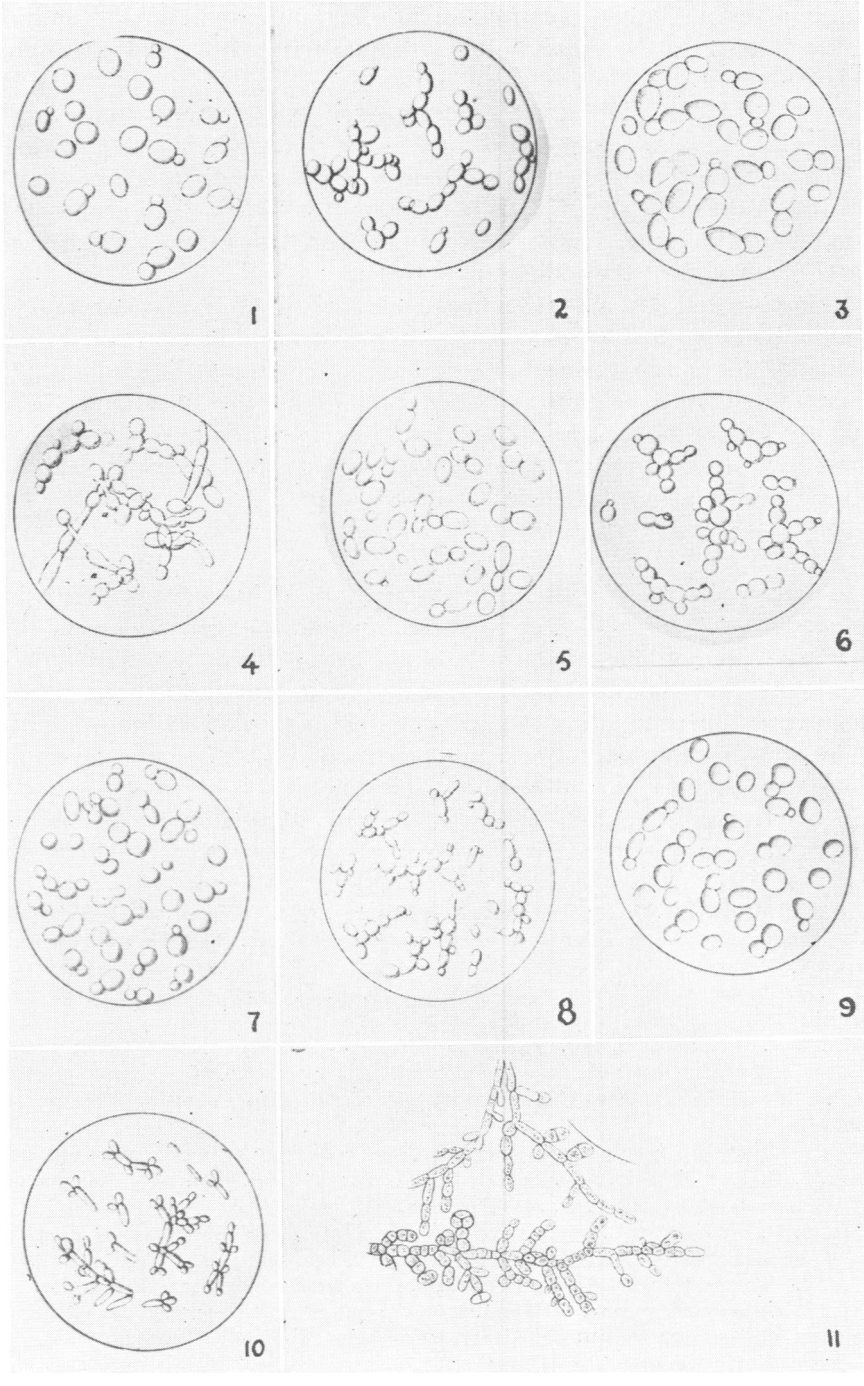

PLATE III

groups in the cell. To support this explanation he showed that the addition of 10^{-3} M cysteine to the polysaccharide medium resulted in yeast phase growth exclusively.

By adding 10^{-2} M cysteine to the glucose-glycine-yeast extract culture medium, Nickerson (110) also accomplished the M → Y conversion in a mutant strain of *C. albicans* 806, which grows in the filamentous phase under conditions in which only the Y phase is found with normal strains. Under these conditions the endogenous respiration was increased as compared to controls which grew in the M phase without the addition of cysteine.

Wickerham and Rettger's (172) taxonomic study of *Candida albicans* (*Monilia albicans*) disclosed that an abundance of oxygen resulted in a decrease in hyphal production for cultures grown on corn meal agar, whereas blastospore growth was enhanced. In some of the *Candida* strains incubated at 30 and 37 C the tendency for the blastospore to aggregate in clusters disappeared; the blastospores grew along all points of the hyphal fragments and appeared to be larger than the normal appearing blastospores.

C. Effect of age

Another factor which appears to be important in Y → F transformation is the age of the culture. When a yeast colony grows on agar, after some time a pseudomycelium of long cells penetrates the agar (Pisova, 116). Lindegren and Hamilton (87) grew yeast colonies on malt-extract agar and found a growth of pseudomycelium penetrating the agar, this being thickest at the edges of the colony, apparently where oxygen was most abundant. The outer layer of the colony was found to be autolyzed, and this autolyzed layer contained the only asci found, suggesting that autolysis supplies essential nutrients on which

PLATE IV

FIG. 1. Culture 141.201 (unclassified yeast culture). (a) Elongated cells with groups of buds from beneath the medium in an old glucose agar culture. (b) Cells from the surface of the medium.

FIG. 2. *Parasaccharomyces candida* comb. nov. Cells from young beerwort culture (Culture 229).

FIG. 3. *P. candida* comb. nov. (a) Elongated cells developed in old agar cultures beneath the surface of the medium; the attached globular cells have large refractive globules filling most of the cell interior. (b) Cells from surface of old culture showing large refractive globules.

FIG. 4. *Cryptococcus aggregatus* sp. nov. Budding cells from young beerwort culture.

FIG. 5. *C. aggregatus* sp. nov. Cells from old agar cultures, showing peculiar aggregation of buds about the enlarged, globular cells.

FIG. 6. *P. candida* comb. nov. (a) Portion of septate mycelium from beneath the surface of the medium. (b) Cells from the surface of old agar cultures.

FIG. 7. *Parasaccharomyces ashfordi* sp. nov. Cells from young beerwort culture.

FIG. 8. *P. ashfordi* sp. nov. (a) Moniliform clusters of cells developed beneath the surface of an old agar culture. (b) Cells from the surface of the same culture.

FIG. 9. Young cells of culture 138.102. (unclassified yeast culture).

FIG. 10. Old cells of culture 138.102.

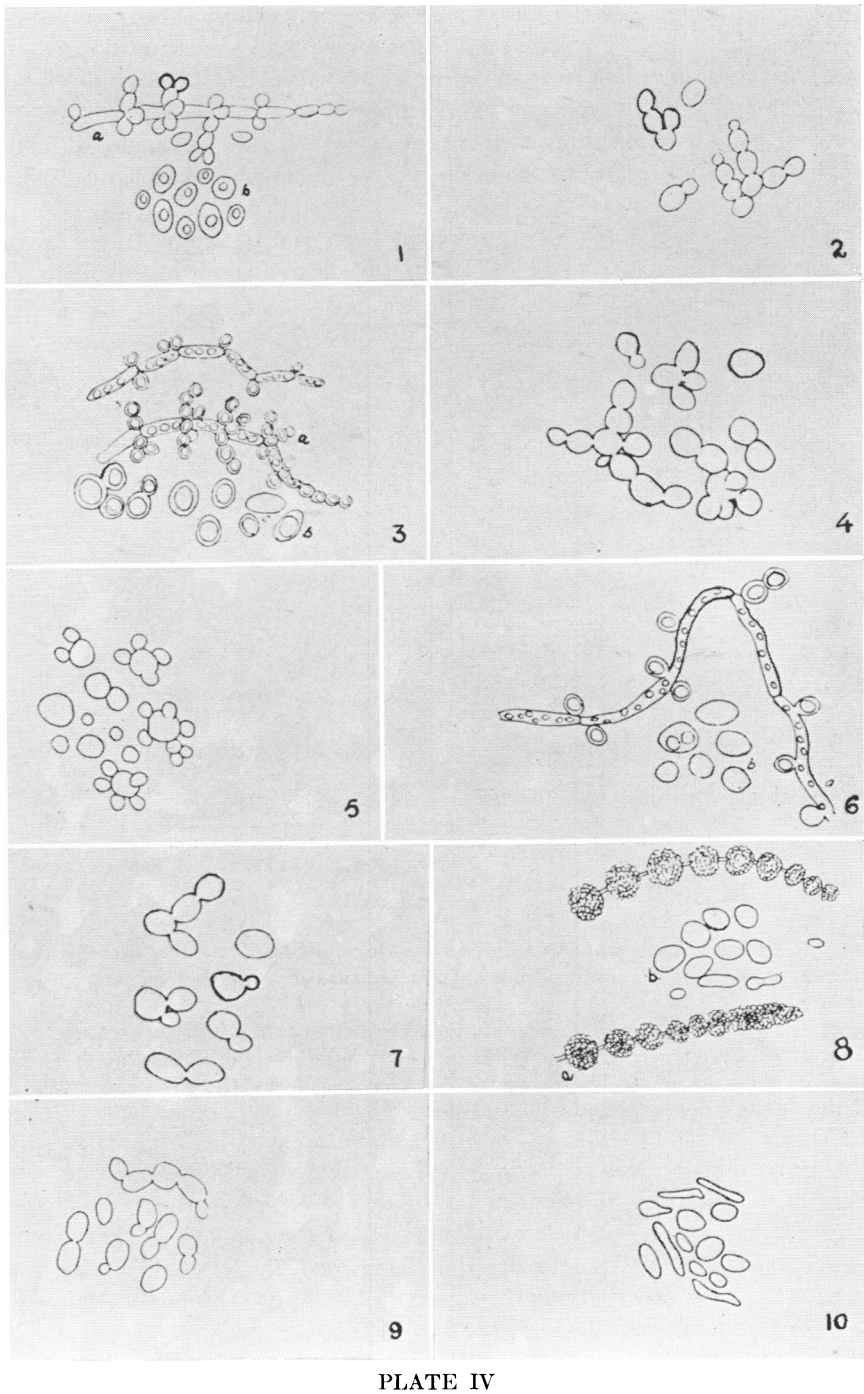

PLATE IV

sporulation depends. The presence of a pseudomycelium at or near the same site where the autolysis occurs might also suggest a correlation between these two. This is essentially the same observation which had been made by Reess (127) (See *Introduction* and note figures 2 and 3 in plate I).

It is a general observation that variation in cell size will increase with age and that the usual trend in such cases is towards larger and longer cells (Lindegren, 86).

The Y → F effect in aging cultures has been clearly demonstrated by many workers (note figure 9 in plate I). All of Hansen's (64) cultures incubated over

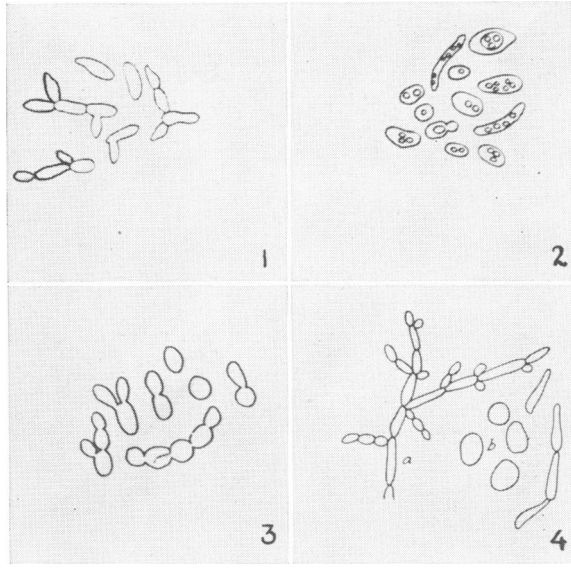

PLATE V

FIG. 1. *Cryptococcus verrucosus* sp. nov. Budding cells from a young beerwort culture.

FIG. 2. *C. verrucosus* sp. nov. From old agar culture showing the pronounced granular condition.

FIG. 3. *Parasaccharomyces thomasi* sp. nov. Cells from young beerwort culture.

FIG. 4. *P. thomasi* sp. nov. (a) Elongated cells forming a false mycelium beneath the surface of the agar slant. The elongated cells are formed by the elongation of terminal buds. (b) Cells from surface of same culture.

extended periods of time show markedly elongated cells and present a vivid picture of this dimorphism phenomenon (plate II). Often the yeast cells resemble the cf- forms of Levan which will be discussed later in this review (compare figure 4, plate II with Levan's drawings in plate IX). Aderhold's (1) drawings also support these observations (figure 11, plate III) as do those of Anderson (2) (plate IV) and Stelling-Dekker (151). Stelling-Dekker also demonstrated that with the same temperature and time of incubation, aging would affect the cell morphology differently in different media. Thus, *Saccharomyces fragilis* Jorgensen grown in wort at 25 C for 3 days appears as oval budding cells, but under similar conditions on wort agar the cells have elongated and formed into filaments.

What changes in aging cultures explain the tendency to F formation have not yet been clearly defined. Lowered pH and depletion of available carbon compounds are doubtless among the more important ones. Pisova's (116) observations on the formation of a pseudomycelium by yeast cultures indicated that Y → F changes were especially pronounced at high sugar concentrations in the medium.[4] A *Candida*-like organism isolated by Berger *et al.* (15) from a case simulating chromoblastomycosis grew in the yeast phase at pH 7 but in a pseudomycelial phase with branching filaments at lower pH values. Scherr (145) also

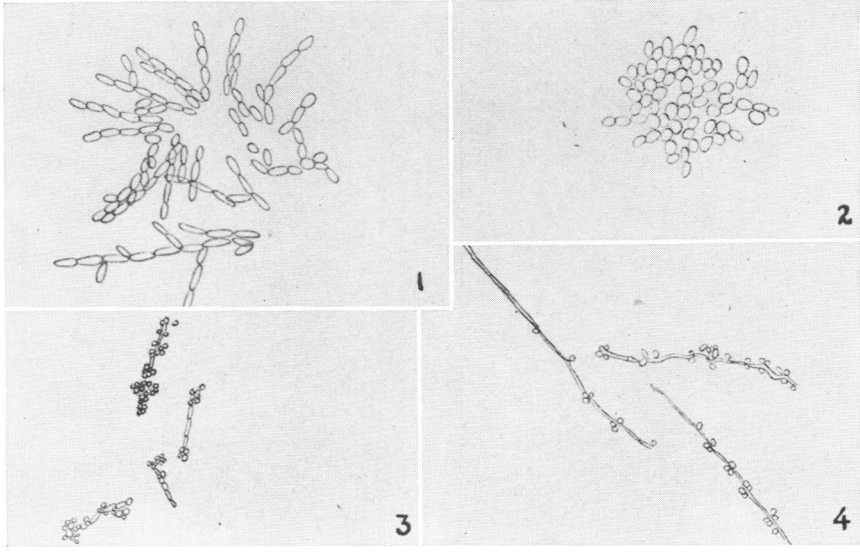

PLATE VI

FIG. 1. *Saccharomyces cerevisiae*, camera lucida drawing of cells from slide agar culture treated with penicillin. 440 ×.

FIG. 2. Same, from agar culture with penicillin plus M/100 cysteine. 440 ×.

FIG. 3. *Candida albicans*, camera lucida drawing of cells from agar slide cultures with M/100 cysteine; note marked shortening of cells and close spacing of blastospore clusters. 300 ×.

FIG. 4. Same, from agar slide cultures with M/1,000 cobaltous acetate; cells markedly elongated, and blastospores widely separated. 300 ×.

reported an increased Y → F effect at lower pH values when *S. cerevisiae* was grown in the presence of tryptophan (figure 4, plate VIII).

It might appear that certain factors conducive to sporulation also enhance the Y → F effect. After 4–5 days on carrot slices, cells of *S. cerevisiae* begin to sporulate, but many cells can also be found with typical filament formation that is characteristic of the dimorphism phenomenon (figure 2 and 3, plate I). That filament formation also appears to occur in the early stages of spore germination is shown quite clearly by Reess' (127) figures (figure 4, plate I).

[4] Nickerson's (109) findings with *C. albicans* appear to be in contradiction to these, but a comparison may not be in order since organisms of dissimilar fermentative properties were used by Pisova and Nickerson.

D. Effects of miscellaneous agents

Various other agents have been shown to enhance Y → F transformation. Segal (146, 147) described abnormal forms of yeasts resulting from their exposure

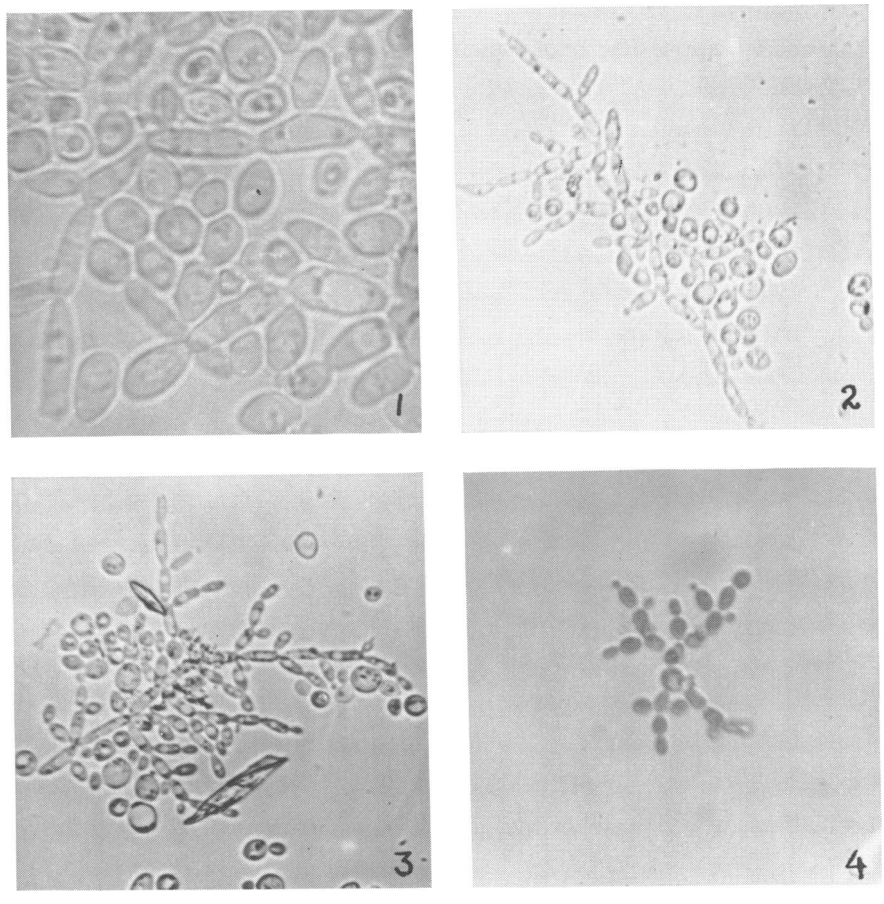

PLATE VII

FIG. 1. *S. cerevisiae* exposed to 0.204 mg/ml of 9,10-bishydroxymethyl-1,2-benzanthracene (BB) in broth for 4 weeks at room temp. Elongated cells occur in the form of filaments. 970 ×.

FIG. 2. *S. cerevisiae* exposed to 0.29 mg/ml of BB in broth for 15 days at room temp. Numerous yeast phase cells are still evident. 440 ×.

FIG. 3. *S. cerevisiae* exposed to 0.29 mg/ml of BB in broth for 21 days at room temp. Yeast phase cells are still evident and crystals of the carcinogen are also visible. 440 ×.

FIG. 4. *S. cerevisiae* exposed to 0.0204 mg/ml of BB in broth for 10 hr at room temp. This figure demonstrates cessation of cell division without the occurrence of cell elongation. 440 ×.

to fusel oil and higher alcohols. Abnormal budding resulted, giving rise to cell chains, but these abnormal forms disappeared when the yeasts were subcultured in ordinary nutrient media free of these agents. Yeasts exposed to the same

alcohols in water produced no unusual forms, suggesting that the cells were affected only while in the process of multiplication.

Cobalt treated cells of *Candida albicans* have been shown by Nickerson and Van Rij (112) to reveal long mycelial fragments with no crosswalls and very

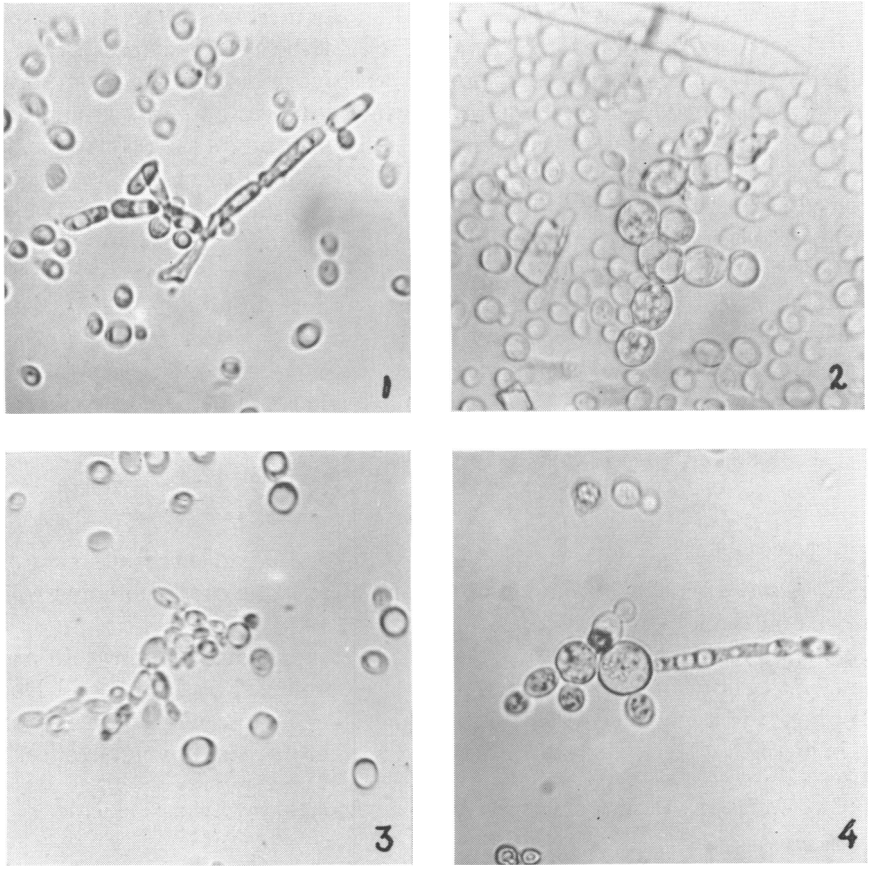

PLATE VIII

FIG. 1. *S. cerevisiae* exposed to 0.0204 mg/ml of BB in broth culture incubated with shaking for 99 hr at room temp. 440 ×.

FIG. 2. *S. cerevisiae* exposed to 0.00002 mg/ml of BB in broth culture incubated for 195 hr at room temp with shaking. Note giant yeast cells and crystals of BB. 440 ×.

FIG. 3. *S. cerevisiae* in broth exposed to 0.01 mg/ml of α-naphthalene acetic acid after 2 days' incubation at room temp. Note that the cell cluster contains yeastlike and elongated cells. 440 ×.

FIG. 4. *S. cerevisiae* exposed to 0.01 M L-tryptophan HCl in synthetic medium after 10 days at 20 C. 440 ×.

wide spacing between blastospore clusters (figure 4, plate VI). A 10^{-2} molar concentration of cysteine reversed the enhancement of Y → M transformation exhibited by the cobalt ions; oxine (8-hydroxyquinoline) had a reversing action similar to that of cysteine. An examination of other metal ions by these workers

revealed that $CuSO_4$, $MnCl_2$, $NaHAsO_3$, and Na_3AsO_4 were without effect on Y → M formation. Only boron ($K_2B_4O_7$) showed a significant effect, but to a much smaller extent than cobalt. Cells of *C. albicans* and *S. cerevisiae* exposed to penicillin became more elongated (112), the latter growing in the form of filaments (figures 1 and 2, plate VI).

The review of Pratt and Dufrenoy (118) on the mechanism of action of penicillin clearly indicates that the attack is on SH-bearing enzymes. Pratt and Dufrenoy (117) suggested that in penicillin sensitive organisms the gram positive complex of the magnesium ribonucleate, which involves an —SH group, accelerates the action of the penicillin molecules in inactivating —SH groups. The inactivated —SH groups are essential links in a chain of metabolic processes involved in growth. The fact that penicillin kills only multiplying bacteria (21, 68) might be explained on the basis of the interference by penicillin with —SH groups which are essential to cell multiplication.

A detailed investigation was made by Kinsey and Grant (69, 70, 71, 72, 73, 74) on the effect of mustard gas and divinyl sulfone on yeast cells. Glutathione was found to reverse the deleterious effects of these compounds on respiration and cell cytology. A reduction in cell glutathione could be demonstrated after exposure to these agents. Cytological damage was mainly confined to the production of elongated cells and pear shaped cells.

E. Effect of carcinogenic chemicals

Yeasts have frequently been used as experimental organisms for the study of the effects of carcinogenic compounds on organisms. One of the common effects has been on the Y → F transformation.

Giant cells and increased morphological differentiation of cells within a culture of *Saccharomyces ellipsoideus* Hansen were produced by Dodge and Dodge (42) when it was subjected to a solution of methylcholanthrene (1/50th of saturation in distilled water). Dodge (41) reported a slight increase in volume of carbon

PLATE IX. Outline drawings of cf- forms induced by various aliphatic chemicals:

FIGS. 1–3, ethyl ether; 4–7, acetone; 8, chloroform; 9, urethan; 10, methanol; 11, propanol; 12–13, normal butanol; 14, isobutanol. All figures 400 ×.

ACKNOWLEDGMENTS

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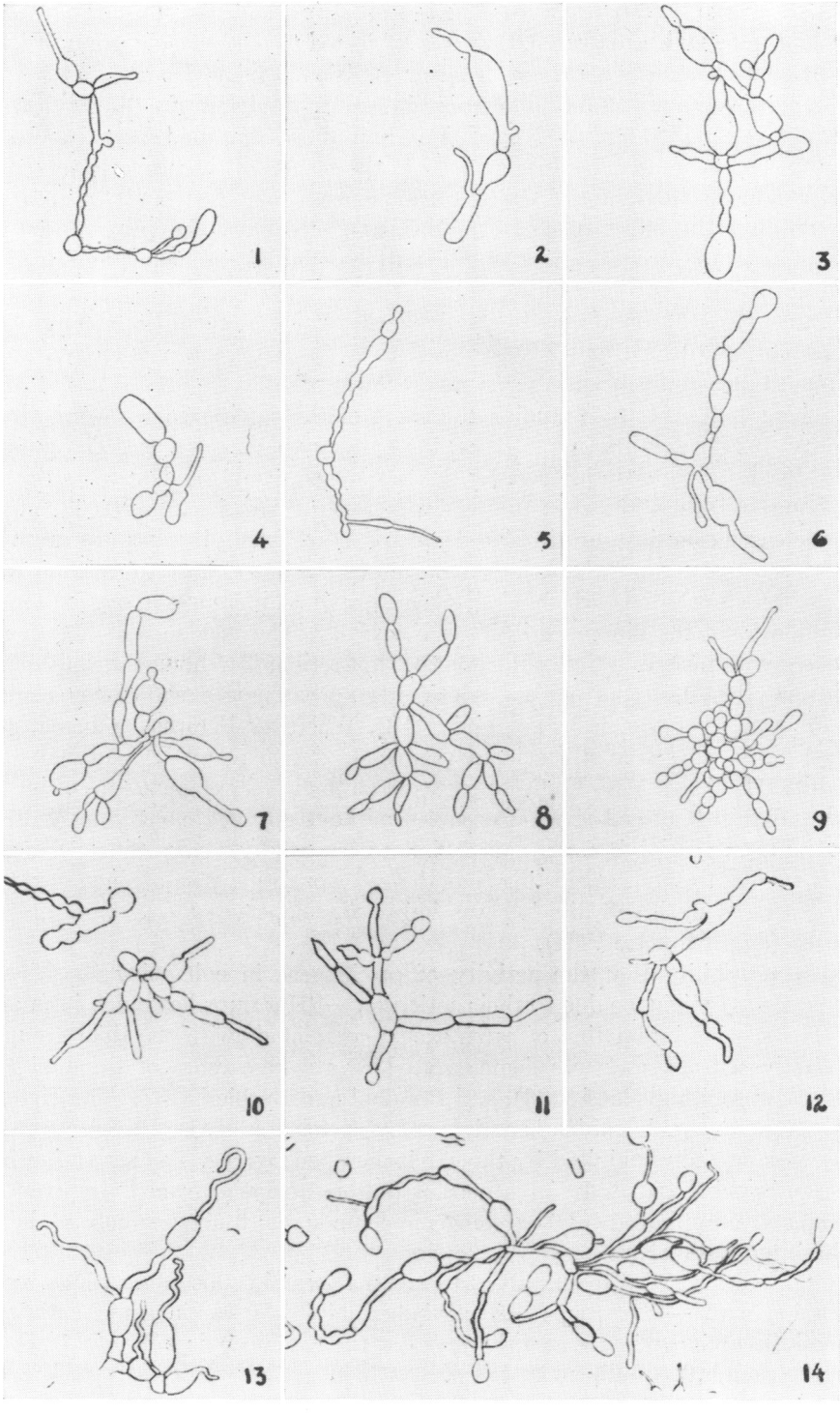

PLATE IX

dioxide given off after exposure of cells of *S. ellipsoideus* to solutions of methylcholanthrene and benzpyrene. Cultures subjected to methylcholanthrene yielded occasional giant cells and more frequent division. Dodge, Dodge, and Johnson (43), however, found small differences in the dry weights of cultures that had been grown under standard conditions and those that had been exposed to methylcholanthrene. The discrepancies in the results of these two papers might well be due to the factor of concentration since Cook, Hart, and Joly (30) had shown that the effect of 1,2,5,6-dibenzanthracene on the proliferation and respiration of *S. cerevisiae* can be stimulative or inhibitory, depending upon the concentration of the carcinogen.

Bauch (7, 8, 9) found greatly enlarged cells, which he classified as mutants, in yeast cultures which had been exposed to 3,4-benzpyrene and methylcholanthrene; 1,2,5,6-dibenzanthracene was without effect.

Scherr (143, 144, 145) studied the effect of the carcinogen 9,10-bishydroxymethyl-1,2-benzanthracene (BB) on the cytology of *S. cerevisiae*. The presence of F forms, apparently induced by the carcinogen, could be detected after only 10 hours of incubation when the cells were exposed to as little as 0.0002 mg/ml of the carcinogen in a broth. The degree of Y → F activity was less when the yeast cells were exposed to the carcinogen in stationary test tubes than when flasks were incubated with shaking, suggesting that differences in oxygen tension might explain the difference in Y → F activity. The incidence of F forms increased with increased time of incubation, but a time (315 hours) was ultimately reached when the control flask containing no carcinogen showed as many F forms as any of the flasks containing BB. Wherever F forms occurred, some yeast phase cells were still evident (figures 1, 2, 3 in plate VII and figure 1 in plate VIII). More Y → F effects occurred at the lower temperatures of incubation. Inhibition of cell-division occurred without any cell elongation (figure 4, plate VII), resulting in forms called "inflorescences" by Scherr. Single elongated cells were not found, suggesting that the cell elongation phenomenon could not be expressed until cell division was impaired. Giant yeast cells were also observed (figure 2, plate VIII).

The possibility that the activity of carcinogens in enhancing the Y → F phenomenon might be due to their action on —SH groups has much in favor of it. The specific interaction of carcinogenic compounds with the thiol groups of SH-activated enzymes was demonstrated by the researches of Rondoni (135, 136), Rondoni and Bassi (138), and Rondoni and Barbieri (137). The digestion of gelatin by papain, which is activated by cysteine or BAL (British Antilewisite), was partially inhibited by the carcinogen benzpyrene. The activating function of cysteine and BAL for cathepsins (autoproteolysins from horse liver) was completely suppressed by 9 carcinogenic compounds; 6 noncarcinogens did not inhibit autoproteolysis.

Crabtree (33, 34) also was able to show that certain substances which readily combine with —SH groups can partially inhibit the carcinogenic activity of chemical carcinogens. He also showed that the carcinogenic action of 3,4-benzpyrene and 1,2,5,6-dibenzanthracene on mouse skin was greatly retarded by

maleic and citraconic anhydrides. These anhydrides apparently act by combining with SH-containing groups and by interfering with sulfur (S) metabolism. Crabtree suggested that the primary phase of the carcinogenic process is the combination of carcinogens or their derivatives through S linkages to cell constituents. On the basis of considerations of the chemical reactivity of the carcinogenic hydrocarbons, Wood and Fieser (184) also suggested that a coupling between carcinogen and S-containing cell constituents was the first possible stage in their biological action.

F. Effect of irradiation

Lacassagne (77) found that cells of *Saccharomyces ellipsoideus* exposed to radiations between 2800 and 3800 A could be characterized into one of three susceptible types:

(a) Those that died immediately.

(b) Those that died after a certain period of delay.

(c) Those that exhibited a retarded rate of cell division but ultimately recovered their ability to reproduce.

The experiments of numerous workers (for example, Wyckoff and Luyet, 185; Glocker, 53) indicated that irradiation of *S. cerevisiae* by X-, cathode, or ultraviolet rays resulted in the death of cells in a manner which supported the multiple hit-to-kill hypothesis. A specific action of cathode and X-rays, but not of ultraviolet rays or formaldehyde vapor, was to injure some factor essential to reproduction. Thus, there was found, with the former two agents, a high percentage of yeast cells which, following irradiation, could pass through one budding, but no further. The cells apparently lost their ability to multiply long before they were killed, giving rise to the development of many giant cells and two-celled colonies. No reference was made to elongation of these cells. The failure of ultraviolet rays to interfere with the cell division mechanism under conditions of the above experiments is significant for the "space" concept of essential —SH groups, which will be discussed in a later section. This failure, however, is contrary to the observations of Lacassagne (77). Latarjet (79) also found that ultraviolet rays could stop cell division in yeasts but that the amount of energy required was 240 times that which was effective using X-irradiation.

In accord with this spacial concept, Giese and Swanson (52) demonstrated that irradiation of yeast cells with ultraviolet increased the endogenous respiration and decreased the exogenous respiration. Carbohydrate synthesis from added sugar was more readily stopped than respiration. These workers suggested that the irradiation caused changes which made it possible for the enzymes to use the intracellular substrate for respiration. They interpreted the decline in exogenous respiration following irradiation as the result of enzyme destruction, since in an excess of nutrient the enzyme concentration is probably the limiting factor.

Using radium, Rochlina (133, 134) obtained variants of *S. cerevisiae*. Among these was a stable race composed of cells with numerous projections which he considered to be undeveloped buds with thin walls, but they might well have been sprouting hyphae from the yeast cells.

Exposure of yeast cells to radium irradiation by Bauch (10) and Nadson and Rochlin (100) occasionally resulted in abnormally long cells. Bauch found some giant cells that appeared to be similar to the Gigas cells previously found by this author to be induced by camphor and other compounds (7, 8). Four transplants of these large cell cultures with cells approximately 1.5 to 1.6 times as large as normal cells bred true for almost a year. Seventy-six cultures of these giant cell isolates reverted to normal appearance after serial transfer. Bauch preferred the view that these Gigas cells were due to a nonhereditary phenotypic modification, in spite of the marked stability of four of his cultures.

A discussion of the effect of ultraviolet radiations on growth and respiration of yeasts will also be found in the treatise by Ellis and Wells (45).

III. RELATIONSHIP TO THE CF-REACTION

Bauch (6), using camphor, was the first person to produce experimentally a high incidence of the filamentous forms in yeast, a reaction which has come to be known as the camphor (cf-) reaction. The investigations of Levan and Sandwall (83) and Levan (81, 82) established that the cf reaction was not by any means limited to camphor since most of 50 organic compounds, many of which had narcotic properties, elicited the camphor reaction in yeast cells. Of the compounds examined that demonstrated camphor activity, 18 were aliphatic, 6 alicyclic, 14 aromatic, and 2 heterocyclic. The few bi- and polycylic aromatic substances tested showed no activity, with the exception of α-naphthalene acetic acid, which "showed certain tendencies to inducing the camphor reaction". The most notable camphor effect was caused by camphor, for which the percentage occurrence of cf- forms increased in solutions up to 0.007 molar, in which concentration almost all living cells were such forms.

The outstanding features of the camphor reaction as shown by Levan and others are:

(a) The cells tend to hang together in colonies.

(b) The reaction is incomplete since normal cells are always found intermingled with cf- forms, even at the highest concentrations tested which still permit cellular growth.

(c) The morphologies of the affected cells are consistent only in that cell elongation and the inhibition of cellular division are pre-eminent. Aside from this, cells affected by different chemicals show irregularly swollen cell aggregates, long filamentous cells, and numerous grotesque looking cells of varied sizes and shapes. Note plate IX, which contains figures reproduced from Levan's (82) paper.

(d) There is an almost consistent inverse relationship between the cf- activity of the chemicals tested and their water solubility.

(e) The cf- reaction induced by different chemicals is further enhanced by low temperatures (9–20 C) and impeded by higher temperatures (30 C). Occasionally it has been observed that low temperatures alone favor cf- growth.

(f) The cf- reaction is reversible in that cf- cells begin to show normal budding processes if the concentrations of the cf- agents begin to fall below their thresh-

old values for cf- activity or if the yeast cells acquire a resistance to the cf- chemicals.

The reversibility of the cf- reaction is deserving of particular mention because the relative ease of reversing Y → M formation among the yeastlike pathogenic fungi is significant in the pathogenesis of diseases caused by these organisms.

Subramaniam (154), exposing cells of *S. cerevisiae* growing in wort medium to acenaphthene for 6 hours, found that "long threadlike mycelial growths" became very common. These elongated forms were similar, morphologically, to the cf- forms of Levan.

The cf- reaction is obviously closely allied to or identical with the Y → F and Y → M activity demonstrated by the various agents discussed in this review. The only possible distinguishing feature of the cf- cells is their grotesque shapes. Levan (personal communication) examined a copy of figure 4, plate VIII which represents a reaction which was described by Scherr (145) as an example of a Y → F reaction and was of the opinion that it represents an instance of the cf- reaction.

IV. RELATIONSHIP TO DISSOCIATION AND LIFE CYCLE PHENOMENA

Y → M transformations in yeasts have been observed in phenomena that have been described as dissociation. Fabian and McCullough (46) and Wickerham and Fabian (171), studying dissociation in a number of yeasts under various cultural conditions, designated four distinguishing types of yeasts:

(a) The smooth form ("S"), the most commonly observed form of the yeasts studied.

(b) The rough form ("R"), occurring in the form of slender, elongated, or irregularly shaped cells.

(c) The gonidial form ("G" from Hadley *et al.*, 61) which produces very small yeast cells, either spherical or rod shaped, but forms colonies which are large, thin, and spreading.

(d) The transitional, or "T" form, which is of normal size and may be elongated or oval, but which invariably reverts to the "G" form.

The studies of Fabian and McCullough (46) on the effect of temperature on *Saccharomyces cerevisiae* Saaz, *S. cerevisiae* Froberg, *S. ellipsoideus*, *Willia anomala*, and *Zygosaccharomyces mandshuricus* indicated that lower temperatures of incubation in malt broth and in nutrient broth permitted the appearance of only the "S" forms of all of the foregoing yeasts. These data are in direct contradiction to all the work previously discussed, in which the general law appeared to be that a lowered temperature of incubation enhanced the F or "R" form of the yeast. Thus, it is puzzling to find that after 4 weeks of observation at temperatures of 9, 17, and 23 C all of these yeasts were reported to be exclusively in the "S" phase; increased periods of incubation enhanced the "R" phase only at temperatures of 29 and 37 C.

Similar studies on "R" and "S" dissociation by *C. albicans* were also made by Negroni (103, 104, 105) and Negroni and Loizaga (106). These studies support the contention of these workers that "S" ⇌ "R" variation in *C. albicans*

may be likened to "S" $\rightleftharpoons$ "R" variation among the bacteria as evidenced by comparable findings with respect to differences in colonial morphology, agglutination by trypaflavine, and serum specific agglutination. They also reported differences in pathogenicity between comparable amounts of "R" and "S" phase cultures. Thus, a quantity of "S" cells which would kill a rabbit in 4 or 5 days when inoculated intravenously had no such deleterious effect when an equivalent amount of "R" cells was injected by the same route.

However, by adding 0.25% lithium chloride to nutrient broth and adjusting to pH 7, these workers found that "R" forms would appear in all of the above cultures in a relatively short period of time (for the cultures listed, 12, 14, 6, 4, and 6 days, respectively). "R" forms could also be induced within 24 hours after the "S" form of *S. cerevisiae* Saaz was transferred to brilliant green broth.

The addition of alcohol (sterilized by refluxing) to malt extract broth also enhanced the formation of "R" forms; the concentration was a critical factor. Thus, they found 10% "S", 85% "R", and 5% "G" forms when *S. ellipsoideus* was exposed to 5% alcohol for 1 week at 20 C, but 100% "G" forms when it was exposed to 15% alcohol under comparable conditions. After 2 weeks, this same culture showed 10% "S" and 90% "R" in 5% alcohol, but 10% "R" and 90% "G" in 15% alcohol.

The three forms of *S. cerevisiae* Saaz were found to display a marked degree of immunological specificity as determined by agglutination reactions. They also differed in their fermentation reactions in eleven sources of carbon tested, both in matter of degree and in types of products formed.

Mickle and Jones (94) reported that dissociation from smooth to rough colonies of *Candida albicans* could be accomplished using 0.25% lithium chloride or 3% immune rabbit serum. The early growth of smooth forms on Sabouraud's agar showed round or slightly oval budding cells. Rough colonies were composed of elongated cells, some having bizarre shapes, and many approaching in appearance mycelial filaments. Fourteen strains of 18 studied gave rise to variants which appeared to be permanent and which showed distinguishing features with respect to colonial morphology, cell cytology, and pathogenicity. Mickle and Jones concluded that these variants were stable rough states of *C. albicans* as they are known to occur among the bacteria. The phenomena which we are considering here as Y $\rightleftharpoons$ M transformations also appear to have been described as the production of "variants" or "mutants".

Lepeschkin (80) reported that the mycelial stage induced in *Schizosaccharomyces pombe* and *S. mellacei* remained constant during numerous generations; he was unable to secure reversion to the original yeast phase. Nadson and Krassilnikov (99) also reported the occurrence of spontaneous variants in a yeastlike fungus, *Guilliermondella selenospora*. Five races emerged, of which one was characterized by abundant mycelium, one was entirely yeastlike and formed no mycelium, and the other three showed somewhat intermediate characters.

Punkari and Henrici (119) described the occurrence in *Torulopsis pulcherrima* of spontaneous variations in color (red and white) and texture (smooth and rough). Roughness was correlated with the development of a mycelium in both

the red and white variants. Mackinnon (88, 89, 90) found spontaneous variants in *Candida albicans* which he classified as "membranous" and lethal. The membranous variants were characterized by the elongation of blastospores to a degree which could be described as filamentous. As might be anticipated, these changes were also reflected in colonial morphology, the trend being towards wrinkled, spiky, and hard colonies. The term membranous is descriptive of the type of growth of this variant in glucose broth media. The filaments tended to aggregate into large clumps, and these changes in cell and culture morphology were accompanied by a decrease in virulence. The biochemical properties apparently were unchanged. The lethal variant grew poorly, showed a near complete loss of virulence, and produced scant, if any, mycelial growth. Mackinnon reported that the same strain of *C. albicans* can vary to the membranous or lethal types in a reversible fashion. These membranous variants of Mackinnon are similar to filamentous variants of yeasts reported by Lepeschkin (80), Punkari and Henrici (119), and Nadson (97, 98).

A study of the phenomenon of dissociation in asporogenous yeasts by Redaelli *et al.* (126) led these workers to postulate the theory that this type of dissociation ("R" $\rightleftharpoons$ "S") is one of transitional variation (not mutation) in a population of cells composed of both types. Populations of various clones have a potentiality of variation to the "R" or "S" forms. However, a clone composed only of "S" cells is incapable of this type of dissociation as is one of "R" cells. In order to dissociate to the "R" type, an "S" clone must have a few "R" cells present; likewise an "R" clone must have a few "S" cells present before dissociation can take place from "R" $\rightarrow$ "S". These workers could shift the "R" and "S" phases by using hypo-, iso-, and hypertonic solutions of sucrose. This work closely resembles that of Braun (18) with *Brucella* dissociation.

Discussions and reviews of morphological variation and dissociation in yeasts will be found in Cavallero (20), Diddens and Lodder (39), and Mackinnon (91).

Bauch (9) obtained true-breeding races of yeasts with the mitotic poisons, acenaphthene, narcotin-hydrochloride, sodium cacodylate, adrenaline, heteroauxin, and camphor, but not with colchicine.

More recently, Connell and Skinner (29) reported on the spontaneous mutation to the filamentous stage by cultures of *Cryptococcus*. Of 131 *Cryptococcus* cultures isolated from the human body, 17 produced typical mycelial growth after 2 to 3 years of subculturing. These transformed cultures were morphologically indistinguishable from members of the *Candida* genus and were stable in that at no time were they found to revert back to the yeast, nonfilament forming phase.

An association of Y $\rightleftharpoons$ M transformations with the life cycles of yeasts is suggested by observations of haploid and diploid yeast cultures. This might also be taken to imply a relationship to genetic mechanism. One of the characteristics of a haplophase yeast culture is the "roughness" of its colonies as compared to a diplophase culture. The temperature of incubation is of major significance when differentiating haplophase from diplophase colonies on the basis of smoothness of colonies since, as might be anticipated from the discussion on the effect

of temperature on cell morphology, diplophase colonies may have a rough topography when grown at a relatively low temperature (Winge and Laustsen, 182). The usual roughness of haploid cultures is attributed by Lindegren (86) to the aggregation of the cells into clusters, called by him "rosettes". Such aggregation of yeast cells into masses has also been reported for *Pityrosporum ovale* (14).

A detailed study of the genetics of this property of flocculation has been made by Thorne (165) for a number of yeasts. Thorne found that the cells could easily be separated from the clusters by micromanipulation but that they reaggregated as soon as the restraining force was removed. This suggested that these aggregates of cells were formed by forces of surface attraction. However, Thorne was able to demonstrate this phenomenon with diploid strains of yeasts, and his studies supported the contention that this property of aggregation or flocculation is under genic control, though he found such genes to be very unstable. Scherr (144, 145) also reported the occurrence of such clusters of cells. Since his cultures were in the diploid state and the cells of these clusters could not readily be separated by micromanipulation (the cells were attached to each other), he termed such clusters "inflorescences" to differentiate them from the groups of cells described by Lindegren and Thorne.

Specific genetic control of the dimorphism phenomenon is supported by the studies of Winge and Laustsen (181). They recorded the segregation of two nonlinked genes governing growth (N) and elongation of the cells (L). The species studied was NnLl, and the sudden appearance of NNLl led to the suggestion that n had mutated to N. Winge and Laustsen still reserve the possibility that conjugation between spores from two different asci had occurred.

Subramaniam *et al.* (155) proposed that the smoothness or roughness of a colony depends upon the presence of one or more of the three alleles, Smooth, Lace, or Rough. Colonies were classified in three degrees of smoothness (Smooth I, II, III) and two degrees of roughness (Rough I, II). They also reported the occurrence of reverse mutations among these alleles and of "mass mutations" due to what was believed to be an increased incubation temperature during the months of August-September.

Y $\rightleftharpoons$ M transformations have frequently been found to follow regular patterns. Nadson (98) summarized a common pattern of morphological variation in yeasts, indicating that this pattern is found whether the variations are spontaneous or induced. He stated that there is a tendency for the normally globular yeast with smooth colonies to give rise to forms which are cylindrical or sausage shaped, then to a pseudomycelium, and finally to a true mycelium, these trends being accompanied by an increasing roughness of the colonies.

Henrici (149) depicted this transition in the following steps:

Yeast	→	Submerged mycelium	→	Aerial mycelium with spores	→	Sterile woolly aerial mycelium	→	Faviform growth

The so-called "black yeasts" offer another example of Y $\rightleftharpoons$ M formation. These yeasts have been isolated from soil, air, and other sources but most frequently from dairy products. Upon first isolation they grow as typical yeasts, both with reference to colonial texture and cell cytology. Deep colonies in agar will soon sprout out filaments of a mycelium which ultimately develop clusters of budding cells similar to those found in *Candida*. Maurizio and Staub (92) have described this transition in some detail. Ultimately, the mycelium develops even on surface culture, giving rise to an aerial mycelium with branched chains of conidia. These yeasts thus can pass through the first three Y $\rightarrow$ M transition stages of Henrici and finally grow as a mold conforming to the genus *Cladosporium*.

In general, changes tend to take place in the direction of the arrows although in many cases they are readily reversible by appropriate changes in conditions of culture. There are also examples where a fungus which is usually (normally) mycelial may appear in the yeast phase under appropriate conditions. Thus, the transition to the yeast phase by *Mucor racemosus* has been known for some time (Dodge, 40; Gaumann and Dodge, 50, p. 95). In submerged culture the hyphae break up into oidia which may multiply by a typical yeastlike budding process. In addition, these sprouting hyphal segments and yeastlike cells have the ability, in common with the true yeasts, to ferment sugars to alcohol. Although pathogenicity of *M. racemosus* for animals is a moot question, the resemblance of the hyphae with gemmae of *M. racemosus* to those of *Haplosporangium parvum* or *C. immitis* is a very strong one.

Certain of the fungi causing ringworm may occur in the last three stages of the Y $\rightarrow$ M cycle, but those belonging to the dermatophytes have not been shown to enter the typical yeast phase. *Blastomyces dermatitidis* might possibly be credited with being able to pass through all of the foregoing stages.

V. PATHOGENICITY

As has been pointed out, many of the yeastlike fungi occur in the tissues of the host almost entirely in the Y form although under most conditions they develop *in vitro* in the M form. It is not uncommon, however, to find the filamentous phase in the tissues, mainly during the latter stages of an infection, *e.g.*, in systemic moniliasis, especially in the kidneys.

One yeastlike fungus which does not ordinarily show this dimorphism is *Cryptococcus neoformans*. From infected tissue and when cultured on Sabouraud's agar at 20 C and 37 C, it shows budding yeastlike cells (Todd and Herrmann, 166; Benham, 12). Occasionally the buds may hold together, producing a chain of cells, but there is nothing in the nature of a true mycelium (13). In old cultures made from primary isolated material, occasional short germ tubes may be found growing from the yeast cells, but these are lost on transfer and the fungus subsequently reproduces by budding only.

With the yeastlike fungi that show dimorphism, either the Y or the M form may be pathogenic when it is injected. Baker (4) demonstrated that the mycelial form of *B. dermatitidis* was as effective as the yeast form in causing death of

mice when weighed equivalent doses were inoculated intraperitoneally. However, the lesions always contained the yeast form, even when the mycelial form had been injected. These results, together with similar ones obtained by other workers, indicate that the host tissues produce factors which favor the transformation of the M stage into the Y stage or the selection of Y forms over the M forms, depending upon the explanation which is to be accepted for the phenomenon.

Although the body temperature of a host (approximately 37 C) probably plays a major role in maintaining the yeast phase *in vivo*, it is probable that other factors are of importance. When the mycelial phase of the fungus becomes evident in the later stages of an infection, it would appear that some factor in the host's physiology which normally favors the Y forms has diminished in function. The organisms which cause deep mycoses are subject to a more constant temperature *in vivo* than those which cause superficial infections (hair, skin, nails). Since the organisms of the latter group exhibit mycelial growth and grow well *in vitro* at temperatures from 25 C to 38 C (113), it is possible that their inability to invade the deeper tissues may be correlated with the absence of an intracellular mechanism responsible for $Y \rightleftharpoons F$ conversion, in the presence of bodily conditions that will suppress mycelial growth.

Some attempts have been made to explain the better adaptation of the Y forms to growth in the tissues. Baker, Mrak, and Smith (3) have postulated that in the Y phase the fungus is able to produce the greatest number of reproductive structures with the least synthesis of new protoplasm; in other words, that the environmental conditions imposed by animal tissues restrict mycelial development, which is less profitable from a reproductive standpoint. It is not yet clear whether a hyphal fragment, essentially the reproductive unit in the mycelial phase of the yeastlike pathogenic fungi, has a greater mass than a yeast phase cell and also a comparable potentiality for invading a susceptible host.

Nickerson and Edwards (111) showed that the oxygen consumption of the Y form, on a dry weight basis, is about 5 or 6 times that for the M phase at 36 C. They concluded that "while the M stages of the organism considered are known to be infectious, it is highly probable that their virulence is, in some way, associated with the fact that, at the temperature of an animal host, $M \rightarrow Y$ will occur, resulting in a greatly increased energy potential available to the invading organism."

Will any yeastlike pathogenic fungus which is capable of growth in the Y phase be capable of producing systemic infections? Experience with the genus *Trichosporon* might be taken to indicate that the answer is negative. Although it cannot be said that with *Trichosporon* dimorphism exists, as it does, for example, with *Blastomyces* or *H. capsulatum*, nevertheless it does have blastospores and arthrospores, shows mycelial growth, and, under certain conditions, predominates in the yeast phase, although not exclusively so. *Trichosporon* has been found to be the etiological agent in hair infections, and no reports are known to the authors indicating that invasion of the deep organs is possible (see Dodge, 40).

If a dermatophyte could be induced to enter the yeast phase, would it invade the deep tissues? Certainly attempts by workers including Sulzberger (156) and Brocq-Rousseau *et al.* (19) to induce systemic infections with dermatophytes have led to consistent failure. Inoculations by these workers of large doses of spores of *Trichophyton* and *Microsporum* into guinea pigs did not cause an infection in any of the internal organs.

Based on the observation that the Y forms of the yeastlike pathogenic fungi are best adapted to development in the tissues, Scherr (144, 145) postulated the theory that any agent which would tend to transform the established yeast phase to the mycelial phase *in vivo* might serve to arrest the multiplication of the pathogen. To test this theory, he tried a number of compounds which have plant growth stimulating properties (auxins) on mice systemically infected with *C. albicans*. The results were negative. These results, however, should not preclude further tests of the theory. In the first place, the choice of *C. albicans* may have been a poor one since this organism perhaps does not produce typical dimorphism, having the concomitant existence of both phases in a culture that has been grown at a constant temperature, with budding yeast cells on a true hyphal stalk, the so-called blastospores. In addition, while the auxins appear to stimulate Y → F transformation *in vitro*, there is little evidence that they may have the same effect *in vivo*. Perhaps other substances should be used to test the theory.

VI. CONCEPTS FOR DIMORPHISM MECHANISM

Four explanations of dimorphism will be discussed briefly. These explanations are not mutually exclusive but in fact may be only different facets of a single explanation. Furthermore, it is, we believe, possible that some cases of dimorphism may be explained by one of these theories and others by one of the other theories, or, more probably, that two or more of these theories, taken together, may be necessary to explain a single case of dimorphism.

A. Mutation and selection

Considerable evidence for the theory based on mutation and selection has been presented in an earlier section of this review. The regularity and rapidity with which the transformations occur under specific environmental conditions might at first appear to cast doubt upon this theory. On the other hand, this could readily occur if mutations are relatively frequent and both Y and M forms are regularly present so that selection may become quickly operative.

The action of selection may be complicated by the fact that if Y forms arise from M forms by a process of budding, then conceivably both Y and M forms would still be present since budding, unlike binary fission, leaves the integrity of the parent cell relatively intact. The presence of F and Y cells within the same cluster of cells is not unusual (figure 3, plate VIII), but a number of generations seemingly clears the culture of F forms under conditions which favor the development of the Y forms.

B. Growth rate mechanism

Considerable evidence has accumulated to indicate that Y $\rightleftharpoons$ F transformations may be explained by the theory of Hinshelwood (67). Hinshelwood designated the factors favoring division and elongation as D and L, respectively. He asserted that environmental factors may affect either of these factors independently of the other. According to this theory the formation of filaments depends upon the comparative rates of elongation and division. Filament formation may, therefore, be a result of the inhibition of the rate of division or of stimulation of the rate of elongation.

Whether the effect is primarily on the cell division or the cell elongation mechanisms of yeasts is difficult to determine. Scherr (145) has presented the following evidence to indicate that the primary effect is on the cell division mechanism:

(a) The cell-division mechanism may be impaired without any cell elongation occurring, giving rise to inflorescences.

(b) Cell elongation has not been observed to occur in single cells when cultures have been subjected to conditions which enhance Y $\rightarrow$ F formation.

(c) Elongated and yeast phase cells occur within the same cluster of cells in a manner suggesting that the effect on cell division has occurred previous to the effect on cell elongation.

(d) In filaments of elongated cells, the younger terminal cells of the filament are consistently less elongated than the older cells (figures 2 and 3, plate VII).

With the cf- reaction which we consider as probably being identical with Y $\rightarrow$ F transformation, the effect has commonly been thought to be due to an inhibition of the cell-division mechanism. This observation was particularly emphasized by Levan (81) who stated that, "it was found that the significant feature of the reaction was not so much the change in cell shape as the *hanging together in colonies of the cells.*"

Little is known about the effect of transforming substances on the stimulation of cell elongation as distinct from the inhibition of cell division. If such stimulation does occur, does it act by increasing the rate of mitosis to the point where cell division cannot keep up with it or by increasing the size (length) of the individual cells? This question can be answered only by extensive cytological work. With bacteria, many workers have shown that filaments may arise by the occurrence of cell multiplication (mitosis) without concomitant cell division. On the other hand some substances, such as auxins, have been postulated to act by increasing the plasticity of the cell so that it may become elongated, even without any increase in volume.

Scherr (145) attempted to determine if α-naphthalene acetic acid had a stimulating effect on the growth rate of *S. cerevisiae*. As it was impossible to obtain Y $\rightarrow$ F transformation without, at the same time, having some toxic effect of the α-naphthalene acetic acid, the results were difficult to interpret. At any rate, no evidence could be obtained for a stimulatory effect. The work of Dodge, Dodge, and Johnson (43) that showed no differences in dry weights of cultures exposed to methylcholanthrene as compared to controls has already been mentioned.

It should be emphasized that the occasional occurrence of filaments in a yeast culture should not be interpreted as Y → F transformation. Goldschmidt (54) has described such nonhereditary variations as merely exhibiting "a simple symmetrical fluctuation around a mean".

C. Inactivation of —SH groups

Much of the data recorded in Section II supports the concept that inactivation of essential —SH groups enhances the Y → F or Y → M effect and this material need not be repeated here.

Although the evidence implicating —SH groups in the mechanism responsible for cell division is convincing, it is not yet clear as to which —SH group or groups are involved. The work of Scherr (144, 145) attempting to implicate the —SH groups involved in the reduction of serine to cysteine has progressed only a little past the speculation stage. Cell division, growth, and multiplication are processes which doubtless require many enzymes for their orderly execution, a large number of which may be —SH activated. A list of enzymes requiring —SH groups for their activation which has been compiled by Elliott (44) indicates the wide scope of —SH activity.

> Systems responsible for pyruvate oxidation, dismutation, decarboxylation and acetoacetate and acetylmethyl-carbinol formation; hexosemonophosphate, phosphoglyceraldehyde, glycerol, α-ketoglutaric, succinic, malic, and alcohol (yeast) dehydrogenases; synthesis of α-ketoglutarate; adenosinetriphosphatase; phosphoglucomutase; phosphorylase; hexokinase; pancreatic amylase; β-amylase of barley; stearate, oleate (bacteria), and acetate oxidases; β-hydroxybutyric dehydrogenase; pancreatic lipase and esterase; cerebrosidase; D-amino acid oxidase; monoamine oxidase; L-glutamic dehydrogenase; transaminase; urease; cathepsins; papain; bromelin; haemolysins; lysozyme; choline oxidase; cholinesterase; and choline acetylase.

One of the difficulties in elucidating the mechanism responsible for dimorphism in microorganisms is that it is apparently so labile that many agents of divergent chemical and physical activity are capable of interfering with it. However, the compounds which have been found to enhance the Y → F, Y → M and cf- effect or effects in microorganisms may all be compounds which are detoxified in the same way when cells are exposed to them. For example, cysteine is one of the few amino acids which may be used by an organism for detoxification (Fieser and Fieser, 47). Fieser and Fieser pointed out that, at least for higher animals, the chloro-, bromo-, and iodo-derivatives of benzene are all excreted as derivatives of mercapturic acid, in which the amino group of the cysteine residue is acetylated. Naphthalene and anthracene are detoxified by some animals in the same way.

The work of O'Connor (115) has shown that fluoride and iodoacetate, because of their action on —SH groups, inhibit aerobic glycolysis in embryonic chick midbrain tissue and that such an inhibition is accompanied by a decrease in the rate of cell division. This association between cell division and aerobic glycolysis is in accord with the association found in normal chick embryo development

(114). These findings agree with those of Rapkine's previous studies (120, 121, 122, 123, 124, 125) showing that iodoacetate affects carbohydrate metabolism because of its effect on the —SH groups (Dickens, 37), more specifically on triosephosphate dehydrogenase. O'Connor (115) therefore suggested that spindle formation and function is associated with a reversible denaturation of protein controlled by oxidation and reduction of the contained —SH groups.

Conclusions from O'Connor's work might not be properly applied here, but if O'Connor's findings are assumed to be applicable to yeasts, then studies of the effects of various materials on yeast respiration through their effects on —SH groups may be significant with respect to the process of cell division. In accord with O'Connor's work it might be expected that yeast cells undergoing limited aerobic glycolysis and/or an increased endogenous respiration might be less susceptible to the effects of agents whose action is on —SH containing groups and thence on the cell division mechanism. This view, interestingly enough, has been borne out by the work of Selzer and Baumberger (148). These workers showed that the endogenous respiration of *S. cerevisiae* is not influenced by metallic mercury in the suspending medium for the cells, but that the exogenous respiration system is markedly inhibited. They suggested that the —SH groups, which are specifically inhibited by mercury, of the endogenous respiration systems are located within the interior of the cell, while the —SH groups concerned in the exogenous respiration system are located in or near the cell wall.

Bernheim (16) similarly found that the exogenous respiration of *Blastomyces dermatitidis* was sensitive to M/100 sodium cyanide, whereas the endogenous respiration was relatively insensitive. Evidence in support of the spatial separation of the endogenous and exogenous systems also comes from the works of Stier and Stannard (152), Winzler and Baumberger (183), and Spiegelman and Nozawa (150). An excellent discussion of this spatial concept for yeasts will be found in Nickerson's (107) text.

Cook, Perisutti, and Walsh (32) demonstrated that phenylmercuric nitrate would depress the respiration of *S. cerevisiae*. That such a depression could be directly correlated with the action of phenylmercuric nitrate on essential —SH groups was shown by reversal of this respiratory depression by cysteine and homocysteine; cystine and methionine were without such an effect. The ratio of cysteine to phenylmercuric nitrate required to antagonize the respiratory depressant effect of the latter suggested that equivalent amounts of cysteine and Hg^{++} were involved. The depressant effect of phenylmercuric nitrate on various oxidases and dehydrogenases (Cook *et al.*, 31) may be traced to the interaction of this compound with the —SH groups in these enzymes.

Cook, Perisutti, and Walsh (32) also showed that cysteine itself may act as a respiratory depressant on *S. cerevisiae*. Approximately 50% depression was found after 1 hour for molar concentrations of L-cysteine HCl of 3.2×10^{-3} to 1.3×10^{-2}; concentrations less than 10^{-4} M were without detectable effect.

The significance of —SH groups for the cell division mechanism appears to be borne out also by observations on animal cells. A study made by Gregory and Castle (55) on the development of rabbit eggs implicates —SH groups in the

cell division and general growth rate processes. After 40 hours of development, eggs from large size races of rabbits produced a larger number of cells in the process of division than did eggs of small size races. The suggestion was made, based on Hammett's (63) postulate, that the "—SH group is the essential chemical stimulus to growth by increase in cell number," that a greater amount of —SH groups in the nucleus may be responsible for the more rapid growth rate of eggs and embryos from large-race matings. The importance of the —SH group in the cell proliferation of *Amoeba proteus* has also been well substantiated by the work of Chalkley (22), Voegtlin and Chalkley (167), and Chalkley and Voegtlin (23, 24).

The significance of the —SH groups for the cell division and growth mechanisms of animal cells is discussed in great detail by Needham (102). This chemical group has been termed by Needham a "nuclear inductor elect" because of his belief that it may be the intermediary between genes and the phenotype.

The works of numerous investigators support the contention of Nickerson and Van Rij (112) that "*cell division, as a process distinct from growth*, depends upon the maintenance of functional intracellular sulfhydryl groups."

D. Auxin concept

The theory that auxin may be involved in the dimorphism phenomenon in yeasts, as proposed by Scherr (144, 145), merely extends the SH-inactivation concept.

Using the coleoptile test, Robinson and Stier (131, 132) assayed the amount of auxin in yeast cultures grown in William's medium plus various concentrations of peptone. They found that with increasing concentrations of peptone the rate of cell multiplication increased, but the rate of auxin production per cell decreased. The addition of increasing amounts of sucrose resulted in increasing the rate of production of auxin, so that with 10% sucrose and 0.1% peptone over 1,000 auxin units per ml of centrifuged medium were obtained. The amount of auxin in the medium was higher than that obtained in the cells themselves by Kögl and Kostermans (76). With a high auxin concentration in the medium many of the yeast cells occurred in the form of filaments. The suggestion was made by these workers that the increased concentration of auxin might have been responsible for the F forms of the yeast.

The work of Tatum and Bonner (158) and Tatum, Bonner, and Beadle (159) suggests the view that tryptophan is synthesized by the coupling of serine and indole through splitting out a molecule of water. This scheme and its ramifications have been graphically illustrated by Foster (48).

Scherr (145) used this as the basis for a postulated explanation of the action of the carcinogen 9,10-bishydroxymethyl-1,2-benzanthracene in producing a Y → F transformation. He thought that if its action was to make —SH groups unavailable for cysteine synthesis by some blocking action, then this would result in the accumulation of serine and tryptophan in the yeast cells, followed by a further shift in the direction of production of 3-indole acetic acid (heteroauxin). He further speculated that the accumulation of heteroauxin might re-

sult in the elongation of yeast cells since elongation of plant cells is a characteristic effect of auxins (Went and Thimann, 170). In support of his hypothesis, Scherr found:

(a) The auxin activity of filtrates from cultures containing numerous filamentous cells of *S. cerevisiae* was higher than that of filtrates from cultures that were predominantly in the yeast phase.

(b) The addition of tryptophan to the medium induced the formation of a relatively large number of filamentous cells. Thimann (160, 161) has shown that the yield of auxin in cultures of *Rhizopus* was determined by the amount of tryptophan present. This origin of heteroauxin is also supported by the work of Wildman, Ferri, and Bonner (173) and Wildman and Muir (174). The work of Kögl and Kostermans (76) has shown that the auxin content of yeasts may be attributed almost entirely to 3-indole acetic acid.

(c) Increasing the hydrogen ion concentration of cultures containing tryptophan enhanced the Y → F phenomenon considerably. At acid levels the free acid form of heteroauxin would be released from an inactive complex (Bonner, 17; Thimann, 162).

(d) The effect of α-naphthalene acetic acid on the growth of *S. cerevisiae* appeared to be that of increasing the number of F forms without affecting the rate of cell multiplication; a comparable phenomenon occurs in higher plants exposed to auxins.

Although few other studies directly bear on this concept, some support may be gained from the work of Thimann (163), who studied the action of a number of —SH inhibitors, using as a test method the effect on the amount of curvature and growth of slit stems of peas. Arsenite and the organic arsenical mapharsen (3-amino-4-hydroxy-phenylarsenoxide) inhibited growth strongly, as did iodoacetate and parachloromercuribenzoate, which is highly specific for —SH groups (Hellerman, Chinard, and Dietz, 66). At concentrations which produced 50% inhibition of growth, arsenite, iodoacetate, and parachloromercuribenzoate did not exert a detectable effect on the coleoptile respiration. Ryan, Tatum, and Giese (139) had already demonstrated that the growth of *Neurospora* is inhibited by iodoacetate in a manner both quantitatively and qualitatively similar to that of the coleoptile sections in the work by Thimann (163).

The observations of Thimann, that the inhibition of growth by —SH inhibitors occurs in the presence of auxin, appears contradictory to the concept propounded by Scherr that —SH inactivation may lead to auxin production in *S. cerevisiae.* However, Thimann and Bonner (164) inhibited the growth processes of *Avena* coleoptiles elongating in auxin and sucrose, and slit internodes of *Pisum* stems curving in auxin alone, with the lactones coumarin and protoanemonin. That both of these compounds acted by reacting with an SH containing enzyme was supported by the fact that BAL prevented the growth inhibition caused by these compounds, and at subinhibiting concentrations the growth of the test plants was promoted in each case. This observation lends support to the auxin concept for Y → F formation since it was shown by Thimann and Bonner that more auxin was produced, at least as evidenced by the *Avena* and *Pisum* tests, in the

presence of subinhibiting concentrations of the —SH inhibiting lactones, coumarin and protoanemonin.

A major flaw in the auxin theory is that compounds known to have plant growth activity have failed to excite a consistent, strong, Y → F effect in cultures of *S. cerevisiae;* this was found to be so with β-3-indolepropionic acid, 3-indole acetic acid, and α-naphthalene acetic acid (Scherr, 144).

It is becoming more apparent that the mechanism of cell division, growth, and multiplication need not be of a unique type for different species and genera. Indeed, it is more than likely that there exist common or closely related factors pertinent to these mechanisms among numerous plant and animal cells. A clearer understanding of these mechanisms might conceivably contribute to a clearer insight into the pathogenesis of various diseases, perhaps to an extent which will permit more efficacious techniques of therapy.

All morphological variations from the normal need not be necessarily considered involution or degeneration forms. This is well substantiated by much of the data reported in this review. The origin of stable mycelial races, for example, from a yeast phase culture could be a clue to the phylogeny of that species or might indicate a portion of the life cycle not frequently observed in the laboratory. It might even indicate one potentiality of further evolution.

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