Studies of the Dimorphism Mechanism in *Saccharomyces cerevisiae* [1) 2)]

by

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Introduction

The pathogenic fungi may be divided into two groups. Those which can invade only the superficial skin are called "dermatophytes"; those which can infect not only the skin but also the deeper organs usually belong to the group of yeastlike fungi. There are a number of yeastlike pathogenic fungi which occur in a yeast (Y) phase *in vivo* and under special conditions *in vitro*, but which usually grow in a mycelial (M) phase *in vitro* when incubated at room temperature. This characteristic has been termed "dimorphism". The dermatophytes do not show dimorphism.

Since the dermatophytes exhibit mycelial growth and grow well *in vitro* at temperatures from 25° C to 38° C (NICKERSON and WILLIAMS, 1947) it is possible that their inability to invade the deeper tissues may be correlated with the absence of an intracellular mechanism responsible for yeast to filament (F) (Y → F) conversion in the presence of bodily conditions that will suppress mycelial growth. Thus attempts by SULZBERGER (1929) and BROCQ-ROUSSEU et al. (1926) to precipitate a systemic and deep infection with dermatophytes that do not exhibit dimorphism failed. 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.

BAKER, MRAK, and SMITH (1943) have suggested that if the parasitic condition is considerd as abnormal for the devlopment of the mycelial form of the fungus, possibly the development of the fungus has been reduced to

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[1]) A portion of this work has been presented before the Genetics Society of America meeting in Columbus, Ohio, Sept. 11, 1950 (SCHERR, 1950).

[2]) This report is taken from a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Kentucky, Lexington.

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a form which produces the greatest number of reproductive structures with the least synthesis of new protoplasm. In other words, the environmental conditions imposed by animal tissues restrict mycelial development, which is less profitable from a reproductive standpoint. It should logically follow from this that any agent which would tend to transform the established yeast phase *in vivo* to the mycelial phase might serve to arrest the multiplication of the pathogen. This, presumably, would occur because of a metabolic blocking action within the parasitic cell, resulting from an enhancement of mycelial formation by the introduced agent on the one hand and a tendency to maintain the yeast phase by the bodily mechanisms on the other.

Because of the postulated possibility of treating infections due to yeastlike pathogenic fungi with Y → F enhancing substances, it was felt that a more extensive investigation of the effects of various compounds in inducing Y → F formation *in vitro* might be profitable. *S. cerevisiae* was chosen for this investigation for the following reasons:

1. It could be handled more easily because it is non-pathogenic.
2. Cause and effect of Y → F formation could be more easily correlated because it does not normally form a filamentous phase.
3. It was felt that, because *S. cerevisiae* is closely related antigenically and phylogenetically to a number of the yeastlike pathogenic fungi, many observations made using the former might be readily applicable to the latter.

HANSEN (1886) showed that *Saccharomyces* strains incubated at a temperature of approximately 20° C grow in characteristic round, budding cells, while a lowered temperature of incubation gives rise to growth in the form of filaments. Even before HANSEN, as LEVAN (1947) points out, brewers had observed such so-called involution forms (filaments) in aging yeast cultures. The sketch of a yeast from an old wine film reproduced by LAFAR (1911) from ADERHOLD (1894) shows the identical type of filament formation later described by many other workers. LEPESCHKIN (1903) observed the formation of a mycelium by *Schizosaccharomyces Pombe* and *Schizosaccharomyces mellacei*. The mycelial stage remained constant during numerous generations and this worker was unable to secure reversion to the original yeast phase. LEPESCHKIN's drawings of these filamenents are nearly identical with those of ADERHOLD (1894).

ANDERSON (1917) reported a decided change in the form of yeast cells in old cultures. In many cases the cells had elongated while remaining attached to each other, forming a hyphal thread constricted at the ends of the cells. In these old cultures giant cells, frequently ten times the diameter of the normal cells, were also present.

ZIKES' (1920) studies on the effect of temperature on yeast morphology indicated that lower temperatures give rise to elongated cells in different yeast genera and that there is also a tendency at lower temperatures for yeast cells to hang together in colonies. He found, in addition, that the normal appearance of the yeast cells was restored after incubation at an optimum temperature. ZIKES termed these aberrent forms "fluchtige Varietaten", or unstable variants. RICHARDS (1928) observed that cultures of *Saccharomyces 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) when compared to cultures incubated at either side of this temperature range.

Early work on the occurrence of spantaneous and induced variations in yeasts showed that some of the more commonly occurring variants developed as mycelial races from normal unicellular ones. NADSON and KRASSILNIKOV (1932) reported the occurrence of spontaneous variants in a yeastlike fungus,

Guilliermondella selenspora. 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 (1933) 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. ROCHLINA (1934, 1936) obtained variants of *S. cerevisiae* by using radium. Among these was a stable race composed of cells with numerous projections which were considered to be undeveloped buds with thin walls, but they might well have been sprouting hyphae from yeast cells.

MICKLE and JONES (1940) 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 of these approached the appearance of mycelial filaments.

NADSON (1937) 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 are accompanied by an increasing roughness of the colonies.

SEGAL (1938, 1939) described abnormal forms of yeasts resulting from their exposure 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. Yeasts exposed to the same alcohols in water produced no unusual forms, indicating that the cells were affected only while in the process of multiplication.

A fuller discussion of and numerous other references relative to this topic will be found in HENRICI's (1941) review.

A number of studies have been reported on the effect of carcinogenic chemicals on yeast cells. Giant cells and increased morphological differentiation of cells within the colony of *Saccharomyces ellipsoideus* Hansen were produced when the cultures were subjected to a solution of methylcholanthrene at a concentration of 1/50 of saturation in distilled water (DODGE and DODGE, 1937). DODGE (1940) found that cultures of *S. ellipsoideus* subjected to methylcholanthrene yielded occasional giant cells and more frequent division. DODGE, DODGE, and JOHNSON (1941), however, found only small differences in the dry weights of cultures that had been grown under standard conditions and those that had been exposed to methylcholanthrene. COOK, HART, and JOLY (1939) showed 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.

ROBINSON and STIER (1941) assayed, using the coleoptile test, the amount of auxin in yeast cultures grown in various media and found that 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.

BAUCH (1941), using camphor, was the first person to experimentally produce a high incidence of the filamentous forms in yeast. Later, BAUCH (1942) found greatly enlarged cell mutants in yeast cultures which were ex-

posed to 3,4-benzpyrene and methylcholanthrene; 1 : 2 : 5 : 6-dibenzanthracene was without effect.

The investigations of LEVAN and SANDWALL (1943) and LEVAN (1944, 1947) established that the camphor (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 were alicyclic, 14 were aromatic, and 2 were heterocyclic. The few bi -and polycyclic aromatic substances tested showed no activity, with the exception of alpha-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 increases in solutions up to 0.007 molar, in which concentration almost all living cells are such forms.

The outstanding features of the camphor reaction were:

1. The cells tended to hang together in colonies.

2. The reaction was incomplete, since normal cells were always found intermingled with cf-forms, even at the highest concentrations tested which would still permit cellular growth.

3. The morphology of the affected cells showed consistency only in that cell elongation and the inhibition of cellular division were pre-eminent. Aside from this, cells affected by different chemicals showed irregularly swollen cell aggregates, long filamentous cells, and numerous grotesque-looking cells of varied sizes and shapes. Note fig. 1 and 2, which are reproduced from LEVAN's (1947) paper.

4. There was an almost consistent inverse relationship between the cf-activity of the chemicals tested and their water solubility.

5. The cf-reaction induced by different chemicals was further enhanced by low temperatures (9°—20° C) and impeded by higher temperatures (30° C). Occasionally it was observed that low temperatures alone would favor cf-growth.

6. The cf-reaction was reversible in that cf-cells would begin to show normal budding processes if the concentrations of the cf-agents began to fall below their threshold value for cf-activity or if the yeast cells acquired a resistance to the cf-chemicals.

SUBRAMANIAM (1945) exposed cells of *S. cerevisiae* growing in wort medium to acenaphthene for 6 hours and found that "long threadlike mycelial growths" (fig. 3) became very common.

NICKERSON and VAN RIJ (1949) were able to shift the Y → M equilibrium toward the Y phase using organisms which very readily formed a mycelium. They found that a concentration of 10^{-2} molar of cysteine inhibited mycelial formation in cultures of *Candida albicans, Candida tropicalis, Trichosporon capitatum,* and *Hansenula anomala.* That this phenomenon could not be explained solely by redox potential differences was demonstrated by exposing these cultures to a 10^{-2} molar concentration of ascorbic acid, with no resultant inhibition of Y → M. It also appeared that some specificity was required of the — SH donor, since sodium thioglycollate was not as effective as glutathione, which in turn was less effective than cysteine, in inhibiting Y → M.

NICKERSON and VAN RIJ (1949) also showed that the characteristic effect of cysteine on *C. albicans* was to produce very short hyphal cells with very close spacing of blastospore clusters. A 10^{-2} molar concentration of cysteine reversed the enhancement of Y → M exhibited by subfungistatic concentrations of penicillin and cobalt ions. The Y → M promoting effects of cobalt were also antagonized by oxine (8-hydroxy quinaline). An examination was

made of other metal ions by these workers, but $CuSO_4$, $MnCl_2$, $NaHAsO_3$, 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 that shown by cobalt. Cells of *S. cerevisiae* exposed to penicillin became more elongated and grew in the form of filaments. Again, cysteine antagonized the effect of the penicillin.

The work of these authors supports their contention that "*cell division, as a process distinct from growth,* depends upon the maintenance of functional intracellular sulfhydryl groups".

Materials and methods

The culture of *S. cerevisiae* used in this study was originally isolated from a commercial pressed yeast cake by Dr. MARY HARDIN of the Department of Bacteriology, University of Kentucky, and has been carried as a stock strain in that department. Its identification was confirmed in this study by culturing in a synthetic medium (≠ 20 medium) containing different compounds as sole sources of carbon. Raffinose, sucrose, maltose, d-galactose, and glucose were utilized as sole sources of carbon; lactose, inulin, dulcitol, d-mannitol, d-sorbitol, d-xylose, d-arabinose, cellobiose, and melibiose were not utilized. Urea was utilized as a sole source of nitrogen. This is in accord with the STELLING-DEKKER (1931) scheme of classification.

The synthetic medium referred to as ≠ 20 medium is essentially a modification of WICKERHAM's (1946) medium.

One liter of the finished medium contains:

A.	1) KH_2PO_4	1 g
	2) $(NH_4)_2SO_4$	5 g
	3) $MgSO_4 . 7H_2O$	0.5 g
	4) NaCl	0.1 g
	5) $CaCl_2 . 2H_2O$	0.1 g
	6) Biotin	20 μg
	7) Inositol	2000 μg
	8) para-Aminobenzoic acid	200 μg
B.	9) Vitamin A	167.0 U.S.P. units
	10) Vitamin D	33.4 U.S.P. units
	11) Vitamin C (ascorbic acid)	1.67 μg
	12) Vitamin B_1 (Thiamine HCl)	11.1 U.S.P. units
	13) Vitamin B_2 (Riboflavin)	13.3 μg
	14) Vitamin B_6 (Pyridoxine HCl)	3.3 μg
	15) Niacinamide	166.8 μg
	16) Pantothenate acid	66.7 μg
	17) Carbon source	0.1 molar concentration
	18) Tap water to volume	

The salts indicated in group A were made up in a stock solution at twice the desired final concentration. Stock solutions of biotin, inositol, and para-aminobenzoic acid contained 5 μg/ml, 1 mg/ml, and 0.1 mg/ml, respectively. The ingredients in group B compose the commercial vitamin preparation known as "Vi-Syneral", manufactured by the U.S. Vitamin Corp. One ml of the Vi-Syneral was diluted 1 : 25 with distilled water to yield a stock solution. All solutions were stored in a refrigerator when not in use.

To prepare, for example, 500 ml of this medium, 250 ml of stock solution A, and 2ml of botion, 1 ml of inositol, 1 ml of para-aminobenzoic acid, and

4 ml of Vi-Syneral stock solutions were added to a 500 ml volumetric flask. The amount of carbon source added, depending on the compound used, was such as to yield a final concentration of 0.1 molar. Tap water was added to the mark and the completed medium was sterilized by filtration through a Seitz filter.

Although WICKERHAM (1946) included the trace elements boron, copper, iodine, iron (ferric), zinc, manganese, and molybdenum in his medium, he indicated that the addition of trace elements appeared to have little influence on growth even when a number of fastideous yeasts were tested. He concluded that sufficient trace elementes are present as impurities in other ingredients to supply the necessary ions.

All tap water referred to in this report was Lexington city water. An analysis of this water, made during Sept. 1948, was kindly provided by Mr. E. E. JACOBSON of the Lexington Water Co. It indicates approximately its usual composition.

Color	< 5
pH	7.7
Turbidity	$< 20 = 1$
Total solids	191 ppm
Dissolved solids	191 ppm
Suspended solids	0
Cl	4 ppm
$CaCO_3$	93 ppm
Alkali $CaCO_3$	77 ppm
Alkali as OH	0
Alkali as HCO_3	77 ppm
Ca	26 ppm
Mg	7 ppm
SO_4	14 ppm
Fe+++	0.1 ppm
Mn	0.0 ppm
Zn	0.0 ppm
Pb	0.0 ppm
Cr	0.0 ppm
Cu	0.0 ppm

The medium referred to in this report as S.G. medium was used by REILLY, HARRIS, and WAKSMAN (1947) for growing cultures of *Streptomyces griseus* (hence S.G.). It gave excellent growth with the yeast used in this study. Its composition is:

glucose	1.0 %
peptone	0.5 %
meat extract	0.5 %
sodium chloride	0.5 %
tap water	

The pH after sterilization in the autoclave at 15 lb. pressure for 15 min. was between 6.7 and 6.8. No adjustment was made. To make S.G. agar, 1.5 % Bacto agar was added.

The *Avena* coleoptile test used for auxin activity assay was essentially that given by BOYSEN-JENSEN (1936). The oat seeds were all *Avena sativa*, kindly provided from a single harvest by Dr. DAVID REID of the Department of Agronomy of the College of Agriculture at the University of Kentucky. This test was performed in a closed closet maintained at approximately 92 %

relative humidity. The only source of illumination reaching the oat seeds during the test came from a 60 watt frosted bulb encased in a cylinder containing 3 thicknesses of red cellophane.

The seeds were freed from their husks and were soaked in water for 2 to 3 hours. After this period they were laid out, groove downward, on wet filter paper in a Petri dish in the darkened closet. The seeds were allowed to germinate for about 30 hours, during which time they were intermittently exposed to the red light for a total of 15 minutes. The seedlings were then planted in glass holders of the type regularly used for this test (see WENT and THIMANN, 1937 ,p. 29), with the roots at the surface of tap water contained in a trough. After 48 hours the extreme tips of the seedlings were out off without cutting through the primary leaf. The primary leaves were then pulled so that they broke at the base and they were partially drawn out. Small blocks of agar, 40 × 20 × 20 mm, which had been impregnated with the compound to be tested, were then placed on one side of the cut surface of the leaf and held in place by capillarity. Ninety minutes after application of the agar blocks, the seedlings were photographed and the angle of curvature of each stem was measured with a protractor from photographic enlargements.

The greater the angle of positive curvature, the greater the auxin activity of the material in the agar blocks, within certain limits.

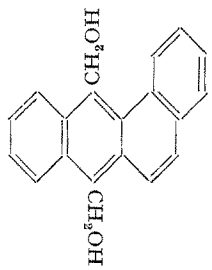
Unless indicated otherwise, all photomicrographs in this report were made in aqueous mount, unstained, and with the light microscope. The magnification indicated refers to the microscope magnification, but most of the photos have been magnified considerably beyond that by the use of a photographic enlarger during the printing of the positives.

Experimental

The Effect of 9 : 10-Bishydroxymethyl-1 : 2-benzanthracene (BB) on the Cell-Division Mechanism of *Sacchraromyces cerevisiae*

a) In S.G. broth cultures incubated with shaking

In an earlier study (SCHERR, unpublished data), the carcinogen 9 : 10-bishydroxymethyl-1 : 2-benzanthracene (BB) (I) was being used in an attempt



(I) 9:10—Bishydroxymethyl—1:2—benzanthracene

to induce biochemical mutants in *S. cerevisiae*. The carcinogenic properties of this compound have been reported by BADGER and COOK (1940). It has been successfully used as a mutagenic agent by SCHERR and WEAVER (1949). Shelved in the laboratory for some time was a small centrifuge tube which contained a culture of *S. cerevisiae* in S.G. medium (see *Materials and Methods*) to which had been added BB to a final concentration of 0.204 mg/ml. A loopful from this culture, examined microscopically, showed the presence of large numbers of cells growing elongated in the form of filaments (fig. 4).

The entire sediment from this centrifuge tube was transferred to a 125 ml

erlenmeyer flask containing 30 ml of S.G. broth and 5 ml of a stock carcinogen suspension (2.04 mg/ml in distilled water). This culture, designated 274D, was incubated at room temperature 22° C ±2°)[1] and examined periodically for 37 days. Microscopic studies of this and subsequent cultures were made in the following way.

The culture was well shaken by hand and 0.2 ml of the suspension, aseptically removed, was transferred to a 12 ml conical centrifuge tube, which was then spun for approximately 5 minutes at approximately 1500 rpm. The yeast cells are brought down with little difficulty. The supernatant was discarded and a portion of the sediment was transferred by loop to a slide and examined in wet mount under a cover glass. The results of the examinations made on culture 274D are described in table 1 and recorded photographically in fig. 5—10.

Not all of the yeast cells were visibly affected by the carcinogen in the transition from Y → F phase (fig. 8 and 9). Microscopic examination showed that the F forms occurred in packets, interspersed with normal-appearing budding yeast cells (fig. 5, 6, 10). This observation was particularly emphasized by LEVAN (1944), who, in referring to the camphor reaction, indicated 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*".

The older forms exhibited branching to a degree which made it difficult to ascertain which cells of the filament were the oldest (see, for eaxmple, fig. 6). It was also observed that inhibition of cell division[2] could occur without

TABLE 1. *Culture 274D of S. Cerevisiae exposed to 9 : 10-Bishydroxy-methyl-1 : 2-Benzanthracene in S.G. Broth at Room Temperature*

Time of incubation (days)	Legend
8	Frequent strings of cells having the appearance of "racquet mycelium. Most of the cells, however, are typical budding yeast cells.
12	F forms more numerous, but still no more than approximately 10 % of culture.
15	F forms don't appear to be getting more numerous, but it appears that those which had been formed are increasing in length (fig. 5).
21	Trend of growth indicated after 15 days incubation appears to be substantiated. Note fig. 6, which represents a typical field.
26	No noticeable change in morphology. Fig. 8 shows a filament resembling a racquent mycelium.
37	Culture composed predominantly of F forms (fig. 10). Crystals of carcinogen still present.

[1]) The room temperature recorded here will not necessarily be identical with room temperatures reported in subsequent work because of seasonal changes occurring during the course of this study.

[2]) In this report "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 on *S. cerevisiae* reported 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.

cell elongation taking place, so giving rise to strings of yeast phase cells. This is especially evident in fig. 7.

In order to confirm and extend the observations implicating BB in Y → F activity the following was performed.

The suspension of yeast cells to be used as an inoculum was prepared by inoculating *S. cerevisiae* into 30 ml of S.G. medium contained in a 125 ml erlenmeyer flask. This flask was then incubated at room temperature (23° C ± 2°) with shaking for 48 hours. Shaking was accomplished with a reciprocating shaker having 68 occillations per minute and a full stroke of $2\frac{1}{2}$ inches. This culture was then centrifuged, the supernatant was discarded, and the residue cells were suspended in 25 ml of sterile buffered distilled water (BDW) (American Public Health Association, 1948). This suspension was disseminated in 0.1 ml amounts into 125 ml erlenmeyer flasks, each of which contained 27 ml amounts of S.G. medium and 3 ml amounts of a serial dilution of BB suspended in distilled water, to give a series of flasks containing final concentrations of 0.00002 to 0.204 mg/ml of BB. Table 2 outlines the method used. The control flask contained no carcinogen. The flasks were all incubated at room temperature with shaking. The aberrent forms were scored at 0 and after 2, 10, 22.5, 27, 99, 195, 315, and 555 hours of incubation. Plate clounts were made up to and including 27 hours of incubation.

In scoring the different types of aberrent forms found, the same technique was employed as previously outlined. The elongated cells which occur in chains were scored as filaments (F). Such filaments have been illustrated in fig. 9.

TABLE 2. *Serial Dilution of* 9 : 10-*Bishydroxymethyl*-1 : 2-*Benzanthracene in S.G. Broth Inoculated with Saccharomyces Cerevisiae*

S.G. Medium Flask	(ml)	BB in distilled water Dilution	(ml)	Final Conc. of BB in S.G. (Mg/ml)
1	27	stock	3	0.20400
2	27	1 : 10	3	0.02040
3	27	1 : 100	3	0.00204
4	27	1 : 1000	3	0.00020
5	27	1 : 10000	3	0.00002
6	30	—	0	0.00000

Although short filaments of cells were occasionally found (fig. 11), a culture of elongated cells of *S. cerevisiae* in which the inhibition of cell division was not also a concomitant phenomenon was not observed. The converse is by no means true, since the inhibition of cell division has been frequently observed without any accompanying cell elongation (fig. 12). I have called such a group of cells an "inflorescence" (Infl), using a term borrowed from the botanists. All cells scored as inflorescences fail to show evidence of cell elongation.

The relative number of inflorescences and filaments are estimated and recorded as follows:

—	none found
±	rare
+	few
++	frequent
+++	many
++++	very many

The results of this experiment are recorded in table 3 and plotted, in part, in the following graph. This graph only records the data up to and including 27 hours of incubation.

Filaments appeared in the cultures containing BB after 10 hours of incubation and increased in frequency up to the end of the experiment. Caution

TABLE 3. *The effect of* 9 : 10-*Bishydroxymethyl*-1 : 2-*Benzanthracene* (*BB*) *on Saccharomyces Cerevisiae in S.G. Medium incubated at room temperature with shaking*

Time of incubation (hours)	Conc. of BB (mg/ml)	Plate Count			Infl*	F**
		Dilution	No. of colonies	Count per ml		
0	0.00000[1]	10^4	32 20	2.6×10^5	—	—
2	0.20400	10^3	149 140	1.5×10^5	—	—
	0.02040	10^3	98 120	1.1×10^5	—	—
	0.00204	10^3	122 109	1.2×10^5	—	—
	0.00020	10^3	170 187	1.8×10^5	—	—
	0.00002	10^5	4 10	7.0×10^5	—	—
	0.00000	10^4	22 10	1.6×10^5	—	—
10	0.20400	10^5	54 31	4.3×10^6	—	±
	0.02040	10^5	22 102	6.2×10^6	±	+
	0.00204	10^5	28 35	3.2×10^6	±	+
	0.00020	10^5	46 31	3.9×10^6	±	+
	0.00002	10^5	83 99	9.1×10^6	—	—
	0.00000	10^5	61 59	6.0×10^6	—	—
22.5	0.20400	10^5	77 89	8.3×10^6	—	++
	0.02040	10^5	70 90	8.0×10^6	—	++
	0.00204	10^5	98 72	8.5×10^6	—	++
	0.00020	10^5	110 91	1.0×10^7	—	+

[1]) Counts at zero hours of incubation were determined only on the control. Since all flasks received equal aliquots of the cell suspensions of *S. cerevisiae* it is assumed that, within experimental error, equal numbers of cells were distributed.

* - Inflorescenses

** - Filaments

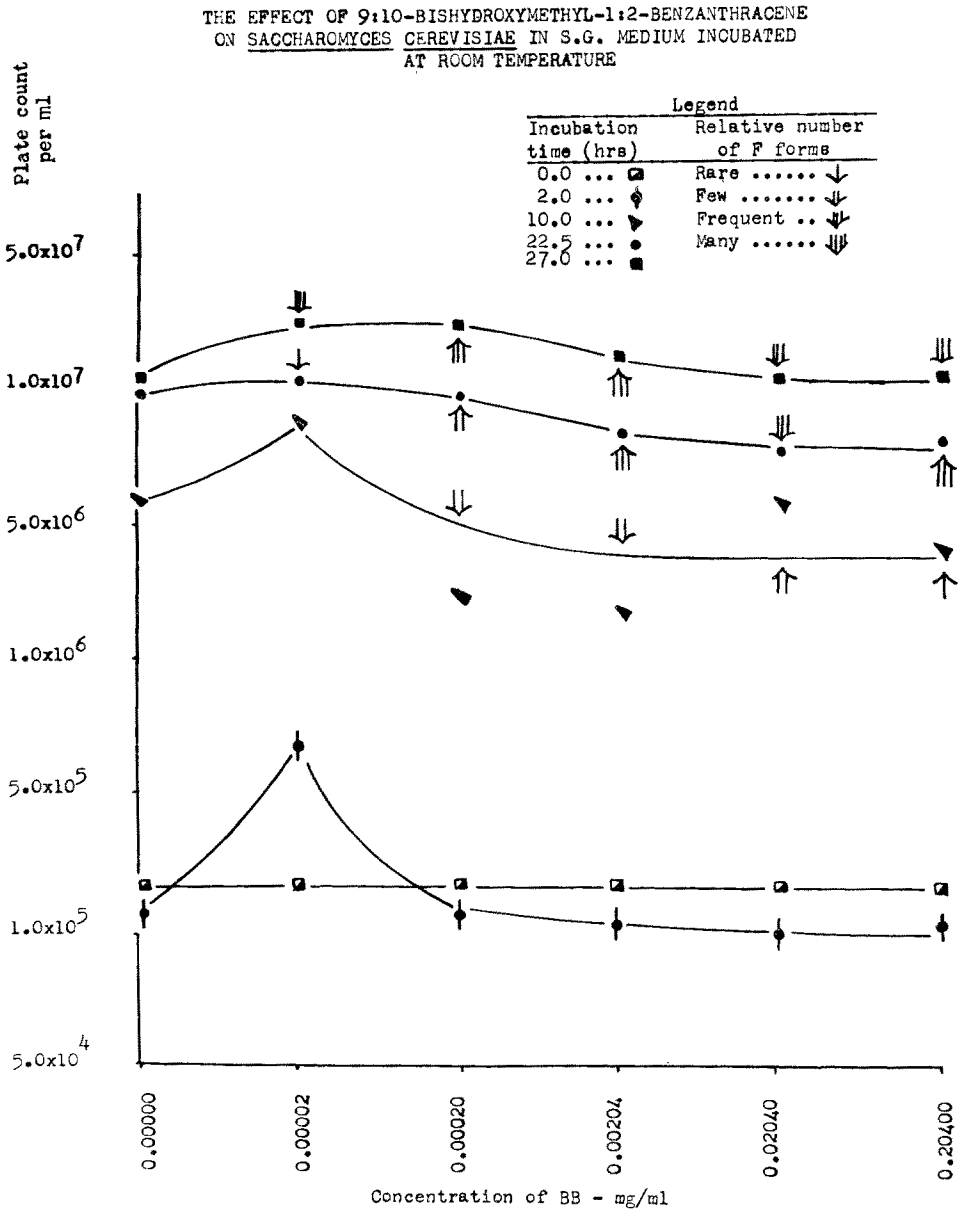
TABLE 3 (con'd)

Time of incubation (hours)	Conc. of BB (mg/ml)	Plate Count			Infl	F
		Dilution	No. of colonies	Count per ml		
	0.00002	10^5	163 141	1.5×10^7	—	±
	0.00000	10^5	131 110	1.2×10^7	—	—
27	0.20400	10^5	175 141	1.6×10^7	—	++
	0.02040	10^5	185 199	1.9×10^7	—	++
	0.00204	10^5	220 265	2.4×10^7	—	++
	0.00020	10^2	333 321	3.3×10^7	—	++
	0.00002	10^5	392 351	3.7×10^7	—	++
	0.00000	2×10^5	74 57	1.3×10^7	—	—
99[1]	0.20400				—	+
	0.02040				—	++
	0.00204				—	+++
	0.00020				—	+++
	0.00002				—	+++
	0.00000				—	±
195	0.20400				—	+
	0.02040				—	+
	0.00204				—	+
	0.00020				—	++
	0.00002				—	—
	0.00000				—	±
315	0.20400				—	+
	0.02040				—	++
	0.00204				—	++
	0.00020				—	—
	0.00002				—	++
	0.00000				—	++
555[2]	0.20400	Very large isolated clumps of F forms present. These clumps macroscopically visible and settle easily to the bottom of the flask where they appear as pellets about the size of the head of a pin. Normal-appearing yeast cells are still very much in evidence, with occasional asci being seen.				

[1]) From this point all flasks were incubated at room temperature without shaking. Plate counts were discontinued.

[2]) All flasks had been discarded except that containing 0.20400 mg/ml of BB.

must be used in interpreting the comparative effects of the different concentrations of BB, since its solubility is low. SCHERR (1949), using a technique developed by DAVIS, KRAHL, and CLOWES (1942), determined the solubility



to be 0.00102 mg/ml in distilled water. The solubility in S.G. medium has not been determined. Note that beyond a certain concentration of the carcinogen (between 0.00020 and 0.00002 mg/ml), which is probably the point of maximum solubility in this medium, the relative number of filaments remained essentially the same. The low solubility of BB probably also results in an appreciable lapse of time between the addition of the carcinogen to the

medium and the point at which the soluble and insoluble fractions reach equilibrium. This factor was not evaluated. Undoubtedly the higher concentrations used in this experiment contained the same amount of soluble BB plus some of the undissolved compound. This is suggested by the straight line sections of the curves for the higher concentrations of BB for the various periods of incubation as indicated in the above graph.

After 2 hours of incubation the growth in the flask containing 0.00002 mg/ml of the carcinogen was nearly 5 times as great as in the control. With larger amounts of carcinogen and longer periods of incubation this stimulating effect decreased or disappeared. At this concentration of BB (0.00002 mg/ml) frequent filaments were not seen previous to the 27th hour of incubation. TOPLEY and WILSON (1946) indicate that there is a concentration for nearly all salts, usually very low, which stimulates microbial growth. This phenomenon may be the explanation for the peak of growth found with the 0.00002 mg/ml concentration of BB after 2 hours of incubation.

Counts on the control culture and on the cultures containing higher concentrations of BB were slightly reduced after 2 hours of incubation, indicating death of some yeast cells, probably due to the shock of being placed in a new environment. The counts on the cultures containing the higher concentrations of BB remained slightly below those of the control after 10 and 22.5 hours of incubation but were slightly above those of the control after 27 hours.

Caution must be used in interpreting these data as indicating that the growth of the yeast was either inhibited or stimulated by the carcinogen BB. Each filament of yeast cells is composed of numerous cells and such a unit would, of course, give rise to a single colony; thus plate counts furnish a poor basis for comparing growth in cultures containing variable numbers of filaments. Attempts to break up the filaments by shaking were unsuccessful and attempts to separate the cells by micromanipulation indicated that they adhered to each other tenaciously. This observation was also made by LEVAN (1947) for cf-forms of yeast cells.

The highest incidence of F forms was found after 99 hours of incubation, yet many Y forms were still evident (fig. 13 and 14). Some decrease in F forms could be noted after 195 and 315 hours. Though it is possible that this decrease in the number of F forms after 99 hours of incubation is a real effect, this seems unlikely. Such a decrease could occur if the rate of growth of the F forms were to become markedly slower than that of the Y forms after a certain period of incubation, if the F forms were to autolyze at a faster rate than the Y forms, or if F $\rightarrow$ Y conversion were suddenly to occur. Any one of these possibilities would also require that the suggested mechanism be reversed sometime before 555 hours of incubation, since, at least for the single culture examined after this period of incubation, the number of F forms by far exceeded the number of Y forms, and F formation had progressed to a degree where microscopic pellets containing enmeshed filaments of cells could easily be detected. It seems more likely that the method employed here lacks the refinement of being able to distinguish differences of the order of + or even ++ consistently. A more refined method has been employed in some of the experiments to be described, but since the primary object in this work was to detect major differences in F formation induced by various agents, the method used here, requiring a minimum of time and labor, sufficed. Thus it has been used in most of the experiments recorded here.

As the age of the culture increased the F forms found were increasingly longer and fewer shorter filaments could be found. It appeared as if those filaments which had been formed at the onset of the experiment had become

longer, while few new F forms were being induced with increased time of exposure to the carcinogen. This suggested that there might be a marked variation among the yeast cells in their susceptibility to the agent inducing the formation of F forms or that the F forms represented mutations which were induced during initial exposure of the cells to the carcinogen and that those cells in the yeast phase represented the progeny of the more resistant cells which had survived the initial shock of the toxic and mutagenic effects of the carcinogen. A fuller discussion of these possibilities will be presented later.

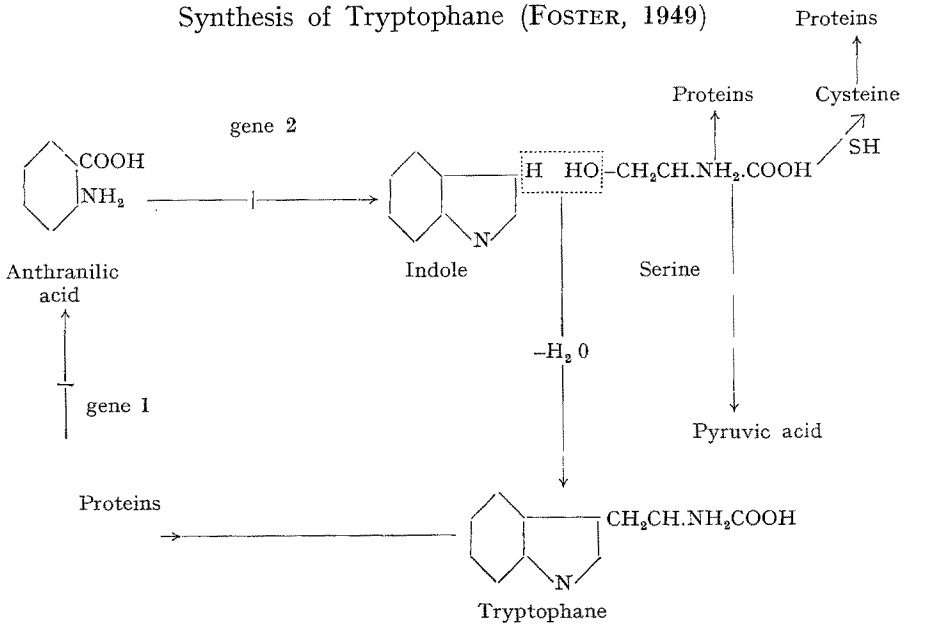
Giant forms of yeast cells became more prevalent in the cultures containing BB (fig. 15). Although these giant forms may also be found in control cultures containing no BB, their incidence appears to be enhanced by the carcinogen and by increased periods of incubation. These forms have not been scored in this work. Numerous other workers have recorded their occurrence under varied conditions (DODGE and DODGE, 1937; SUBRAMANIAM, 1945). Their designation as polyploids by SUBRAMANIAM may well be applicable here, but to determine this would require a genetic analysis.

The fact that gained immediate attention was the enhancement of cell elongation. It is important to note that F forms may be found in the control flask containing no BB after an extended period of incubation. This observation is in accord with those made by other workers whose papers have already been reviewed (ADERHOLD, 1894; ANDERSON, 1917), and will be demonstrated again in most of the experiments reported here.

That an increased concentration of auxin[1] may have been a possible cause of the elongated yeast cells was considered. Such an effect might be anticipated from work which will be briefly reviewed.

The work of TATUM and BONNER (1944) and TATUM, BONNER, and BEADLE (1944) supports the view that tryptophane is synthesized as illustrated in the following scheme.

Synthesis of Tryptophane (FOSTER, 1949)



[1]) The term "auxin" will be used in this report to refer to those substances which bring about the specific growth reaction in which the degree of curvature of *Avena* coleoptiles is affected. This is in accord with the practice of the workers in the field of plant physiology.

If the action of BB were to make -SH groups unavailable for cysteine synthesis by some blocking action, then this would result in the accumulation of serine and tryptophane and a further shift in the direction of production of 3-indole acetic acid (heteroauxin), which stems from an oxidative deamination of tryptophane (THIMANN, 1935a; WILDMAN, FERRI, and BONNER, 1947; WILDMAN and MUIR, 1949). The accumulation of heteroauxin might result in the elongation of yeast cells, since elongation of plant cells is a characteristic effect of auxins (WENT and THIMANN, 1937). It is, in fact, the basis for the *Avena* test for auxin assay.

The specific interaction of carcinogenic compounds with the thiol groups of -SH activated enzymes was demonstrated by the researches of RONDONI (1948a, 1948b), RONDONI and BASSI (1948), and RONDONI and BARBIERI (1950). 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 non-carcinogens did not inhibit autoproteolysis. CRABTREE (1944, 1945) was also able to show that certain substances which readily combine with -SH groups could partially inhibit the carcinogenic activity of chemical carcinogens. He 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 act by combining with -SH containing groups, and their interference with carcinogenicity functioned by interference with sulfur (S) metabolism. CRABTREE suggests 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 (1940) also suggested that a coupling between carcinogen and S-containing cell constituents was the first possible stage in their biological action.

The work of NICKERSON and VAN RIJ (1949) implicating -SH groups in the dimorphism mechanism of yeasts has already been mentioned.

The fact that auxin is elaborated by yeast cultures has been demonstrated by a number of workers (ROBINSON and STIER, 1940, 1941; WENT and THIMANN, 1937, p. 71). The work of KOGL and KOSTERMANS (1934) has shown that the auxin content of yeasts may be attributed almost entirely to 3-indole acetic acid. THIMANN (1935a, 1935b) has shown that the yield of auxin in cultures of *Rhizopus* was determined by the amount of tryptophane present. The reader is referred to the excellent monograph on the plant growth sybstances by WENT and THIMANN (1937) for a comprehensive presentation of this topic. A section on phytohormones is also contained in PINCUS and THIMANN's (1950) volume on *The Hormones*.

If the action of BB in enhancing Y → F formation were on a -SH grouping, then it would be expected that the addition of a sulfhydryl containing compound would antagonize the effect of BB. Accordingly, the following experiment was performed, utilizing cysteine as the sulfydryl-containing compound.

b) In synthetic medium with the addition of cysteine, stationary cultures.

For this experiment a synthetic medium, synthetic medium # 20, (see *Materials and Methods*) which is a modification of WICKERHAM's (1946) medium was used. Such a medium was used so that the quantity and type of S- and -SH containing compounds present during the course of the experiment would be known and controlled.

Into 6 tubes were measured 0.05 ml amounts of a stock suspension of

BB (2.04 mg/ml) in distilled water. To 3 of these tubes were also added 0.5 ml amounts of 0.1 molar l (+) cysteine hydrochloride (PFANSTIEHL). Medium # 20 was added to the experimental tubes to a final volume of 5 ml. Three control tubes were prepared to contain 5 ml of # 20 medium. To all 9 tubes were added 0.05 ml amounts of a suspension of *S. cerevisiae* cells prepared by harvesting the cells by centrifugation from a 24 hour culture grown in 30 ml of S.G. broth incubated at 25° C, washing them with BDW, and then suspending the cells in 10 ml of BDW. Tubes were prepared in triplicate so that incubation could be accomplished at 20°, 30°, and 37° C. The cultures were not shaken during incubation. The Infl and F forms were scored after 5 and 10 days of incubation in the manner previously described.

The amount of growth in the cultures was estimated by using a technique similar to that developed by WICKERHAM and BURTON (1948). By this method the culture is shaken and held before a white card upon which are drawn a number of parallel lines in India ink. The ease with which the lines are observed through the culture liquor is inversely proportional to the amount of growth and is arbitrarily rated as follows:

—	lines are distinctly seen
±	lines very slightly blurred
+	lines slightly blurred
++	lines very blurred
+++	lines barely seen
++++	lines obliterated

The results of this experiment are outlined in table 4.

The dimorphism effect (Y → F) was less pronounced in the # 20 medium stationary cultures than in the S.G. broth cultures incubated with shaking (table 3). The complete absence of F forms in all the tubes, even after 10 days of incubation, is in striking contrast to the previous experiment.

BB and cysteine in combination were more toxic than BB alone, a reaction which may be attributed almost solely to the action of the cysteine. The data are recorded in table 5a. The 0.01 molar concentration of cysteine at the 37° C incubation temperature proved to be more toxic alone than when it was used in conjunction with BB. This suggests that some of the cysteine may have been chemically bound by the BB, at least at 37° C. Here, too, no F forms were found.

A greater number of inflorescences (fig. 16) was found when BB and cysteine were used together than when either was used alone. This suggests that 0.01 molar cysteine may be too toxic to permit sufficient growth for the expression of inflorescences, but that when it is combined with BB some of the cysteine is oxidized (or neutralized in some other way) so that a more optimum concentration for Y → F activity results. A very large portion of the Y → F activity in the BB — cysteine mixture should be credited to cysteine, since BB alone in this medium showed little Y → F activity. The Y → F activity of cysteine presumably might be due to a shift in the tryptophane synthesis resulting from an upset equilibrium: cysteine → serine → tryptophane → heteroauxin.

Support for the idea that some neutralization of cysteine may be attributed to BB stems from the fact that when the concentration of cysteine was reduced tenfold to 0.001 molar, most of the toxic effects found when 0.01 molar concentration was used disappeared and a much stronger Y → F effect occurred (see table 5b). Also, when 0.01 molar cysteine was combined with 0.0204 mg/ml concentration of BB, the Y → F effect was comparable to that which was found with 0.001 molar cysteine alone (compare tables 4 and 5b).

TABLE 4. *The effect of* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene* (*BB*) *on the cell-division mechanism of Saccharomyces cerevisiae cultured in* # 20 *synthetic medium*

Incubation		Control			BB (0.0204 mg/ml)			BB (0.0204 mg/ml) + 1(+) cysteine (0.01 molar)		
Days	Temp (°C)	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F
5	20	++++	—	—	+++	+	—	++	+++	—
	30	+++	—	—	+++	+	—	++	+++	—
	37	+++	—	—	+++	++	—	++	+++	—
10	20	++++	++	—	++++	++	—	++	++++	—
	30	++++	±	—	++++	+	—	++	++++	—
	37	++++	+	—	++++	++	—	+++	++++	—

TABLE 5a. *The effect of* 1(+) *cysteine on the cell-division mechanism of Saccharomyces cerevisiae cultured in* #20 *synthetic medium*

Incubation		Control			0.01 molar 1 (+) cysteine		
Days	Temp (°C)	Growth	Infl	F	Growth	Infl	F
5	20	++++	+	—	++	++	—
	30	++++	+	—	++	++	—
	37	++++	+	—	+[1]		
10	20	++++	+	—	++	++	—
	30	++++	+	—	+++	+++	—
	37	++++	+	—	+		

[1] No estimations of inflorescences and filaments were made if the amount of growth was below ++

It is possible that BB and cysteine both display Y → F activity due to different and possibly unrelated mechanisms and the combined effect is purely an additive phenomenon.

There is the possibility that BB may have been chemically inert when it was used in the # 20 medium cultures. This is unlikely, since there were such marked differences in inflorescence formation between the experiments with 0.01 molar cysteine alone and those in which it was in combination with 0.0204 mg/ml of BB.

The possibility that BB may have been physiologically inert for *S. cerevisiae* in the # 20 medium cultures is suggested by the fact that the inflorescence activity in #20 medium containing BB, when compared to controls lacking it, was very slight and bordered on the limits which could be graded as significant.

It is entirely possible that the more marked Y → F activity displayed by BB in S.G. broth may be attributed to one or more of the following:

TABLE 5b. *The effect of* 1(+) *cysteine and* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene* (*BB*) *on the cell-division mechanism of Saccharomyces cerevisiae cultured in* #20 *synthetic medium*

Incubation		Control			(1+) cysteine (0.001 molar)			BB (0.0204 mg/ml) + (1+) cysteine (0.001 molar)		
Days	Temp (°C)	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F
5	20	++++	+	—	+++	++	±	++++	++	±
	30	+++	+	—	+++	+++	±	+++	+++	—
	37	+++	±	—	++	+++	—	++	+++	—
10	20	++++	++	—	++++	+++	±	++++	+++	±
	30	++++	++	—	++++	+++	±	+++	+++	—
	37	++++	++	—	++++	++++	—	+++	++++	±

1. The modification by BB in S.G. broth of -SH containing compounds outside of the yeast cell, which then enter the cell in their modified form and disrupt some biochemical system controlling the dimorphism mechanism. The synthetic medium #20 contains no -SH groups, but the S.G. broth no doubt does contain -SH compounds.

2. The modification of the -SH containing compounds outside of the cell, thus making them inaccessible to the yeast cell. This might require some modification in the cell's physiology in order to make up for a lack of preformed -SH groups.

3. A higher oxygen tension in cultures in S.G. broth incubated with shaking than in the 5 ml of synthetic medium in a test tube maintained in a stationary position. The formation of auxin from tryptophane proceeds through a biochemical pathway involving an oxidative deamination (THIMANN, 1935a; WENT and THIMANN, 1937).

The possibility of (1) is suggested by the facts that the synthetic medium used here contains no free -SH groups and that BB has little influence on Y → F formation in this medium. If BB were inducing Y → F formation in the S.G. broth by affecting some intracellular system directly, then it might be expected that the cell wall and/or the membrane of the yeast cell would not be impermeable to this carcinogen by dint of being grown in another medium, unless this other medium lacked some essential materials which might result in faulty synthesis of protoplasmic materials within the cell, or unless the composition of the medium itself were a direct factor in affecting this permeability.

The second possibility seems to have little foundation, since cells grown in #20 synthetic medium are not exposed to -SH groups and yet do not show Y → F formation.

The third possibility gets some support from the fact that cells grown in S.G. broth under the same conditions as used for the synthetic medium did not show as much Y → F activity as those in the S.G. broth cultures incubated with shaking. This knowledge was gained from the following experiments.

c) In S.G. broth stationary cultures

The experiment recorded here is a duplicate of the one described in section *b* in every respect except that S.G. broth was substituted for #20 synthetic medium. The results are outlined in tables 6 and 7.

The Y → F activity displayed by BB in stationary test tube cultures using S.G. broth was intermediate between that found in stationary test tube cultures using #20 synthetic medium and that found in S.G. broth cultures incubated with staking. These results cannot be explained entirely on the basis of oxygen tension unless it can be shown that the stationary cultures in S.G. medium had a higher oxygen tension than the cultures in #20 synthetic medium.

The enhanced Y → F activity found when BB and cysteine were used together in the synthetic medium also occurred in this experiment.

The increased F formation induced by BB in the S.G. broth stationary cultures, as compared to that in the synthetic medium cultures, strongly suggests that the presence of -SH groups in a medium can markedly modify the morphological disturbance induced by agents active in the production of Y → F transformations. The confirmation of this depends upon the demonstration of a direct correlation between the amount of -SH groups in a medium and the degree of Y → F activity induced in yeast cells by some agent.

If, as has been suggested by the data in tables 4 and 6, BB reacts in some way with cysteine, then it should be possible to demonstrate this aside from its effect on the growth of yeast cells. Since the most labile portion of the cysteine molecule is probably the -SH group, it was felt advisable to look for a change at this portion of the molecule first.

TABLE 6. *The effect of* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene* (*BB*) *on the cell-division mechanism of Saccharomyces cerevisiae cultured in tubes of S.G. broth*

Incubation		Control			BB (0.0204 mg/ml)			BB (0.0204 mg/ml) + 1(+) cysteine (0.01 molar)		
Days	Temp (°C)	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F
5	20	++++	—	—	++++	±	—	++++	++	++
	30	++++	—	—	++++	+	±	+++	++	+
	37	++++	—	—	++++	+	+	++++	++	±
10	20	++++	—	+	++++	±	++	++++	++	+++
	30	++++	±	+	++++	+	+	++++	+	+++
	37	++++	—	+	++++	±	+	++++	++	++

d) The *in vitro* reaction between 9 : 10-bishydroxymethyl-1 : 2-benzanthracene and 1(+) cysteine in sterile #20 synthetic medium.

The method used for determining -SH groups was taken from SIGGIA (1949) and is a modification of the KIMBALL, KRAMER, and REID (1921) iodometric technique. This method utilizes a fixed amount of iodine to oxidize -SH groups, which are calculated by backtitrating the unused iodine with sodium thiosulfate, using starch solution as an indicator. The 0.1 N iodine, 0.05 N sodium thiosulfate, and starch indicator solutions were prepared according to the method of HAMILTON and SIMPSON (1948).

The 0.1 N iodine solution was always added as a 10.00 ml quantity, using a burette, to the solution to be examined and back-titrated by burette with 0.05 N $Na_2S_2O_3$ solution, using a starch indicator. Although the standard procedure for the iodometric titration (SIGGIA, 1949) calls for 0.1 N $Na_2S_2O_3$

solution, it was hoped to gain more sensitivity by using 0.05 N. Since temperature corrections for the volume readings of the burettes used in this work were not made, every titration, both for the iodine and the $Na_2S_2O_3$ solutions, was begun at the 0.00 ml marking on the burette.

TABLE 7. *The effect of* 1(+) *cysteine and* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene* (*BB*) *on the cell-division mechanism of Saccharomyces cerevisiae cultured in tubes of S.G. broth*

Incubation		Control			1(+) cysteine (0.01 molar)			(0.001 molar)			BB (0.0204 mg/ml) + 1(+) cysteine (0.001 molar)		
Days	Temp (°C)	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F
5	20	++++	—	—	++++	—	—	++++	—	—	++++	—	—
	30	++++	—	—	+++	+	—	++++	++	—	+++	+	—
	37	++++	—	—	++++	+	—	++++	±	—	++++	+	—
10	20	++++	—	+	++++	+	++	++++	+	+	++++	+	+
	30	++++	±	+	++++	+	+	++++	++	+	++++	++	+
	37	++++	—	+	++++	+	+	++++	+	+	++++	+	±

BB and cysteine, both singly and together, were added to sterile #20 synthetic medium and quantitative determinations of the residual -SH groups were made on 0.1 ml aliquots, using the iodometric technique, immediately before and after incubation for 20 hrs at 20° C and 37° C. In all cases the concentrations of BB and cysteine in the #20 medium were 0.0204 mg/ml and 0.01 molar, respectively.

Controles consisted of titrations of 10.0 ml of 0.1 N iodine and of 1.0 ml of #20 medium, The results are outlined in table 8.

The titres of 0.05 N $Na_2S_2O_3$ for the 10 ml amount of 0.1 N I_2 solution were essentially the same at the start of this experiment and after these solutions had remained at 20° for 20 hours. However, a 1.0 ml aliquot of sterile #20 medium gave a titre with $Na_2S_2O_3$ significantly higher than that for the 10 ml amount of 0.1 N I_2 alone, indicating that one or more oxidizing components in the #20 medium were being reduced by the $Na_2S_2O_3$. The titre for the #20 medium did not change after 20 hours of incubation at 20° C. The titre for the 10 ml amount of I_2 was 20.05 ml of $Na_2S_2O_3$, and for the 1 ml aliquot of # 20 medium plus 10 ml of I_2 it was 20.18 ml. It might, therefore, seem to be necessary to subtract the difference between these two values (00.13 ml) from all the other determinations for BB and cysteine in # 20 medium. This was not done because:

1. The 1 ml amount of # 20 medium when titrated alone is not exactly equal to 1 ml of # 20 medium when it contains BB and/or cysteine.

2. The primary objective of this experiment was to determine whether any oxidation of -SH groups could be attributed to BB. This information could be obtained independently of the titre for the # 20 medium. The purpose in titrating the # 20 medium was to determine if after 20 hours any change in its oxidation-reduction potentialities occurred which might interfere with the iodometric titration of -SH groups. Apparently, such was not the case under the conditions of this experiment.

The titre for cysteine alone was equal to that for cysteine plus BB, in-

dicating that no oxidation of the cysteine by BB occurred, at least not in a quantity that could be detected by this method. The data, however, shows that the quantity of cysteine used could be detected by this method. Some oxidation of the cysteine occurred after 20 hours, the same quantity at both 20° C and 37° C.

TABLE 8. *The in vitro reaction between* 9 : 10-*bishydroxymethyl* 1 : 2-*benzanthracene* (*BB*) *and* 1(+) *cysteine in sterile* #20 *synthetic medium*

Tube No.	Legend [1]	Incubation		0.1 N I_2	0.05 N (ml)	$Na_2S_2O_3$ Average (ml)
		Temp °C	Hrs.			
1	Standardization			10.00	20.05 20.05	20.05
2	0.0204 mg/ml BB in #20 medium		0	10.00	20.00 20.05	20.03
4	0.01 molar cysteine in #20 medium		0	10.00	18.90 18.85	18.88
6	0.0204 mg/ml BB plus 0.01 molar cysteine in #20 medium		0	10.00	18.80 18.90	18.85
8	#20 medium		0	10.00	20.20 20.15	20.18
2	0.0202 mg/ml BB in #20 medium	20	20	10.00	20.05 19.95	20.00
3	0.0204 mg/ml BB in #20 medium	37	20	10.00	20.00 20.05	20.03
4	0.01 molar cysteine	20	20	10.00	19.10 19,10	19.10
5	0.01 molar cysteine in #20 medium	37	20	10.00	19.10 19.15	19.13
6	0.0204 mg/ml BB plus 0.01 molar cysteine in #20 medium	20	20	10.00	19.05 19.10	19.08
7	0.0204 mg/ml BB plus 0.01 molar cysteine in #20 medium	37	20	10.00	19.05 19.05	19.05
8	#20 medium	20	20	10.00	20.20 20.20	20.20
1	Standardization	20	20	10.00	20.00 20.05	20.03

[1]) In every case a 1 ml aliquot of each sample was titrated in duplicate.

Some evidence, though admittedly circumstantial, that the reaction between BB and cysteine probably occurs at the -SH portion of the molecule can be gained from the data of the next experiment.

e) The effect of dl-serine and 9 : 10-bishydroxymethyl-1 : 2-benzanthracene (BB) on the cell-division mechanism of *Saccharomyces cerevisiae* cultured in # 20 synthetic medium.

If the reaction between BB and cysteine occurred at some point(s) of the cysteine molecule other than the -SH group, then it is possible that an effect similar to that found in the experiment outlined in section b (table 4) would occur between BB and serine (II), since serine differs from cysteine in structure only for the -SH portion of the cyteine molecule.

$$\begin{array}{l} HO.CH_2CHCOOH \\ \quad\quad\quad\quad | \\ \quad\quad\quad\quad NH_2 \end{array}$$

(II) Serine

Accordingly, the experiment outlined in section b was duplicated, using serine in place of cysteine. The only change made was that twice the quantity of serine was used, since the dl form was being used. The results of this experiment are outlined in tables 9 and 10.

The Y → F activity of the BB-serine mixture was less than the activity found in the BB-cysteine mixture under comparable experimental conditions (compare tables 4 and 9). In addition, the serine, used either alone or in conjunction with BB, was much less toxic to the yeast cells than cysteine was.

Serine showed Y → F activity greater than that found for 0.01 molar cysteine (compare tables 5a and 10), but the comparatively low Y → F activity of the 0.01 molar cysteine, as has already been mentioned, may be attributed to its toxicity. Thus, when 0.001 molar cysteine was used, the toxic effects were decreased and the Y → F activity was on a par with that found for serine (compare tables 5b and 10).

TABLE 9. *The effect of dl-serine and* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene (BB) on the cell-division mechanism of Saccharomyces cerevisiae cultured in #20 synthetic medium*

Incubation		Control			BB (0.0204 mg/ml)			BB (0.0204 mg/nl) + dl serine (0.02 molar)		
Days	Temp	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F
5	20	++++	+	—	++++	+	—	++++	++	±
	30	++++	±	—	++++	+	—	++++	++	—
	37	++++	++	—	++++	++	—	+++	+++	—
10	20	++++	++	—	++++	++	—	++++	++	—
	30	++++	+	—	++++	+	—	++++	++	—
	37	++++	++	—	++++	++	—	+++	+++	—

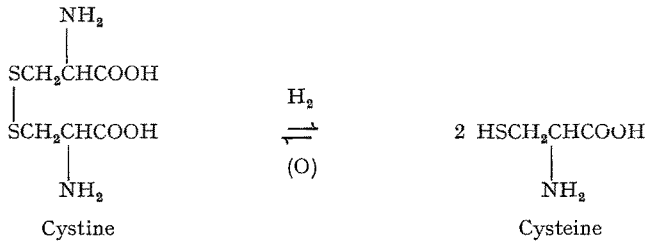
TABLE 10. *The effect of dl-serine on Saccharomyces cerevisiae in synthetic medium # 20*

Incubation		Control			o.o2 molar dl serine		
Days	Temp	Growth	Infl	F	Growth	Infl	F
5	20	++++	+	—	++++	++++	—
	30	++++	+	—	++++	++	—
	37	++++	+	—	++	+++	—
10	20	++++	+	—	++++	+++	±
	30	++++	+	—	++++	+++	—
	37	++++	+	—	++++	++++	±

The essential difference between the molecules of cysteine and serine is that the -SH substituent in cysteine is replaced by an OH group in serine.

The toxicity of 0.01 molar cysteine may be due to some activity of the -SH group, or possibly to the activity of an oxidation product of cysteine (these two possibilities may be essentially the same thing). If the latter speculation is correct, the oxidation product of cysteine would not be serine, since 0.02 molar dl-serine displays very little toxicity when compared to cysteine.

The evidence for the presence of cysteine in proteins is indirect, resting with the identification of -SH groups in some proteins (FIESER and FIESER, 1944). FIESER and FIESER also indicate that the difficulty of isolating cysteine results from the ease with which it is readily oxidized to cystine.



Cystine Cysteine

Accordingly, it was felt advisable to examine the Y → F activity of cystine for *S. cerevisiae* in # 20 medium.

f) The effect of l-cystine and 9 : 10-bishydroxymethyl-1 : 2-benzanthracene (BB) on the cell-division mechanism of *Saccharomyces cerevisiae* cultured in # 20 synthetic medium.

This experiment was a duplicate of the one using cysteine, which is described in section d, except that l-cystine was used in place of l-cysteine. The data are recorded in table 11.

TABLE 11. *The effect of* l-*cystine and* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene* (*BB*) *on the cell-division mechanism of Saccharomyces cerevisiae cultured in #20 synthetic medium*

Incubation		Control			0.01 molar l-cystine			BB (0.0204 mg/ml)			BB(0.0204 mg/ml) + l-cystine (0.01 molar)		
Days	Temp (°C)	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F
5	20	++++	—	—	++++	++	±	++++	++	±	++++	±	—
	30	++++	±	—	++++	++	±	++++	++	±	++++	+	—
	37	++++	—	—	+++	++	—	++++	+	—	++++	+	—
10	20	++++	±	—	++++	++	+	++++	++	++	++++	±	±
	30	++++	+	—	++++	+++	+	++++	++	+	++++	+	±
	37	++++	+	—	++++	+++	±	++++	+	±	++++	+	+

The .01 molar cystine was much less toxic for the yeast cells than was cysteine under comparable conditions (compare tables 5a and 11). Cystine also showed Y → F activity similar to that of cysteine and serine when their activities are compared using concentrations at which they displayed a low toxicity for the yeast cells (compare tables 5b, 10, and 11). The Y → F activity of cysteine, and of other agents, may be misleading when analysis is made at toxic levels, since it has not been determined whether the inflorescences are more or less resistant than Y phase cells to the toxic effects of the agent being tested.

BB showed somewhat higher Y → F activity than previously found under comparable conditions (compare tables 4, 9, and 11). It would appear that cystine was effective in antagonizing some of the Y → F activity of BB, though to a degree too small to be convincing. However, there definitely did not occur the enhanced Y → F activity found when 0.01 molar l-cysteine (table 6) or .02 molar dl-serine (table 9) was added to BB. The data for cysteine, cystine, and serine do not readily lend themselves to an analysis of the part played by these compounds either alone or in conjunction with BB, in affecting the dimorphism mechanism of *S. cerevisiae.*

g) Discussion

There are a few inferences that can be made from the work on BB reported here. This carcinogen enhances the formation of filamentous forms of *S. cerevisiae* cells grown in S.G. broth cultures incubated with shaking; it enhances the formation of these forms to a lesser extent when the cultures are grown in a synthetic medium contained in test tubes kept stationary during incubation. That this difference in Y → F activity may be due to differences in oxygen tension in the media is suggested by the fact that the Y → F activity for BB in S.G. medium, when the experiment is performed in test tubes, is intermediate between that found in the test tube experiment using # 20 synthetic medium and that found in S.G. cultures incubated with shaking. This point will be discussed in greater detail later.

Two separate morphological entities of yeast cells can be distinguished, inflorescences and filamentous forms. That the cell elongation phenomenon may not be expressed until those cellular processes which give rise to inflorescences occur is suggested by the fact that single elongated cells have not been found and that inflorescences usually occur before filamentous forms, at least under the conditions of the experiments performed here (note, for example, tables 6, 7, 10 and 11).

One fact that stands out clearly in reports of studies on the mechanism of cell division is that -SH groups are involved (HAMMETT, 1930; GREGORY and CASTLE, 1931; GARDNER, 1940; PRATT and DUFRENOY, 1947; NICKERSON and EDWARDS, 1949; NICKERSON and VAN RIJ, 1949; THIMANN, 1949; O'CONNOR, 1950a). The results reported here lend some support to this fact in that BB plus cysteine gave greater Y → F activity than BB plus cystine or serine under comparable conditions (see tables 4, 5b, 9, and 11).

It has been postulated that agents which affect the cell-division mechanism probably act by upsetting a mechanism in which -SH groups are involved. In view of the objective of this study to attempt to arrest parasitic reproduction by inducing F or M formation of the yeastlike pathogens *in vivo*, two alternatives are suggested. One is the use of a substance which oxidizes the -SH groups required for cysteine synthesis, which should result in the accumulation of tryptophane and finally heteroauxin and thus, if auxin were the responsible factor, might induce F formation in the yeast phase cells. The other alternative is the use of auxins themselves in an attempt to affect the Y → F transition.

Since S- and -SH containing compounds are also essential to the metabolism of the parasitized host, it is to be expected that it would be very difficult to interfere with the -SH metabolism of the parasite at levels non-toxic to the host. Also, if it may be postulated that an interference with -SH metabolism disrupts the cell division mechanism, resulting in inflorescences, and this mishap is followed by a series of biochemical reactions which ultimately lead to the formation of auxin and a resulting effect of cell elongation, then it would appear that a simplified and more profitable approach to the induction of filament formation in *S. cerevisiae* might be to examine the properties of the auxins.

The Effect of Auxins on the Cell-Division Mechanism of Saccharomyces cerevisiae

a) The effect of alpha-naphthalene acetic acid (ANAA) on the cell-division mechanism of *S. cerevisiae* in S.G. broth

Alpha-naphthalene acetic acid (ANAA) is one of the substances having plant growth activity. Measured by the avena curvature test, it is approximately 1/40 as active biologically as 3-indole acetic acid; measured by the Pisum split stem curvature, it equals the latter in activity (WENT and THIMANN, 1937, p. 137). The difference in activity measured by these two tests is probably due to the fact that the difference in solubility of the two compounds is more manifest under the conditions of the avena test.

The effect of ANAA on the cell-division mechanism in *S. cerevisiae* was studied as follows:

One gram of commercial powdered ANAA (Kolker Chemical Co.) was dispensed into a tared sterile test tube, to which was added 10 ml of sterile distilled water. This constituted a stock suspension having a concentration of 0.1 g/ml. Two ml of this stock suspension were added to 18 ml of S.G. broth contained in a 125 ml erlenmeyer flask, a concentration of 10.0 mg/ml of ANAA. From this a series of serial dilutions were made in similar flasks of S.G. broth to give the following concentrations.

Flask #	Conc. of ANAA (mg/ml)
1	10.0
2	1.0
3	0.1
4	0.01
5	0.001
6	0.0001
7	0.00001
8 (control)	0.00000

Every flask was inoculated with a 0.1 ml amount of a 24 hour suspension of *S. cerevisiae* grown in S.G. broth and incubated at room temperature (22° C ± 2°) for 26 days. Scoring of F forms and inflorescences was made by the same method as in the previous experiments. The resultant data are compiled in table 12.

The 10.0 mg/ml concentration of ANAA is presumed to be fungicidal, since no growth occurred when the culture containing this amount was streaked out after 2 days of incubation, and no growth could be discerned in the flask even after 26 days of incubation.

After 4 days of incubation all the concentrations of ANAA that permitted growth enhanced the Y → F transition except the 0.0001 and 0.00001 mg/ml ones. After 26 days of incubation all the flasks in which growth occurred, including the control, showed Infl and F forms. The observation here that an extended period of incubation will result in Y → F activity among cells grown in a regular laboratory medium is in accord with that made in the experiments with BB.

TABLE 12. *The effect of alpha-naphthalene acetic acid (ANAA) on the cell-division mechanism of Saccharomyces cerevisiae in S.G. broth*

Time of incubation (days)	Conc. ANAA (mg/ml)	Growth	Infl	F	Remarks
2	10.0	—			Sterility of culture confirmed by streaking out on agar.
	1.0	++	—	—	
	0.1	++++	++	++	
	0.01	++++	++	—	
	0.001	++++	++	—	
	0.0001	++++	—	—	
	0.00001	++++	—	—	
	0.00000	++++	—	—	
4	10.0	—			Infl and F forms similar to those found with BB. Note fig. 18, 19, and 20. Fig. 20 is an unusual type of Infl.
	1.0	+++	++	+++	
	0.1	++++	+++	++	
	0.01	++++	++	—	
	0.001	++++	++	—	
	0.0001	++++	—	—	
	0.00001	++++	—	—	
	0.00000	++++	—	—	
26	10.0	—			
	1.0	++++	++	+++	
	0.1	(contaminated-discarded)			
	0.01	++++	+	+++	
	0.001	++++	+	+++	
	0.0001	++++	++	++	
	0.00001	++++	++	++	
	0.00000	++++	++	++	

The morphology of the Infl and F forms induced by ANAA was essentially the same as those induced by BB, except for one distinguishing characteristic. That is that many of the Infl induced by ANAA contained both typical yeast cells and elongated cells within the inflorescence (note fig. 17 and 19). Such an arrangement was not found in the course of the BB work. There was also the suggestion (fig. 20) that single elongated cells of *S. cerevisiae* were induced by ANAA, but this observation was made so infrequently that this possibility must await further work before it can be considered significant.

An examination of the inflorescences and filaments of the cells resulting from their exposure to ANAA and BB might well lead one to propose the explanation that the cell-division mechanism could not keep pace with growth and cell multiplication, so resulting in the formation of filaments. This explanation for the formation of filamentous cells was proposed by HINSHELWOOD (1946). Within this reasoning one would be forced to conclude that the effect of ANAA and BB on *S. cerevisiae* was to increase its rate of multiplication or delay the rate of cell division.

The delay of cell division has been readily accomplished in many or-

ganisms by irradiations. The increase in the size of cells following irradiation has been explained by LEA (1947) as the result, in most cases, of an inhibition of division while growth continues. Such an increase in size had been observed for bacteria (SPENCER, 1935; LEA, HAINES, and COULSON, 1937), yeast (HOLWECK and LACASSAGNE, 1930; WYCKOFF and LUYET, 1931), protozoa (MOTTRAM, 1926; ROBERTSON, 1933), fungal spores (LUYET, 1932), algae (LUYET, 1934), and other organisms (see Chap. VIII in LEA, 1947).

In order to attempt to assess the effect of the agents used here on the rate of growth (multiplication) of the yeast cells, the following experiment was performed.

b) The effect of alpha-naphthalene acetic acid (ANAA) on the growth rate of *S. cerevisiae* in S.G. broth

To 125 ml erlenmeyer flasks, each containing 18 ml of S.G. medium, were added 0.2 ml amounts of appropriate dilutions of the stock suspension of ANAA, prepared as previously described (section 3a) to yield a series of flasks having the following concentrations of ANAA in mg/ml: 10.0, 0.1, 0.001, and 0.00000 (control). The flasks of medium were inoculated as in the previous experiment and incubated at 20° C for 48 hours. Plate counts were made immediately after inoculation of *S. cerevisiae* into the culture flasks and after 12 and 24 hours; microscopic counts were made after 12 and 24 hours. The direct microscopic counts of cells and of inflorescences and filaments of cells were made in duplicate using a Hellige improved Neubauer Haemacytometer under 440 magnification. Counts of individual cells and of filaments of various lengths were made. An attempt to make another series of counts after 48 hours of incubation failed because it was not possible to accurately count the large numbers of cells present in many of the larger filaments and inflorescences.

The data in table 13a represent the average of the duplicate counts. The table includes summaries of the number of cells and number of clones per ml. The term clone is used here to refer to the total of individual cells plus filaments, a figure which should correspond with the plate count if all the cells are viable. Plate count results are recorded in table 13b.

The 10.0 mg/ml concentration of ANAA was germicidal, sterilizing the culture within 12 hours. The 0.1 and 0.001 mg/ml concentrations appreciably stimulated the production of filaments. As judged by the microscopic method the 0.1 mg/ml concentration resulted in a moderate increase in number of cells produced. The plate counts of the lower concetrations of ANAA, however, were approximately 40 per cent below that of the control. The small size of the plate counts in proportion to the microscopic counts indicates that many of the cells in the cultures were non-viable. The greater differences with the ANAA cultures indicate that all the concentriations of ANAA displayed some toxicity.

The interpretation of results in an attempt to determine whether the effect of the ANAA was merely to inhibit cell division, or whether it also modified the rate of cell multiplication, is difficult. While the number of cells produced by the 0.1 mg/ml concentration was apparently increased, the plate count results show that the culture contained less viable cells. It appears that the increased multiplication may have resulted from less competition with viable metabolizing cells. One is led to believe then that the effect of ANAA on *S. cerevisiae* is to modify the cell division mechanism without any appreciable change in the rate of cell multiplication.

The work of numerous investigators has indicated that auxin permits growth by increasing the plasticity of cells, so that elongation of plant cells

TABLE 13a. *The effect of alpha-naphthalene acetic acid (ANAA) on Saccharomyces cerevisiae in S.G. medium at 20: C*

Incubation time	Conc. ANAA mg/ml		Number of cells per filament																										Total cells per ml	Total clones per ml	Mean filament length
hrs			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	24	26	28	29	30	32	44	45	x10⁶	x10⁶	
12	10.0	No. of filaments	3	2	1			1																							16	7	2.3
	0.1		3	9	5	9	4	6	1	4	3	2	1				1	1													256	49	5.2
	0.001				3	5	4	2	2	2				1																	103	19	5.4
	0.00			14	3	9	2	6	1	1																					134	36	3.7
24	10.0		2	6	1																										17	9	1.9
			5	243	21	88	15	16	7	7	4	3	1	3		1				2		1			1	1	1	1	1	1	1494	403	3.7
	0.001		6	142	14	50	29	17	8	11			1		2			1	1		1		1	1		1					1092	286	3.8
	0.00			135	59	78	21	7	3	1	2			1	1																978	308	3.1

1) The term clone refers to individual cells and to filaments of cells.

may occur with no corresponding increase in weight (see WENT and THIMANN, 1937, for a comprehensive discussion of this topic.) The demonstration that ANAA induced Y → F formation without any significant change in cell capital (mass) requires for its support the demonstration that the average ANAA-induced filamentous cell of *S. cerevisiae* has the same mass as the average yeast phase cell. This could best be accomplished with cultures containing a high percentage of F forms (over 90 %), induced, preferably, by an agent having a minimum toxic effect on the culture. At the present time this has not been feasible.

TABLE 13b. *The effect of alpha-naphthalene acetic acid (ANAA) on Saccharomyces cerevisiae in S.G. medium at 20° C*

Incubation time (hr.)	Conc. ANAA (mg/ml)	Plate count (per ml)
0	all flasks	1.1×10^5
12	10.0	sterile
	0.1	2.5×10^5
	0.001	1.2×10^5
	control	2.5×10^5
24	10.0	sterile
	0.1	5.3×10^6
	0.001	5.2×10^6
	control	9.8×10^6

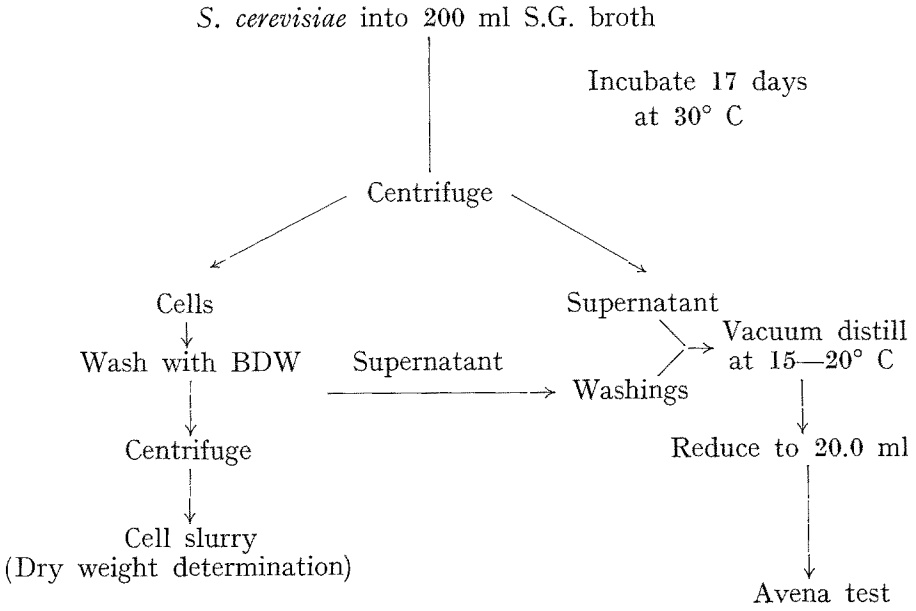
A correlation between prevalence of F forms and auxin content has, however, been shown. It has been possible to demonstrate that the auxin activity of a filtrate from cultures having a relatively large number of F forms of *S. cerevisiae* is greater than the auxin activity of a filtrate from a yeast phase culture, with both cultures containing comparable weights of cells.

c) The auxin activity of filtrates from cultures containing yeast and filamentous forms of *S. cerevisiae.*

Since it was desirable to examine the auxin activity, using the avena test, of filtrates from cultures containing F forms of *S. cerevisiae,* it was inadvisable to use a compound having auxin properties to induce the F forms. The use of BB was decided upon because auxin activity for BB could not be demonstrated with the avena test; BB at some of the higher concentrations used did not display any discernible toxicity above that which could probably be attributed to the shock of cells which frequently precedes the lag phase; and the concentrations of BB which exhibited Y → F activity for *S. cerevisiae* in S.G. broth were small enough so as not to interfere with weight measurements.

To each of two 1-liter erlenmeyer flasks were added 200 ml of S.G. broth. These flasks were autoclaved at 15 lb. pressure for 15 minutes. To one of these flasks was added 2 ml of a stock suspension of BB, to yield a final concentration of 0.0204 mg/ml. Both flasks were inoculated with 0.1 ml amounts of a suspension of cells of *S. cerevisiae* in buffered distilled water (BDW) harvested from 48 hour culture grown on an S.G. agar slant, and incubated at 30° C for 17 days. At the end of this period the numbers of filaments and inflorescences were determined for each culture.

Both cultures were then processed in the following manner. The culture was centrifuged and the supernatant liquid was separated from the cells by decantation. The cells were washed with approximately 50 ml of BDW and this washing was added to the supernatant liquid. The volume of the slurry of cells was measured ,and the dry weight of cells in this slurry was determined by weighing an amount of this slurry in a tared weighing boat and then drying it for 24 hours in an oven set at 110° C. The supernatant liquid plus cell washings was then reduced to a 20.0 ml volume by vacuum distillation at a temperature between 15° and 20° C. One ml of the reduced supernatant liquid was mixed with 1 ml of 3 % agar and the auxin activity was determined as previously described (see *Materials and Methods*). This process is outlined in the following scheme.



The data for this experiment are recorded in table 14. The filtrate from the culture which contained the greater number of F forms had a significantly greater auxin activity than that of the control. This observation confirms that of ROBINSON and STIER (1941). Although it is not possible to determine from the data of this experiment that elongation of cells was not accompanied by an increase in weight, some support is gained for this idea from the fact that the total dry weights of cells in the S.G. broth and in the broth containing BB did not differ by more than approximately 8 %. This could be still more significant if we draw on the data in tables 13a and 13b to reason by analogy that the rate of cell multiplication was not significantly changed.

Since a relationship between auxins and Y → F activity was becoming more evident, further auxins were tested for their activity.

d) The effect of beta-3-indolepropionic acid (IPA) on the cell-division mechanism of *S. cerevisiae*

Beta-3-indolepropionic acid (IPA) is a plant growth regulator and, with 3-indole acetic acid, has been proposed as part of the scheme for tryptophane breakdown (PORTER, 1946). The auxin axtivity of IPA is approximately

1/1000 of the activity of auxin a, auxin b, and 3-indole acetic acid (see table III in WENT and THIMANN, 1937).

TABLE 14. *The auxin activity of filtrates from cultures containing yeast and filamentous forms of Saccharomyces cerevisiae*

		S.G. broth	S.G. broth containing 0.0204 mg/ml BB
Y → F activity:	Growth	++++	+++
	Infl	+	++
	F	±	++
Cells: Vol. of slurry		13.0 ml	30.0 ml
	Gross	51.9431 g	53.5798 g
	Tare	48.5818 g	48.5714 g
Slurry aliquot	Net	3.3813 g	5.0084 g
After 24 hr. at 110° C	Gross	48.6442 g	48.6149 g
	Tare	48.5818 g	48.5714 g
	Net	0.0624 g	0.0435 g
% dry wt. of cell slurry		1.86	0.87
Total dry wt. of cell slurry (Total cells in culture)		0.242 g	0.261 g
Avena test: coleoptile curvatures		+15.0°	
		+ 9.0°	+25.0°
		+ 8.0°	+22.0°
		+11.0°	+17.0°
		+12.0°	+15.0°
		55.0°	79.0°
Mean		+11.0°	+19.8°
Average dev. from mean		± 2.0°	± 3.8°

Five mg of powdered commercial IPA, kindly provided by the DOW Chemical Co., were suspended in 5 ml of #20 synthetic medium contained in a sterile test tube. Serial dilutions were then made, using #20 medium, to yield a series of tubes ranging in concentration of IPA from 1.0 to 0.0001 mg/ml. A control tube contained no IPA. These tubes were inoculated with 0.05 ml amounts of a washed cell suspension of *S. cerevisiae*, incubated at 25° C, and examined periodically for Y → F activity. The results are outlined in table 15.

The IPA did not enhance the Y → F activity to any significant degree in the #20 synthetic medium.

The activity of IPA was also studied in S.G. broth cultures incubated with shaking, by the method previously described. The data for this are recorded in table 16.

The Y → F activity of the IPA in the S.G. broth cultures that were shaken was higher than that found in the #20 synthetic medium.Although F forms did not appear until after 14 days of incubation, the elongated cells were much longer than those found in the previous studies, averaging 2 to 3 times the

TABLE 15. *The effect of β-3-indolepropionic acid (IPA) on the cell-division mechanism of Saccharomyces cerevisiae in #20 synthetic medium at 20° C*

Incubation (days)	Conc. IPA (mg/ml)	Growth	Infl	F
5	1.0	+++	+++	—
	0.1	++++	++	—
	0.01	++++	++	—
	0.001	++++	++	—
	0.0001	++++	++	—
	0.000	++++	++	—
10	1.0	++++	+++	—
	0.1	++++	++	—
	0.01	++++	++	—
	0.001	++++	++	—
	0.0001	++++	++	—
	0.000	++++	++	—

TABLE 16. *The effect of β-3-indolepropionic acid (IPA) on the cell-division mechanism of Saccharomyces cerevisiae in S.G. broth cultures incubated at room temperature with shaking*

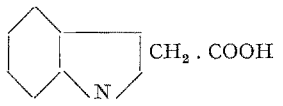
Incubation (days)	Conc. IPA (mg/ml)	Growth	Infl	F
2	10.0	++	+++	—
	1.0	++++	++	—
	0.1	++++	+	—
	0.01	++++	±	—
	0.001	++++	±	—
	0.0001	++++	±	—
	0.00001	++++	±	—
	0.000	++++	±	—
4	10.0	+++	++	—
	1.0	++++	±	—
	0.1	++++	±	—
	0.01	++++	±	—
	0.001	++++	±	—
	0.0001	++++	±	—
	0.00001	++++	±	—
	0.000	++++	±	—
14	10.0	+++	++	—
	1.0	++++	++	++ [1]
	0.1	++++	++	++
	0.01	++++	++	±
	0.001	++++	++	±
	0.0001	++++	+	±
	0.00001	++++	±	±
	0.000	++++	±	±

[1]) Elongated cells were much longer than any previously found.

length of those shown, for example, in fig. 6. These very long cells were found in the cultures containing 1.0, 0.1, and 0.01 mg/ml of IPA, so that 0.01 mg/ml might be considered close to the limit of physiological activity for this compound under the conditions of this experiment. The concentration of 10.0 mg/ml was visibly toxic to the cells and did not result in F formation.

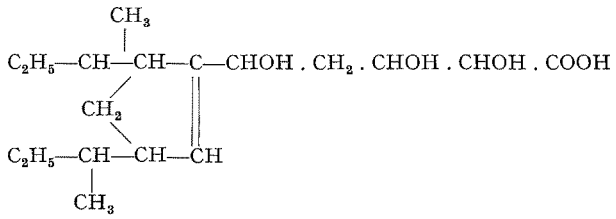
e) The effect of 3-indole acetic acid (IAA) on the cell-division mechanism of *S. cerevisiae*

The isolation of 3-indole acetic acid (III) from yeast cultures and urine was accomplished by SALKOWSKI in 1885(b), but it was not until 1934 that KOGL, HAAGEN-SMIT, and ERXLEBEN, reisolating this substance from urine, demonstrated its auxin activity. It was then named "heteroauxin", the term usually used at the present time in referring to it. The activity of 3-indole acetic acid (IAA) is of the same order as that of auxin a (IV) and auxin b (V), and these three compounds are among those showing the highest auxin

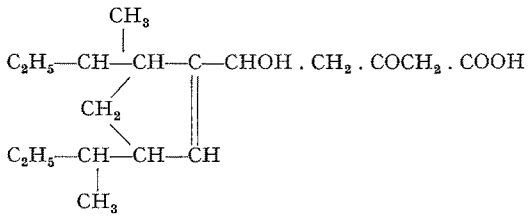


(III) 3-Indole acetic acid (heteroauxin)

activity as judged by the avena curvature test (WENT and THIMANN, 1937, p. 137).



(IV) Auxin a (Auxentriolic acid)



(V) Auxin b (auxenolonic acid)

The Y → F activity of IAA on *S. cerevisiae* was tested exactly as described for IPA in the S.G. broth cultures (see previous section d). The results are recorded in table 17.

The amount of Y → F activity displayed by IAA was small and the time required for its expression relatively long, when compared to what would be expected, considering its known auxin activity, if auxin activity were responsible for cell elongation. A repetition of this experiment using tubes of #20 synthetic medium produced little or no enhancement of Y → F activity (table 18).

One of the major difficulties of the work reported here on the Y → F activity of various agents stems from the fact that many of the chemicals examined are relatively insoluble in the different media used. This is true for BB, ANAA, IPA, and IAA. A more acceptable decision as to the Y → F

activity of these compounds awaits their examination under conditions in which they are in true solution (not mechanical suspension or even colloidal dispersion).

TABLE 17. *The effect of 3-indole acetic acid (IAA) on the cell-division mechanism of Saccharomyces cerevisiae in S.G. broth cultures incubated with shaking at room temperature*

Incubation (days)	Conc. IAA (mg/ml)	Growth	Infl	F
2	10.0	+		
	1.0	++++	±	—
	0.1	++++	±	—
	0.01	++++	±	—
	0.001	++++	±	—
	0.0001	++++	±	—
	0.00001	++++	±	—
	0.000	++++	±	—
4	10.0	++	++	—
	1.0	++++	+	—
	0.1	++++	+	—
	0.01	++++	+	—
	0.001	++++	+	—
	0.0001	++++	+	—
	0.00001	++++	+	—
	0.000	++++	±	—
14	10.0	++++	+++	+
	1.0	++++	++	+
	0.1	++++	++	+
	0.01	++++	++	±
	0.001	++++	++	±
	0.0001	++++	+	±
	0.00001	++++	+	±
	0.000	++++	+	±

TABLE 18. *The effect of 3-indole acetic acid (IAA) on the cell-division mechanism of Saccharomyces cerevisiae in #20 synthetic medium at 25° C*

Incubation (days)	Conc. IAA (mg/ml)	Growth	Infl	F
5	1.0	+++	++++	—
	0.1	++++	+++	—
	0.01	++++	++	—
	0.001	++++	++	—
	0.00	++++	++	—
10	1.0	+++	+++	—
	0.1	++++	++	—
	0.01	++++	++	—
	0.001	++++	++	—
	0.00	++++	++	—

If a soluble precursor of heteroauxin were added to a culture of *S. cerevisiae*, then any Y → F activity which might occur might be associated with auxin formation. Since the work of many investigators has already established that tryptophane is a precursor of 3-indole acetic acid, this compound was next examined for Y → F activity.

f) The effect of tryptophane on the cell-division mechanism of *S. cerevisiae.*

A stock solution of 0.1 molar l-tryptophane (Nutritional Biochemicals Corp.) was prepared by suspending 0.204 g of the commercial crystalline compound in 10 ml of sterile distilled water and, while the solution was warmed to approximately 50° C, adding dropwise an amount of 0.1 normal HCl just sufficient to solubilize the tryptophane. Five tenth ml aliquots of this stock solution of tryptophane were aseptically added to 4.5 ml amounts of #20 synthetic medium. These tubes were inoculated with 0.05 ml amounts of a washed cell suspension of *S. cerevisiae* and incubated at 20° C, 30° C, and 37° C. Y → F activity was assayed after 5 and 10 days of incubation. Table 19 contains the data for this experiment.

TABLE 19. *The effect of l-tryptophane solubilized as the hydrochloride on the cell-division mechanism of Saccharomyces cerevisiae in # 20 synthetic medium*

Incubation Days	Temp (°C)	Control Growth	Control Infl	F	0.01 molar l-tryptophane Growth	0.01 molar l-tryptophane Infl	F
5	20	++++	+	—	++++	++++	++
	30	++++	±	—	++++	++	—
	37	++++	++	—	++++	++	—
10	20	++++	++	—	++++	++++	++
	30	++++	+	—	++++	++++	±
	37	++++	++	—	++++	+++	—

The 0.01 molar tryptophane elicited a strong Y → F reaction in the culture after 5 days of incubation, the strongest activity being found at 20° C. There was a slight increase in Y → F activity after 10 days of incubation over that found after 5 days. The filamentous cells did not resemble those found in previous experiments but resembled germinating yeast phase cells going into hyphael formation (fig. 21).

An attempt was made to determine the concentration of tryptophane required for Y → F activity, using the method described above except that the tryptophane was added to the #20 synthetic medium in the form of a mechanical suspension, no HCl being added to solubilize the tryptophane. Under these conditions, although some Y → F activity was indicated by the presence of inflørescences, no filaments of the type illustrated in fig. 21 could be discovered (see table 20). Nevertheless, no crystals of tryptophane could be found in the cultures, indicating that it had all gone into solution. The effect of acids on the growth of plant tissues is especially pertinent and a portion of WENT and THIMANN's (1937, p. 130) discussion of this topic is quoted here.

"BONNER (1934) has shown . . . that . . . the growth caused by acid . . . increases steadily towards higher acidities. If we allow for the fact that the pH within the tissues is not the same as the pH of the (acid) buffer, then the curve of acid growth against pH follows closely the pH dissociation curve of auxin (pK = 4.8). This suggests that only the free acid form of auxin has

growth-promoting activity, and that the applied acid sets free the auxin acid from the salt form in which it is present (pH of sap = 6.1). That the applied acid is not in itself effective in producing growth is supported by the fact that 2 hours after decapitation, when the auxin content has fallen to about one half . . . the curvatures produced by acid are also reduced to about one half. It is still more clearly borne out by the experiments of (THIMANN, 1935c). He immersed coleoptile sections in acid buffer, which caused temporary growth acceleration: when this had ceased, immersion in fresh acid had no effect because all the auxin in the sections had been used up, but immersion in auxin gave good growth.

Thus it is clear that the action of acid is *through its effect on the auxin in the plant . . .*"

TABLE 20. *The effect of* l-*tryphophane on the cell-division mechanism of Saccharomyces cerevisiae in # 20 synthetic medium*

Incubation Days	Temp (°C)	Control Growth	Infl	F	1-Tryptophane 0.01 molar Growth	Infl	F	0.001 molar Growth	Infl	F
5	20	++++	+	—	++++	++	—	++++	+++	—
	30	++++	+	—	++++	+	—	++++	++	—
	37	++++	+	—	++++	+++	—	+++	+++	—
10	20	++++	+	—	++++	++	—	++++	++	—
	30	++++	+	—	++++	+	—	++++	++	—
	37	++++	+	—	++++	++	—	++++	+++	—

It was therefore necessary to ascertain whether the quantity of HCl required to solubilize the tryptophane would cause the same Y → F activity in the absence of tryptophane. This was done as follows:

Ten ml quantities of #20 medium containing 0.01 molar l-tryptophane were aseptically added to 5 sterile 125 ml erlenmeyer flasks. To this series of ilasks, and to another set which lacked only the tryptophane, were added various quantities of N/1 HCl or N/1 NaOH in order to adjust the pH of the medium to various levels. The pH of the unadjusted medium was 6.65. All the flasks were inoculated with 0.1 ml amounts of a washed cell suspension of *S. cerevisiae* and incubated at room temperature (20° C ± 2°) with shaking. The results are compiled in table 21.

TABLE 21. *The effect of pH on the Y → F activity of* l-*tryptophane on Saccharomyces cerevisiae in # 20 synthetic medium, incubated at room temperature for* 48 *hours*

Control pH	Growth	Infl	F	0.01 molar l-tryptophane pH	Growth	Infl	F
2.70	++	++	—	2.65	++	+++	—
3.15	+++	++	—	3.10	+++	++++	+
6.65 [1]	++++	+	—	6.50 [1]	++++	++	—
8.28	+++	±	—	8.25	+++	±	—
9.00	++	±	—	9.05	++	±	—

[1]) pH of these cultures was unadjusted.

Although the effect of acid was to increase the number of inflorescences compared to those found in the unadjusted culture, the acid plus tryptophane yielded the highest Y → F activity, the maximum activity being found at

a pH of 3.10. It is significant that at the alkaline pH values, irregardless of the presence of tryptophane, only negligible numbers of inflorescences and no F forms were found.

These data are in accord with the hypothesis that l-tryptophane was utilized by the yeast as the precuror of heteroauxin and that the role of a low pH was to release the free indole acetic acid compound from an inactive complex, so that its activity on cells of *S. cerevisiae* became evident.

The Reversibility and Stability of the Y → F Reaction

One of the observations recorded by LEVAN (1947) as a characteristic of the cf-reaction was that the reaction is reversible. That is, if the concentration of the active compound in the medium falls below its threshold value for cf-activity, then cf-cells start to bud off normal-appearing daughter cells, which in turn behave normally. This assumes that the period required to reach the peak of cf-activity is not of such duration as to exhaust the essential nutriments required for cell multiplication.

The following experiment was performed in order to ascertain whether such an effect of "reversibility" can be found in cultures where Y → F formation has been induced. When the experiment with ANAA was being run, 0.1 ml aliquots were removed from the cultures containing the 1.0 and 0.01 mg/ml concentrations of ANAA after 26 days of incubation (see table 12) and streaked on plates containing S.G. agar, in duplicate. These plates were incubated at room temperature for 3 days. No colonies were found that indicated that an Infl or F of cells had bred true. No rough colonies were found and, of the approximately 20 colonies picked at random and examined microscopically, none showed any significant Y → F activity. It was thus established that the vast majority of the Infl and F forms revert to normal growth when the Y → F active agent is depleted.

On the other hand, when yeast phase cells of *S. cerevisiae* were inoculated onto plates of S.G. agar containing 1 mg/ml of ANAA, a characteristic change in colonial and cell morphology occurred. This experiment was performed as follows:

Three plates were poured with S.G. agar containing 1 mg/ml of ANAA. A set of control plates contained S.G. agar but no ANAA. To each plate was added a 0.1 ml amount of an 8 hour culture of *S. cerevisiae* in S.G. broth, which was streaked out. The plates were incubated at 20°, 30°, and 37° C for 8 days, one of each set at each temperature. The colonies were examined microscopically for cell morphology. The results are outlined in table 22.

That the ANAA was toxic to the yeast cells was shown by the diminutive size of the colonies (compare fig. 25 and 26 with fig. 22, 23, 24). There was also pronounced Infl formation at 30° C. No growth occurred at 37°.

One of the colonies from the ANAA-containing plate incubated at 20° C was streaked out onto a fresh S.G. agar plate containing the same concentration of ANAA. This procedure was also carried out with a colony from the ANAA-containing plate incubated at 30° C. The inoculated plates were incubated at the same temperatures at which their sources of inocula had been incubated, again for a period of 8 days. It was found that the growth of *S. cerevisiae* was extremely poor after this transfer and that most of the colonies were of pinpoint size. The cell morphology still showed approximately +++ Infl and + F forms in both plates. Certainly the cells had not become adapted to the toxic effects of ANAA by this serial transfer using an ANAA-containing medium.

The marked toxicity displayed by the ANAA after a serial transfer was made probably indicates that the Y → F effect is limited, i.e., that Infl and

F forms cannot be further enhanced by increasing the concentration of the Y → F agent beyond a certain level, at which toxic effects become manifest and prevent growth completely. These data are in accord with the observation for auxins, that small concentrations may enhance growth (and cell elongation), whereas higher concentrations will prevent growth (FIEDLER, 1936).

TABLE 22. *The effect of* 1.0 *mg/ml alpha-naphthalene acetic acid (ANAA) in S.G. agar on the growth of Saccharomyces cerevisiae after* 8 *days incubation.*

	Incubation Temp. (°C)	Remarks
Control, no ANAA	20	Typical yeast colonial growth (fig. 22); budding cells only
	30	Same as above (fig. 23)
	37	Same as above (fig. 24)
1.0 mg/ml ANAA in S.G. agar	20	Minute colonies, appear dull (fig. 25); Infl +, F —
	30	Same as above (fig. 26); Infl ++++, F —
	37	Sterile

Further evidence that Infl and F are not stable forms resulting from their concentration by selection is gained from the following experiment.

TABLE 23. *The stability of inflorescences and filamentous cells of Saccharomyces cerevisiae induced by* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene (BB) and alpha-naphthalene acetic acid (ANAA) in S.G. broth*

Culture No.	Legend	Growth	Infl	F	Incubation Days	Incubation Temp (°C)
I	0.0204 mg/ml BB	++++	++	++	5	20
II	0.0204 mg/ml BB	++++	++	++	5	30
III	0.1 mg/ml ANAA	++++	+	+	5	20
IV	0.1 mg/ml ANAA	++++	+	+	5	30
V	S.G. broth (control)	++++	+	+	5	20
VI	S.G. broth (control)	++++	±	±	5	30
VII	1 ml I, 0.0204 mg/ml BB	++++	++	++	5	20
VIII	1 ml II, 0.0204 mg/ml BB	++++	+	+	5	30
IX	1 ml III, 0.1 mg/ml ANAA	++++	++	++	5	20
X	1 ml IV, 0.1 mg/ml ANAA	++++	++	++	5	30
XI	1 ml V (control)	++++	+	+	5	20
XII	1 ml VI (control)	++++	+	+	5	30
I		++++	+++	+++	10	20
II		++++	+++	+++	10	30
III		++++	+++	+++	10	20
IV		++++	+	++	10	30
V		++++	+	++	10	20
VI		++++	±	+	10	30

BB and ANAA were suspended in 30 ml amounts of S.G. broth contained in 125 ml erlenmeyer flasks, with resultant concentrations of 0.0204 mg/ml and 0.1 mg/ml, respectively. These flasks were prepared in duplicate so that they could be incubated at both 20° and 30° C. All flasks, plus 2 controls, were inoculated with 0,1 ml amounts of a washed cell suspension of

S. cerevisiae. After 5 days of incubation the cultures were examined for Y → F activity, and a 1 ml aliquot from each was transferred to a flask containing media of the same composition as that from which the 1 ml aliquot was taken. These were likewise incubated for 5 days and also the original flasks were incubated.

Table 23 contains the data for this experiment. It can be seen that the Y → F activity found in the flasks that received the 1 ml aliquot, after 5 days of incubation was no greater than that found in the first set of flasks after the first 5-day period of incubation, whereas the first set incubated for 10 days showed an increase in Y → F activity over that found after 5 days. If the Infl and F forms were genetically stable, a greater proportion of them, as compared to their incidence in a yeast phase culture, would have been carried over in the 1 ml aliquot and they should have predominated after growth in the presence of the supposedly selective agent was permitted. This did not occur.

Discussion and Summary

Certain of the chemicals tested in this study, e.g., BB, exhibited stronger Y → F activity in S.G. broth cultures, both when incubated with shaking and in stationary cultures, than in the synthetic medium. In the S.G. broth, cultures incubated with shaking showed superior Y → F activity (tables 3, 4, and 6).

It is pertinent to this phenomenon to record the work of SELZER and BAUMBERGER (1942), who 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 sulfhydryl 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 (1942) similarly found that the exogenous respiration of *B. dermatitidis* was sensitive to M/100 sodium cyanide, whereas the endogenous respiration was relatively insensitive. Evidence in support of the spacial separation of the endogenous and exogenous systems also comes from the works of STEIR and STANNARD (1936), WINZLER and BAUMBERGER (1938), and SPIEGELMAN and NOZAWA (1945).

The #20 synthetic medium contains no -SH groups, whereas the S.G. broth doubtless does. It is possible that there is a greater degree of endogenous respiation of the yeast cells occurring in the synthetic medium than in the S.G. broth. Whether this difference could be attributed to differences in oxygen tension among S.G. broth cultures incubated with shaking, S.G. broth stationary cultures, and #20 medium stationary cultures is not known.

The work of O'CONNOR (1950b) 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 (O'CONNOR, 1950a). Conclusions from O'CONNOR's work may not be properly applied here, since no comparative metabolic data exist for yeast growing in S.G. broth and in #20 medium. Nevertheless it would be expected that 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. Support for the postulation that cells of *S. cerecisiae* may have a greater

endogenous respiration in #20 medium than in S.G. broth as yet lacks experimental confirmation.

The inhibitory action of iodoacetate on cell division of the midbrain of 6-day-old chick embryos may also be correlated with its ability to combine with -SH groups (O'CONNOR, 1950b). The favorable results obtained with iodide therapy in certain cases of systemic mycotic infections (MARTIN and SMITH, 1939a, 1939b) might profitably be re-investigated with the idea of ascertaining the effect of iodides on the cell-division mechanism of yeasts.

The significance of -SH groups for the cell division mechanism appears, in general, to be borne out on observations with plant and animal cells. The study made by GREGORY and CASTLE (1931) 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 (1930) 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.

Further evidence for the -SH nature of the enzyme(s) which regulate cell multiplication can be gleaned from the work of THIMANN (1949). The action of a number of -SH inhibitors was studied, using the effect on the amount of curvature and growth of slit stems of peas as a test method. Arsenite and the organic arsenical mapharsen inhibited growth strongly, as did iodoacetate and parachloromercuribenzoate. The last compound is a more specific -SH reagent than the others. It was first used by HELLERMAN, CHINARD, and DIETZ (1943). 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 (1944) showed that the growth of *Neurospora* is inhibited by iodoacetate in a manner both quantitatively and qualitatively similar to that in the coleoptile sections in the work of THIMANN (1949). The observations of THIMANN, that the inhibition of growth by -SH inhibitors occurs in the presence of auxin, appears contradictory to the concept propounded here that -SH inactivation may lead to auxin production in *S. cerevisiae*. There is no doubt that there are many -SH activated enzymes in a living system and that they probably could be graded as to their relative susceptibility to -SH inactivating agents. It would be expected that physiological damage would increase progressively with an increase in the concentration of the agents being used. It is pertinent to note here that THIMANN and BONNER (1949) were able to inhibit 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 act by reacting with a SH-containing enzyme was supported by the fact that BAL prevented the growth inhibition caused by these compounds. However, at sub-inhibiting concentrations the growth of the test plants was *promoted* in each case. This observation lends support to the auxin concept for $Y \rightarrow F$ 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 sub-inhibiting concentrations of the -SH inhibiting lactones, coumarin and protoanemonin.

The work reported here lends only little support to the idea that the inactivation of SH-containing compounds resulted in the formation of Infl and F forms of *S. cerevisiae*. The fact that BB plus cysteine gave greater

Y → F activity than BB plus cystine or serine (tables 4, 5b, 9 and 11) suggests that — SH groups were involved, because the reduced S group is the only component of the cysteine molecule not shared by serine and cystine.

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. In addition to the agents already mentioned, Y → F and B → F activity have been attributed to x-rays (EISENSTARK and CLARK, 1947), gamma rays (LEA, HAINES, and COULSON, 1937), radium (SPENCER, 1935), magnesium ions (WEBB, 1949a, 1949b), and many other agents. The many compounds of divergent chemical structure which induce the cf-reaction have already been mentioned (LEVAN, 1947).

Most of the features of the cf-reaction indicated previously are also characteristic for the Y → F activity. LEVAN has had the opportunity to examine copies of fig. 6 and 21 and is of the opinion (personal communication) that both photographs represent instances of the cf-reaction, although fig. 6 may represent a more regular (less narcotized) growth than fig. 21.

The compounds which have been found to enhance the Y → F, Y → M, and B → F formation and the cf-effect in microorganisms, may have that characteristic in common which concerns their mode of detoxification when cells are exposed to them. Cysteine is one of the few amino acids which may be used by an organism for detoxification (FIESER and FIESER, 1944). FIESER and FIESER point 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.

Some evidence from the work reported here, supplemented by the results of other investigators, does, in some measure, support the contention that auxins may be responsible for the elongation of cells of *S. cerevisiae*. Significant points may be summarized as follows:

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

2. Tryptophane (hydrochloride), which is a precursor of indole acetic acid, induced the formation of a relatively large number of filamentous cells in #20 synthetic medium.

3. Increasing the hydrogen ion concentration of cultures containing tryptophane enhanced the Y → F phenomenon considerably, presumably by releasing the free acid form of heteroauxin from the inactive complex.

4. The effect of ANAA 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 plant cells exposed to auxins.

If auxin induces elongated cells in yeasts, then this mechanism might also explain filament (B → F) formation in bacteria. The smooth to rough (S → R) or Y → F variation in yeasts (PUNKARI and HENRICI, 1933) and yeastlike fungi (MICKLE and JONES, 1940) shows such a close parallelism to this type of variation in bacteria that it is very likely that the mechanism governing S → R, Y → M, Y → F, and B → F formations are the same. LIESKE (1921) suggested that unicellular bacteria bear a relation to the actinomycetes similar to that of the yeasts to molds. NOVAK and HENRICI (1933) were able to recover a yellow staphylococcus from Berkefeld-filtered cultures of an actinomycete after incubation for 5 weeks at room temperature. This "staphylococcus" was

morphologically and culturally indistinguishable from other staphylococcus strains grown on ordinary media. On glucose agar the coccoid strain grew in the form of rods and branching filaments, but these reverted to cocci when transferred again to plain agar.

The growth of bacteria has been associated with 3-indole acetic acid for some time (SALKOWSKI and SALKOWSKI, 1880; SALKOWSKI, 1885a, 1885b). *Proteus vulgaris* has been found to produce 3-indole acetic acid (HERTER and TEN BROEK, 1909). HERTER (1908) demonstrated that the 3-indole acetic acid found in the urine of patients came from bacteria that were active in the putrefaction of intestinal contents. The demonstration that typhoid bacilli in the urine of urinary carriers are frequently in the form of long filaments (JORDON and BURROWS, 1945) may well be correlated with the very high heteroauxin content of human urine (KOGL, HAAGEN-SMIT, and ERXLEBEN, 1933).

A study of CHOLODNY and BELTYUKOVA (1939) on the effect of phytohormones on a number of microorganisms, among which were *Bacillus subtilis*, *Bacillus mycoides*, and *Saccharomyces cerevisiae*, showed that often an abnormal enlargment of the cells was produced.

Thus it would appear that the induction of filament formation in bacteria is probably of the same nature as the mechanism responsible for Y → F formation in yeast cells.

One of the ramifications of this work concerns the genetics of Y → F formation: the question as to the inheritance of the factor of resistance or susceptibility of yeasts to agents inducing dimorphism; the relative effects on haploid and diploid yeast cultures of Y → F inducing agents; the genetic factors determining the stability or instability of F forms when they are removed from the Y → F enviornment. The work of LINDEGREN (1944) indicates that the genetic constitution of the cell may be of fundamental significance in Y → F effects. Haploid cultures examined by LINDEGREN showed many extermely thin elongated cells, which are generally absent from diploid cultures. Also, haploid cells tended to grow in clusters because of failure of the full-sized daughter cells to separate. Such clusters sometimes were large enough to settle to the bottom of the tube and they contained long, thin cells resembling a mycelium. Comparable observations have been made on numerous occasions during the course of this work, yet diplophase cultures were employed here exclusively, as indicated by the morphology of cells and colonies and the observation of ascospore formation within F forms.

The failure to precipitate a deep infection, under conditions conducive to F → Y formation (bodily temperature of approximately 37° C), with dermatophytes (F phase) that do not exhibit dimorphism has already been mentioned (SULZBERGER, 1929; BROCQ-ROUSSEU *et al.*, 1926). It may be that Y and F formation results from biochemical pathways that are mutually exclusive and that the transition from one phase to the other is governed by rather restricted nutritional and temperature requirements.

An attempt toward applying the auxin concept for Y → M formation *in vivo* by treating mice systemically infected with *Candida albicans* with compounds having plant growth activity will be reported in a subsequent paper.

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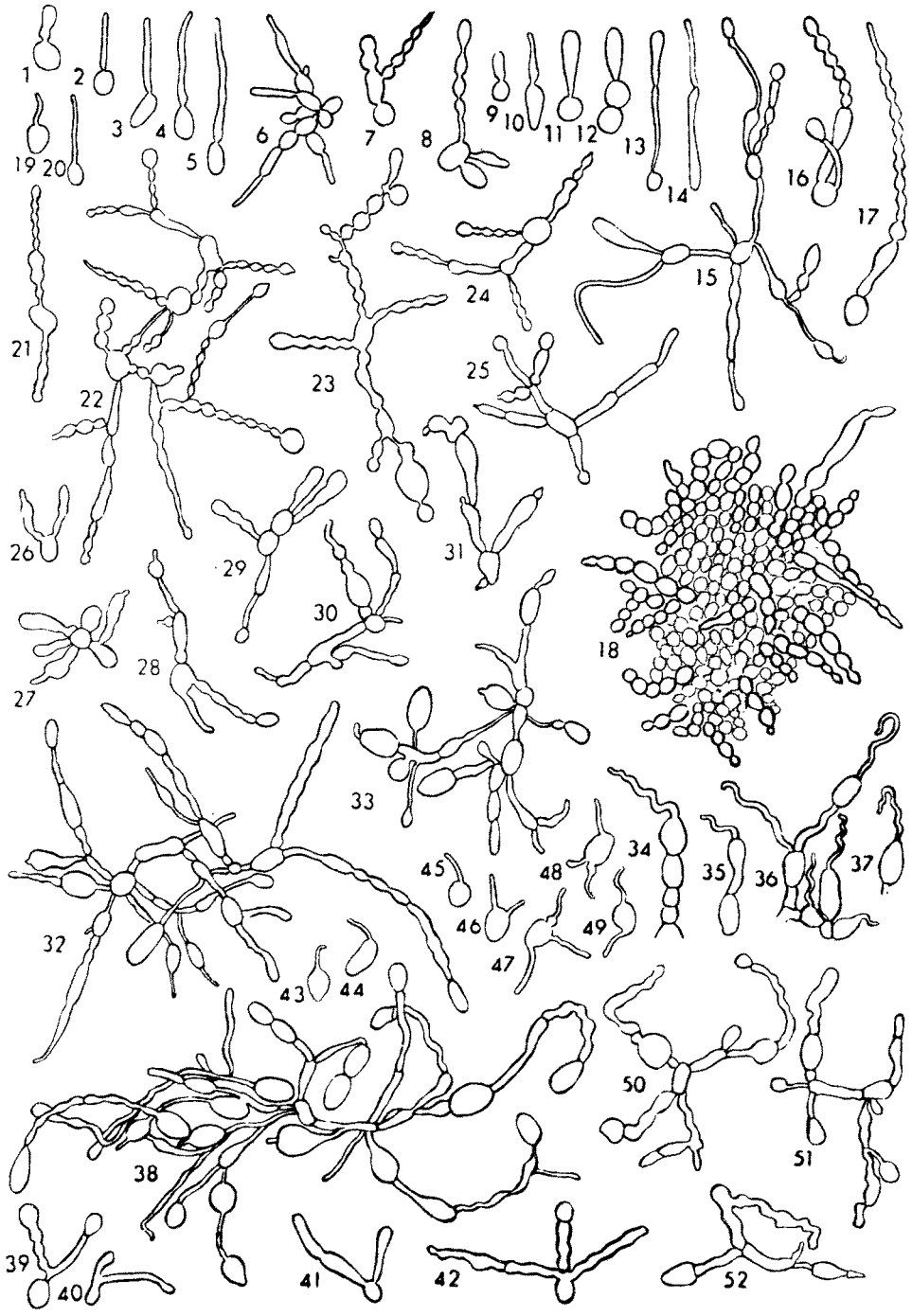


Fig. 1. Outline drawings of cf-forms induced by aliphatic alcohols: methanol (1—14), ethanol (15—18), propanol (19—25), normal butanol (26—37), iso-butanol (38), tertiary butanol (39—42), iso-pentanol (43—52). 400×. Reproduced from LEVAN (1947).

Courtesy Prof. ALBERT LEVAN.

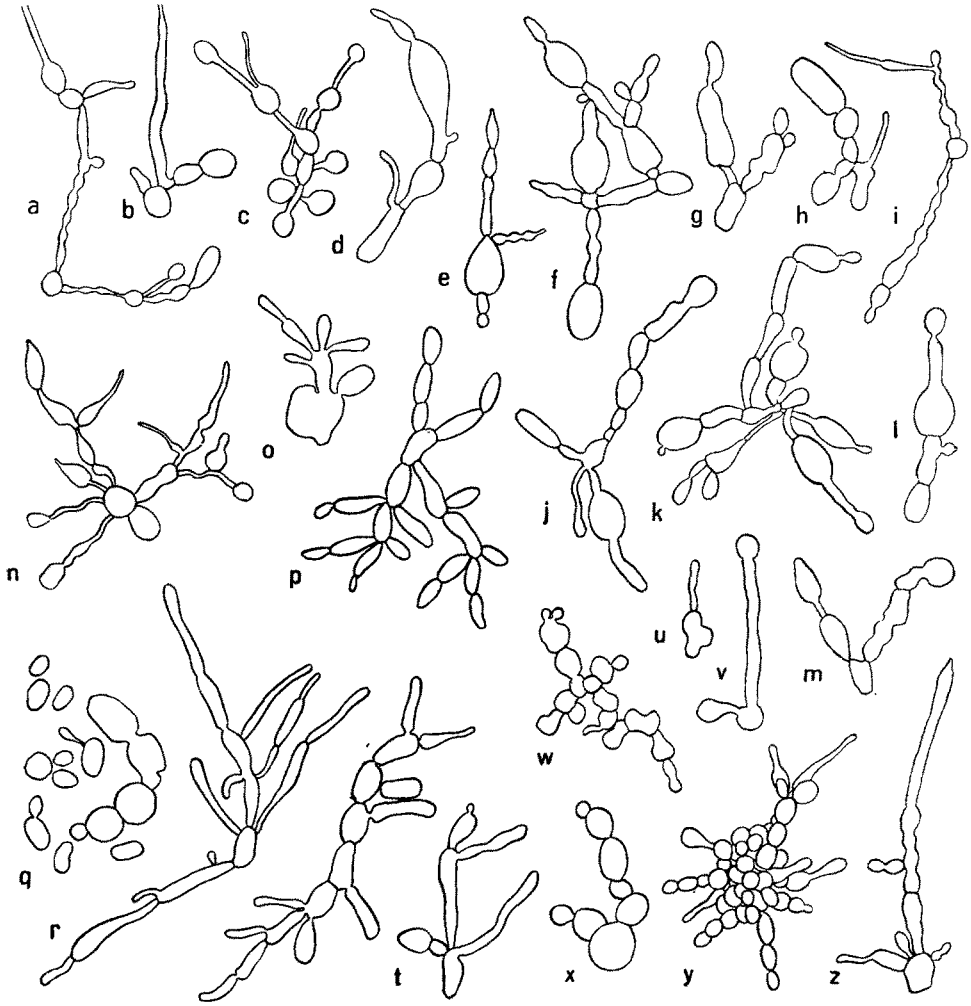


Fig. 2. Outline drawings of cf-forms induced by various aliphatic substances: ethyl ether (a—f), acetone (g—n), chloroform (o, p), paraldehyde (q, w), chloral hydrate (r—t,) urethane (u—v, x—z). 500×. Reproduced from LEVAN (1947).

Courtesy Prof. ALBERT LEVAN.

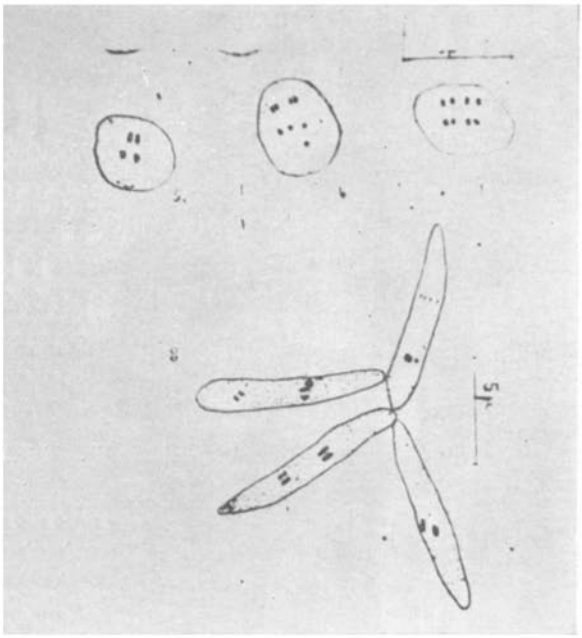


Fig. 3. *S. cerevisiae* exposed to acenaphthene in wort medium for 6 hr. resulted in filamentous cells. The elongated cells and some of the yeast phase cells (5) contain 4 chromosomes, according to SUBRAMANIAM. Reproduced from SUBRAMANIAM. (1945) Courtesy Current Science Assoc., Bangalore, India

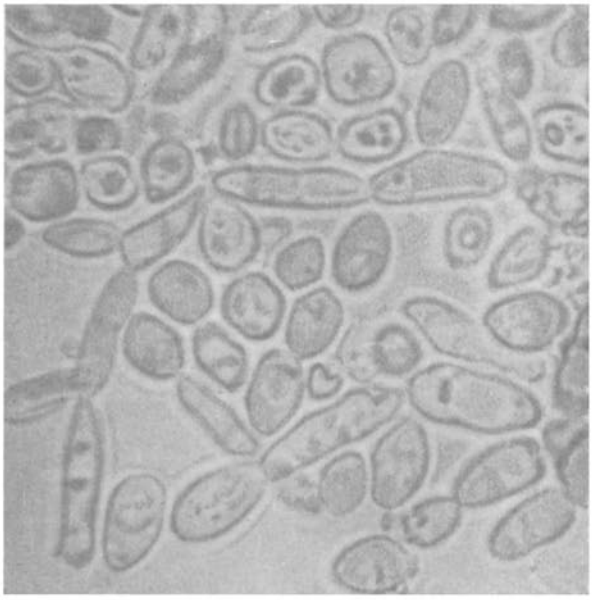
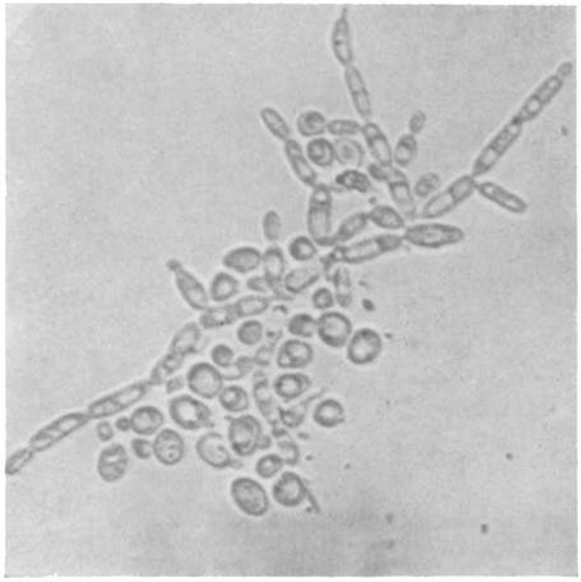


Fig. 4. *S. cerevisiae* exposed to 0.204 mg/ml of 9 : 10-bishydroxymethyl—1 : 2—benzanthracene (BB) in S.G. broth for 4 weeks at room temp. Elongated cells occur in the form of filaments. 970×.



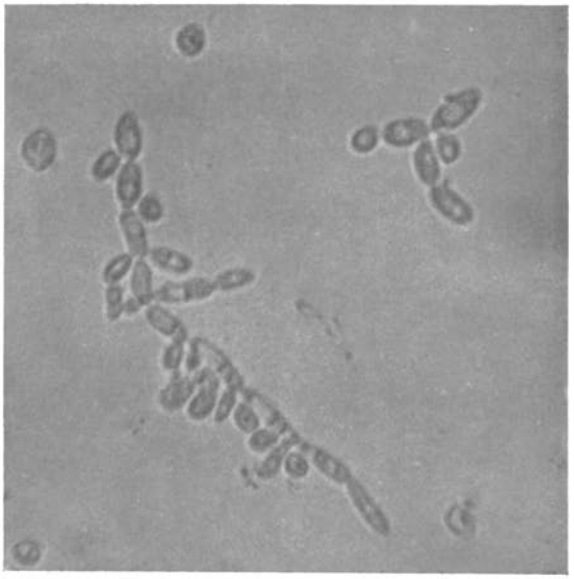


Fig. 5. *S. cerevisiae* exposed to 0.29 mg/ml of BB in S.G. broth for 15 days at room temp. Numerous yeast phase cells are still evident. 440×.

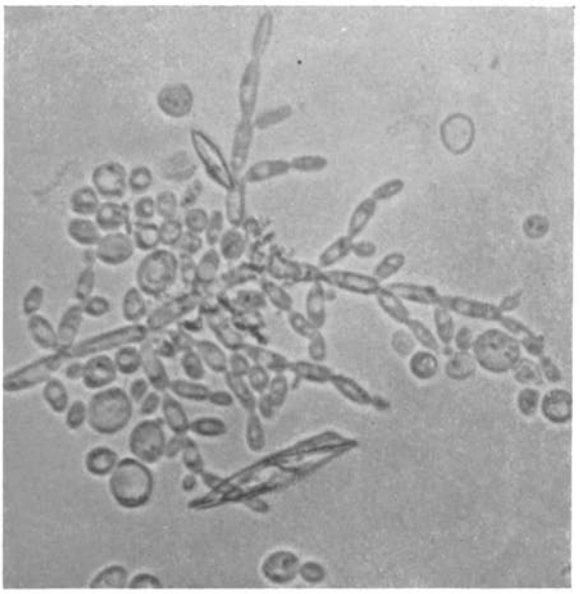


Fig. 6. *S. cerevisiae* exposed to 0.29 mg/ml of BB in S.G. broth for 21 days at room temp. Yeast phase cells are still evident and crystals of BB are also visible. 440×.

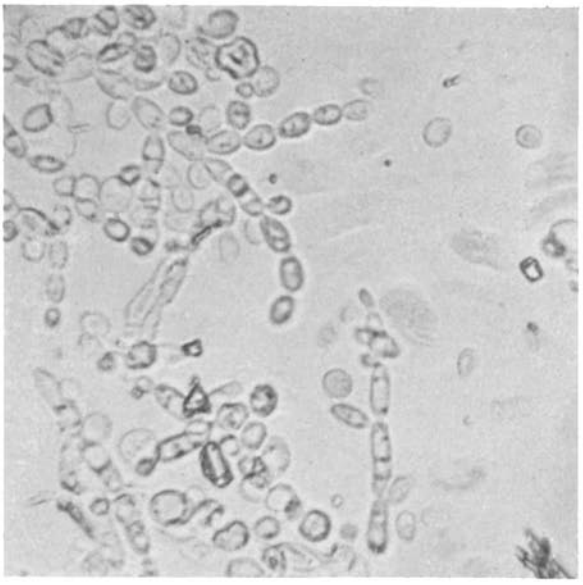


Fig. 7. Same conditions as in fig. 6. Note the strings of yeast cells which have not elongated. 440×.

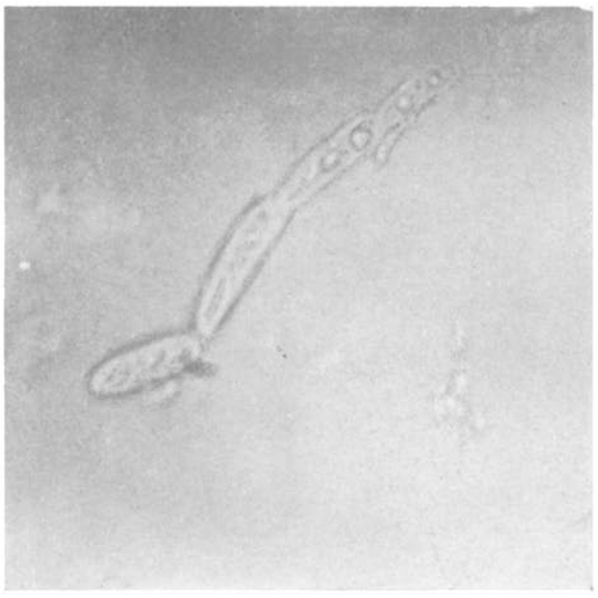


Fig. 8. *S. cerevisiae* exposed to 0.29 mg/ml of BB in S.G. broth for 26 days at room temp. Note the racquet mycelium. 970×.

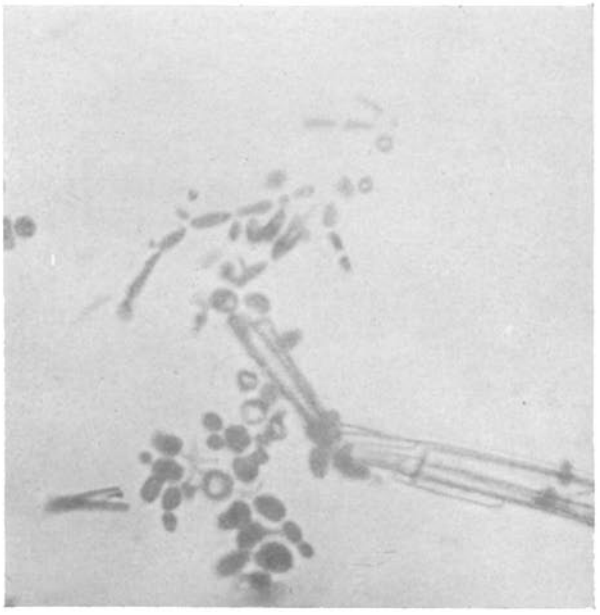


Fig. 9. *S. cerevisiae* exposed to 0.29 mg/ml of BB in S.G. broth for 26 days at room temp. Note the many cells that were unaffected morphologically. 440×.

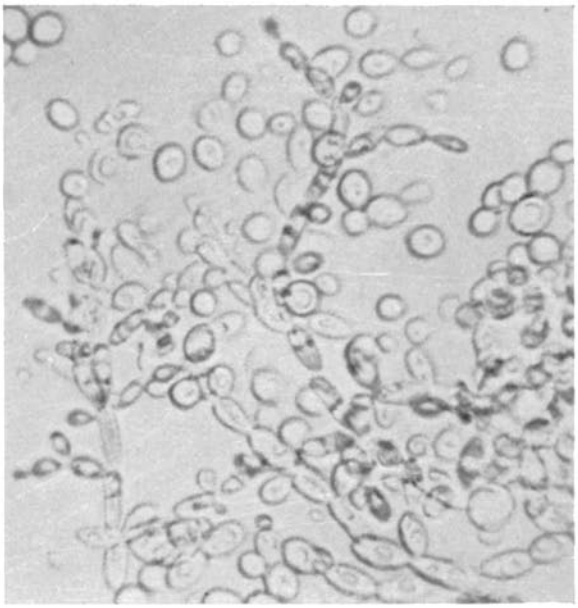


Fig. 10. *S. cerevisiae* exposed to 0.29 mg/ml of BB in S.G. medium for 37 days at room temp. Yeast phase cells are still evident, filaments of elongated cells are very long and occur in packets. 440×.

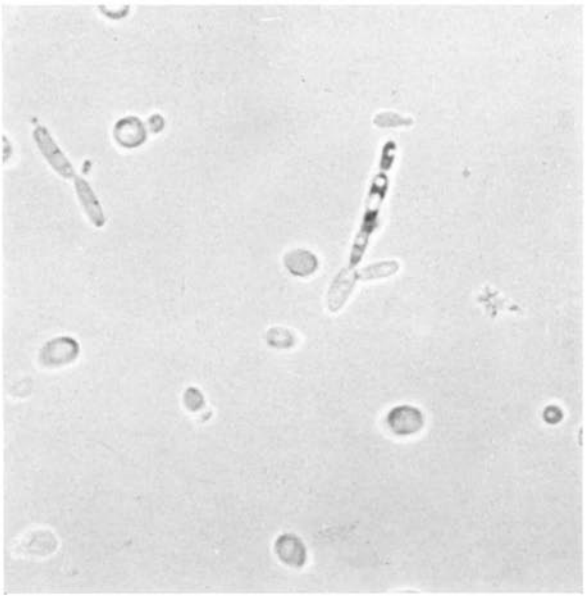


Fig. 11. *S. cerevisiae* exposed to 0.0204 mg/ml of BB in S.G. broth for 99 hr. at room temp. 440×.

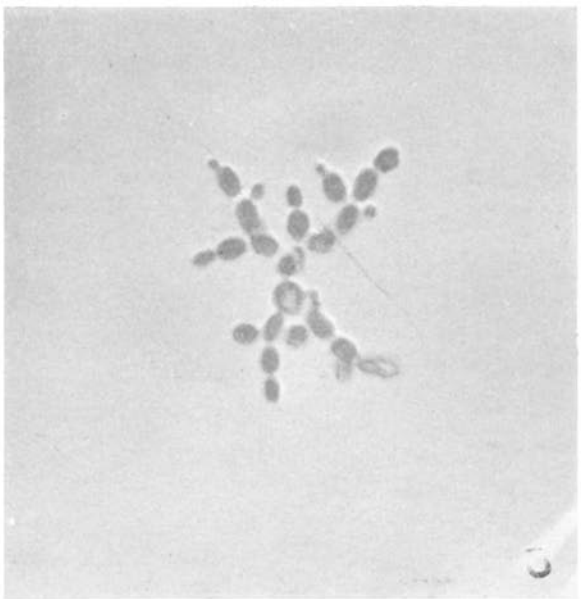


Fig. 12. *S. cerevisiae* exposed to 0.0204 mg/ml of BB in S.G. broth for 10 hr. at room temp. This fig. demonstrates cessation of cell division without occurrence of cell elongation. Stained with cotton blue. 440×.

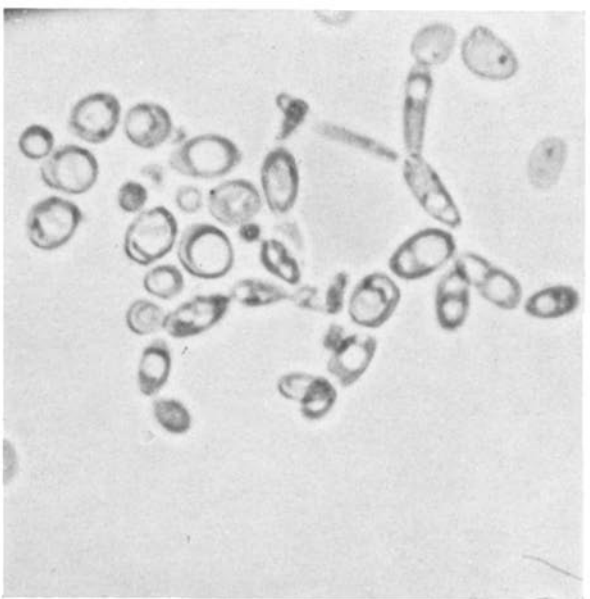


Fig. 13. *S. cerevisiae* exposed to 0.0204 mg/ml of BB in S.G. broth culture incubated with shaking for 99 hr. at room temp. 440×.

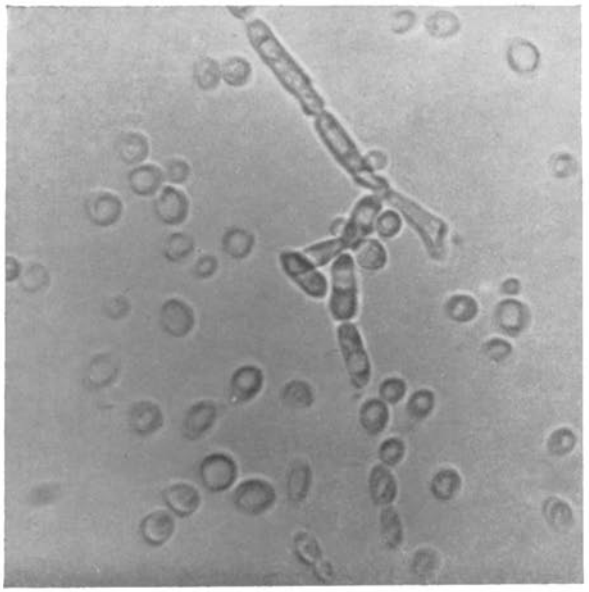


Fig. 14. *S. cerevisiae* exposed to 0.0204 mg/ml of BB in S.G. broth culture incubated with shaking for 99 hr. at room temp. 440×.

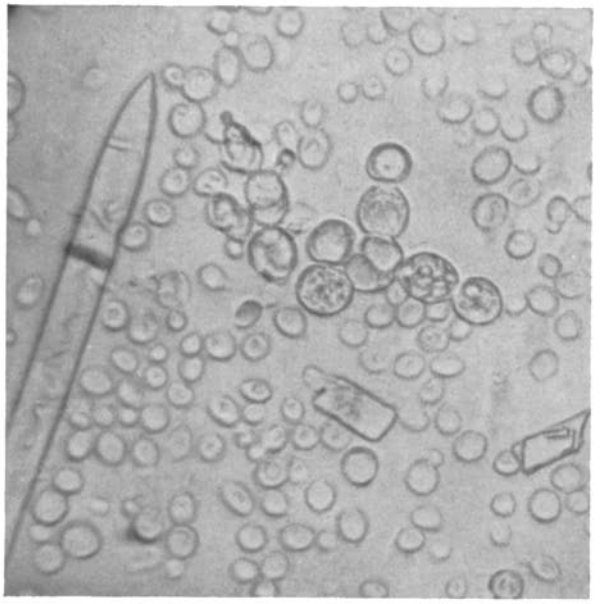


Fig. 15. *S. cerevisiae* exposed to 0.00002 mg/ml of BB in S.G. broth culture incubated for 195 hr. at room temp. with shaking. Note giant yeast cells and crystals of BB. 440×.

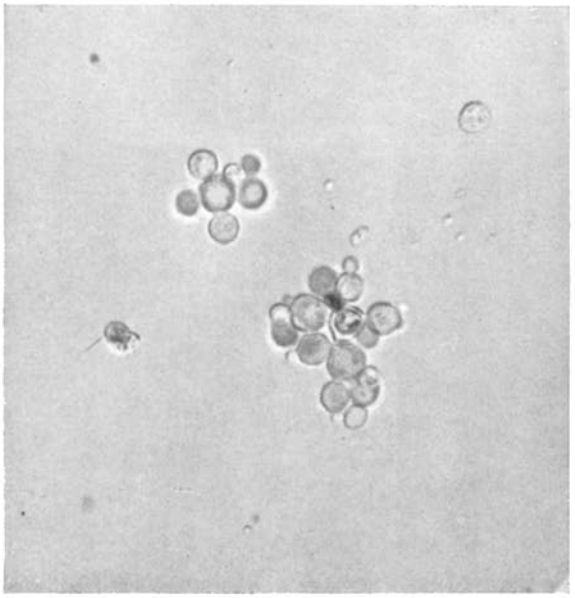


Fig. 16. *S. cerevisiae* exposed to 0.0204 mg/ml of BB plus 0.01 molar l-cysteine in #20 synthetic medium for 5 days. 440×.

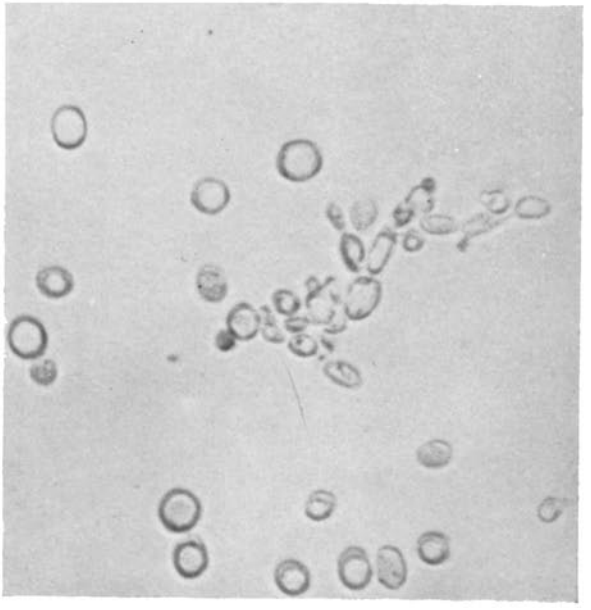
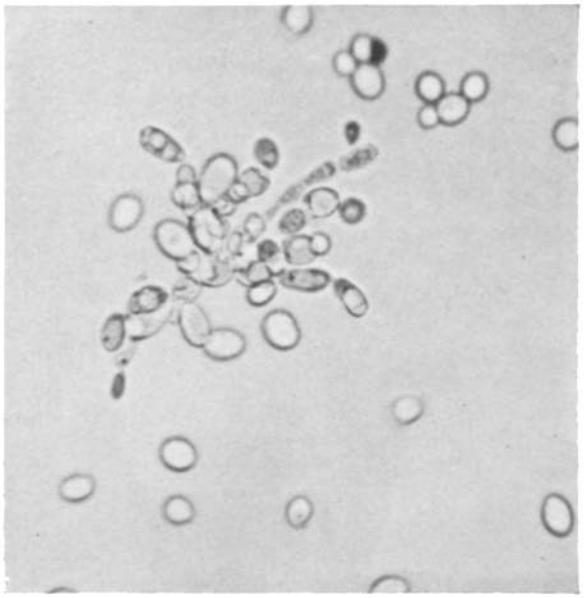


Fig. 17. *S. cerevisiae* in S.G. broth exposed to 0.01 mg/ml of ANAA after 2 days incubation at room temp. Note that the Infl contains yeastlike and elongated cells. 440×.



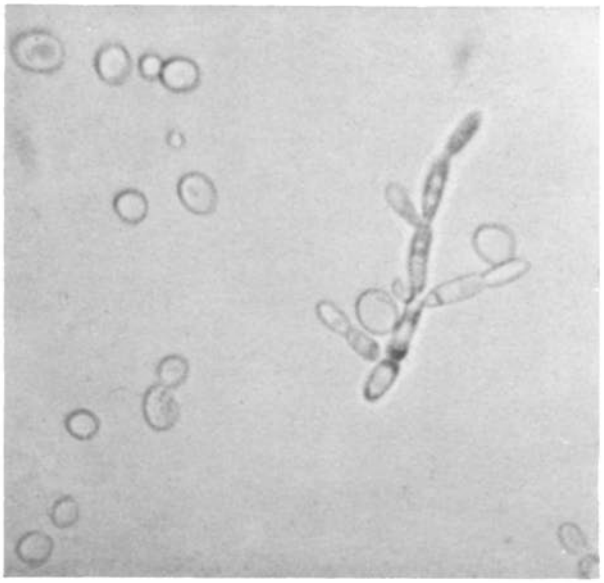


Fig. 18. *S. cerevisiae* in S.G. broth exposed to 1.0 mg/ml of ANAA for 4 days at room temp. 440×.

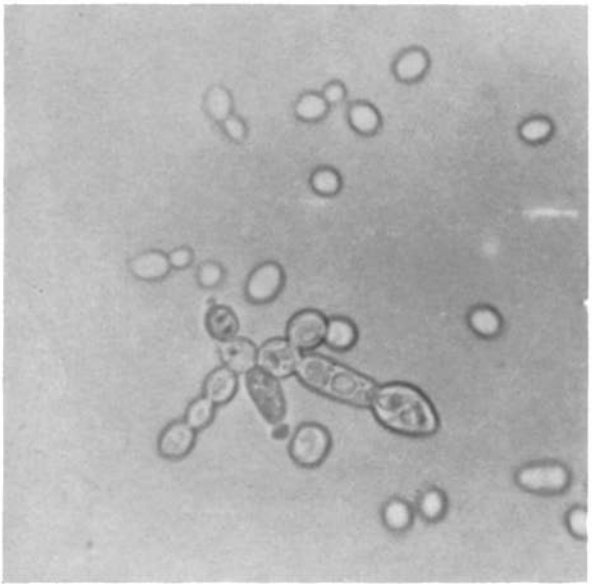


Fig. 19. *S. cerevisiae* in S.G. broth exposed to 1.0 mg/ml of ANAA for 4 days at room temp. The longest filament may be multinucleate. 440×.

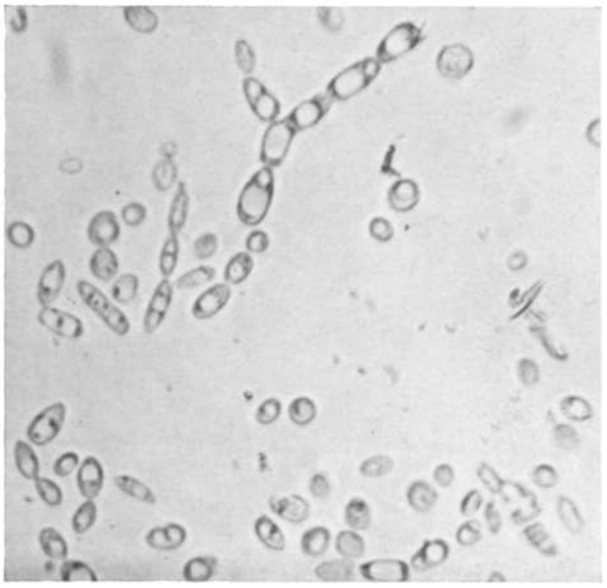


Fig. 20. *S. cerevisiae* in S.G. broth exposed to 0.1 mg/ml of ANAA for 4 days at room temp. 440×.

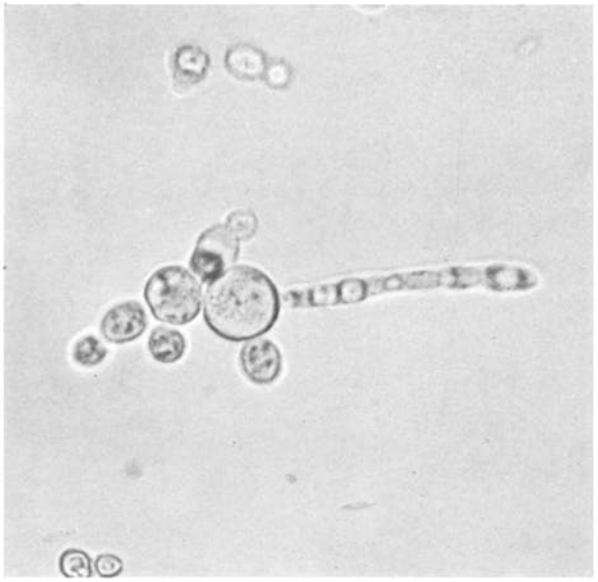


Fig. 21. *S. cerevisiae* exposed to 0.01 molar l-tryptophane HCl in #20 synthetic medium after 10 days at 20° C. 440×.

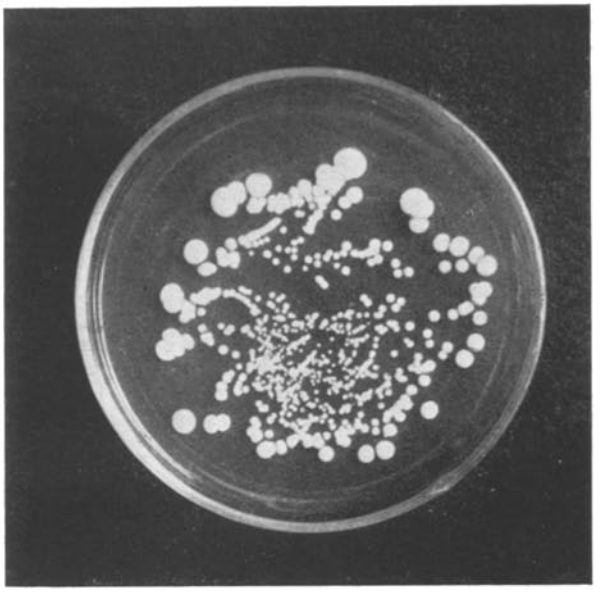


Fig. 22. *S. cerevisiae* on S.G. agar incubated at 20° C. for 8 days.

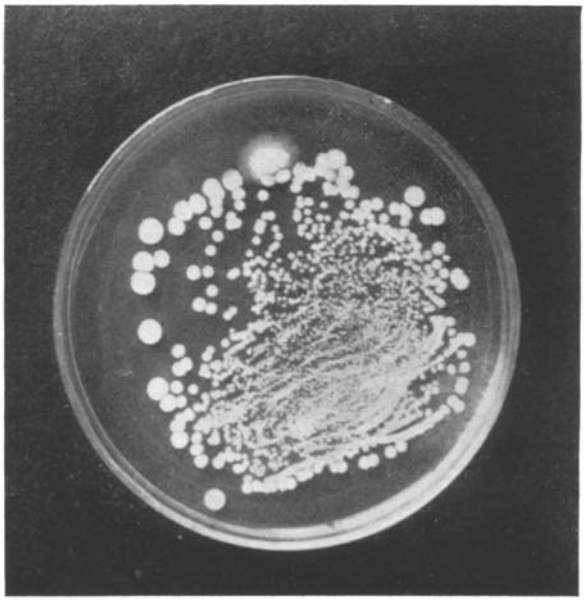


Fig. 23. *S. cerevisiae* on S.G. agar incubated at 30° C. for 8 days.

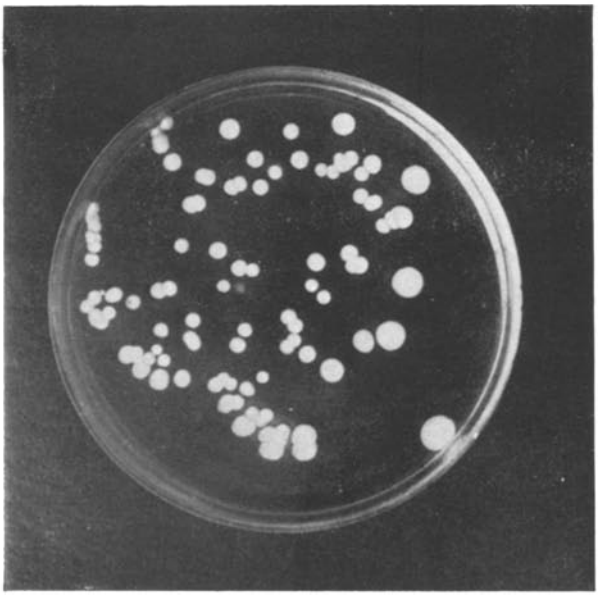


Fig. 24. *S. cerevisiae* on S.G. agar incubated at 37° C. for 8 days.

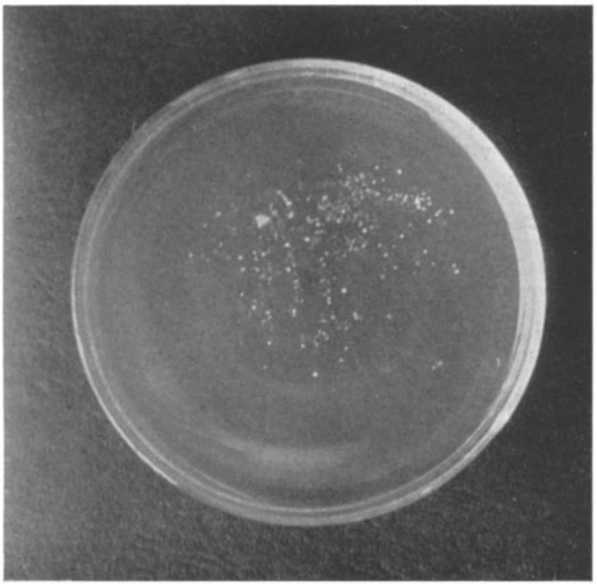


Fig. 25. *S. cerevisiae* on S.G. agar containing 1 mg/ml of ANAA, incubated at 20° C for 8 days.

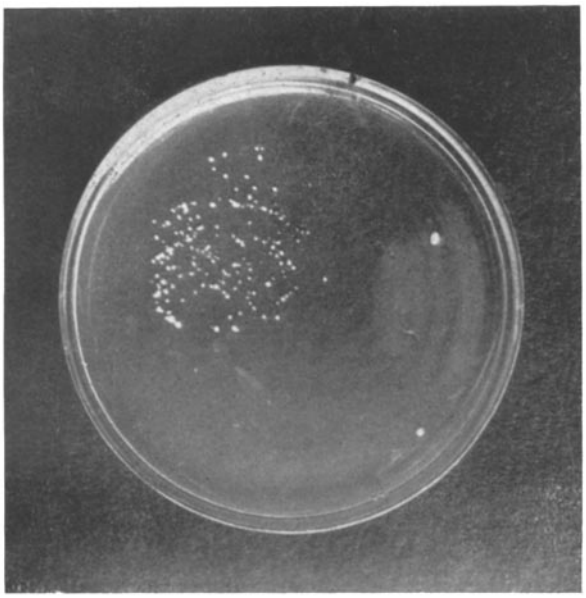


Fig. 26. *S. cerevisiae* on S.G. agar containing 1 mg/ml of ANAA, incubated at 30° C for 8 days.