THE EFFECT OF SULFHYDRYL COMPOUNDS, PENICILLIN, AND COBALT ON THE CELL DIVISION MECHANISM OF YEASTS

by

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The production of elongated cells of microorganisms may be viewed as a result of the differential inhibition of cell division without a concomitant inhibition of metabolic processes necessary for the growth of cells. This concept has been elaborated for bacteria[1, 2] and for yeasts and yeast-like organisms[3, 4, 5, 6]. There is evidence which indicates that differential inhibition of cell division results from the inhibition of a single enzyme complex, specifically active in cell division[3]. The cell division enzyme appears to be sensitive to intracellular sulfhydryl $\rightleftharpoons$ disulfide equilibria, and to be particularly sensitive to the presence of penicillin and cobalt. As is well known, there is a very extensive literature indicating that sulfhydryl groups (-SH) play some special rôle in cell division. RAPKINE[7, 8] has clearly implicated -SH groups in the cell multiplication processes of yeasts. Early studies on the effect of sub-bacteriostatic concentrations of penicillin by GARDNER[9] demonstrated that elongation of bacterial cells into filaments (designated here as B → F conversions) is the first, microscopically demonstrable, effect of penicillin. It is important to stress the extreme specificity of the locus affected by penicillin at such high dilution. Recently PRATT AND DUFRENOY[10] have emphasized this aspect of penicillin action and have demonstrated both an *in vivo* and an *in vitro* enhancement of penicillin by trace amounts of cobalt. The action of cobalt on yeast to mycelial conversion (Y → M) was observed independently, as an outcome of studies on the accumulation of radioactive cobalt by yeasts[11].

The present studies concern the action of sulfhydryl compounds on the inhibition of Y → M (promotion of cell division), and the promotion of Y → M (inhibition of cell division) by penicillin and heavy metals. Preliminary cytological studies on the nuclear conditions accompanying Y → M conversions have also been made. These indicate that nuclear division may continue even though cell division has been inhibited.

MATERIALS AND METHODS

Several species of yeasts, selected from the cultures maintained at the CENTRAAL BUREAU VOOR SCHIMMELCULTURES, Yeast Division, Delft, were employed as test organisms in a study of the effects of a variety of chemical substances on yeast to mycelial conversions (Y → M). The following organisms (strain number indicated) were used:

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Candida albicans,	35.1. 1	*Torulopsis colliculosa*,	24.3. 1
Candida pulcherrima,	35.2. 1	*Torulopsis Molischiana*,	24.5. 1
Candida tropicalis,	35.5. 1	*Torulopsis utilis*,	24.9. 1
Hansenula anomala,	10.1. 1	*Trichosporon capitatum*,	46.5. 1
Saccharomyces cerevisiae,	8.1. 1	*Trichosporon sp.*,	46.7. 1

The slide culture technique[12] was employed to permit microscopic examination of Y → M development. Addition of a cover glass to the agar slide cultures facilitated prolonged microscopic inspection. A potato agar was employed as the basal medium to which all substances to be tested were added; this was prepared as follows:

potato extract	230 ml
tap H_2O	770 ml
glucose	20 g
agar	20 g

The potato extract was prepared by soaking 100 g of washed, peeled, and ground potatoes in 300 ml tap water for several hours at *ca.* + 4° C. The mass was then filtered through cloth and autoclaved for 15 minutes at $1^3/_4$ atmospheres.

All substances to be tested were sterilized separately by heating or by filtration, as appropriate, and added to the potato agar immediately before preparing the slide cultures to give final concentrations as indicated. All slides were inoculated promptly (in duplicate) with three parallel streaks, using 24–48 hours old stock cultures. Microscopic examinations were made after 24 and 48 hours incubation in a humid atmosphere (in sterile Petri dishes) at 25° C. The organic chemicals employed were commercial products, purified when necessary before use; sodium penicillin G was a product of the NEDERLANDSCHE GIST -EN SPIRITUS-FABRIEK; inorganic compounds used were reagent quality.

Cytological studies were made on air dried smears stained for ten minutes with 1% aqueous toludine blue (National Aniline), washed in water, and differentiated in 95% ethanol.

EXPERIMENTAL

The slide culture technique, a modification of the direct agar-microscopy procedure of ØRSKOV[33], is a quite satisfactory method for determining whether or not a given strain of a yeast will develop mycelial or pseudo-mycelial structures. This method is widely employed for purposes of identification of yeasts. The potato agar substrate, usually employed with the slide culture method, permits ready development of M structures by strains of the *Mycotoruloideae*, which are recognized on the basis of their production of such structures. This procedure can also serve as a suitable method for the testing of substances, added to the potato agar, for their effect on Y → M conversions. In this manner we have tested many compounds for ability to inhibit or to promote Y → M conversions in a variety of yeasts.

Maintenance of a single cell condition (Inhibition of Y → M)

As a result of work[4, 5] on the fractionation of filtrates (from cultures of *Trichophyton rubrum*) which exhibited ability to inhibit Y → M in *Candida albicans*, our attention was directed toward the testing of sulfhydryl (–SH) group containing compounds for such properties. As shown in Table I the property of inhibiting Y → M is clearly exhibited by cysteine at 10^{-2} M concentration in potato agar and is apparent, with *C. albicans* and *H. anomala*, at 10^{-3} M. The appearance of cysteine-treated, and of control growths, is shown in Figs 1, 2, and 6a. A simple influence on the redox potential as an explanation for the action of cysteine appears improbable from the data in Table II which show a complete absence of effect with ascorbic acid at 10^{-2} M (Fig. 4a and b). A certain specificity in the nature of the –SH donor may also be inferred from Table III, wherein it is shown that sodium thioglycollate (Fig. 3) is less effective than glutathione, which is in turn less effective than cysteine for inhibiting Y → M. The high

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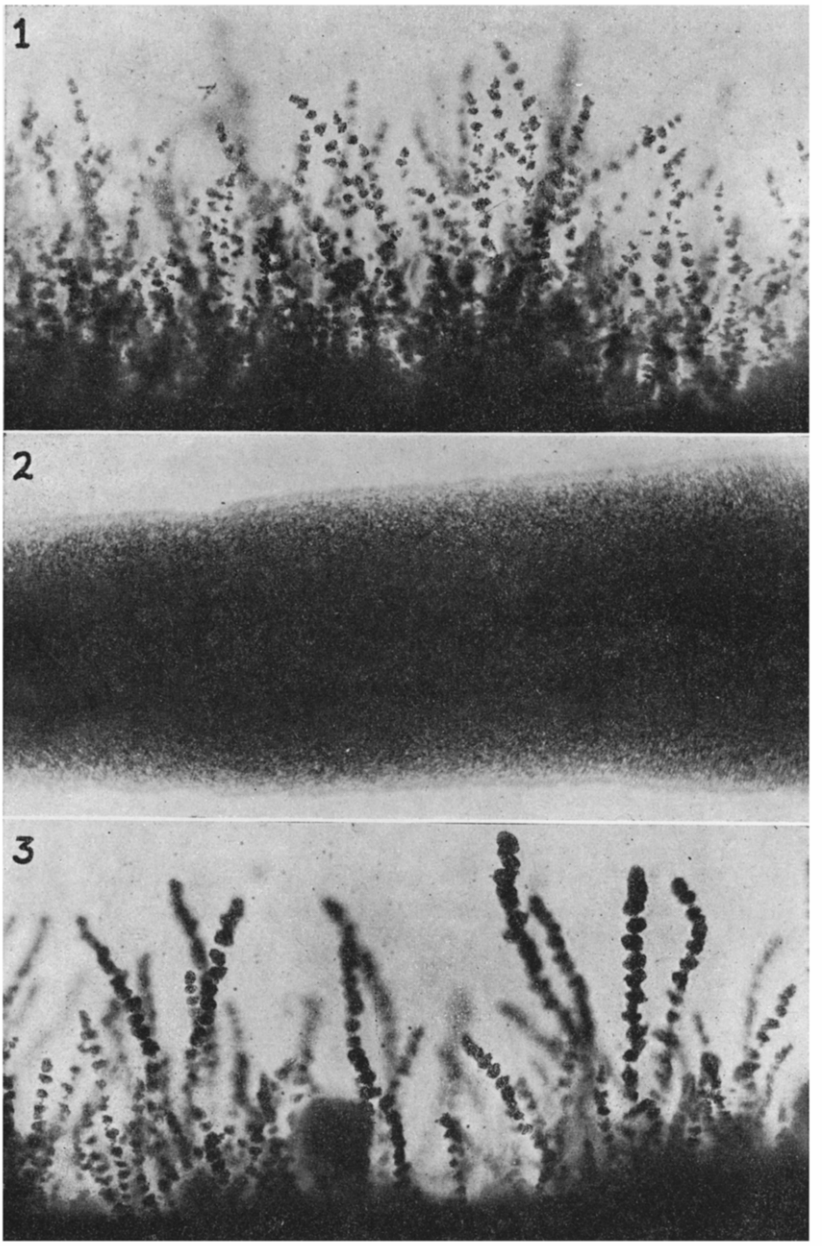

Fig. 1. *Candida albicans*, untreated, from control slide culture; note spacing between clusters of blastospores; 100 ×

Fig. 2. *Candida albicans*, slide culture treated with M/100 cysteine; complete inhibition of M development; 100 ×

Fig. 3. *Candida albicans*, slide culture treated with M/100 sodium thioglycollate; note close spacing of clusters of blastospores; 100 ×

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activity of potassium dithiooxalate is notable. While this compound exhibited considerable growth-inhibitory action when tested against yeasts that do not normally form M structures (*Torulopsis Molischiana*, *T. colliculosa*, and *Saccharomyces cerevisiae*), it adversely affected the growth of only one of the yeasts listed in Table III.

Inhibition of mycelial formation may also be viewed as the maintenance of cells in a single cell condition. In this light we may also consider the action of cysteine to be one of favouring, or promoting cell division. This concept of association of -SH compounds with cell division may serve to clarify many earlier observations on the dimorphism of *Candida albicans* in which various metabolic phenomena were implicated. As is well known (see HEWITT[13] and KLUYVER[14]), the metabolism of carbohydrate substrates by yeasts is associated with a fall in the redox potential of the medium (reflecting, in all probability to varying degrees, conditions in the intracellular environment). This intracellular reducing situtation will undoubtedly shift the *in vivo* equilibrium, $-SH \rightleftharpoons -S-S-$, toward the left. Early researches of LINOSSIER AND ROUX[15], and FINEMAN[16], on Y → M

TABLE I

EFFECT OF CYSTEINE ON MAINTENANCE OF SINGLE CELL CONDITION AND INHIBITION OF Y → M CONVERSIONS IN DIFFERENT YEASTS

— = NONE OBSERVABLE; +, ++, AND +++ INDICATE SLIGHT, MODERATE, AND PRONOUNCED, RESPECTIVELY

Organism	Control (Potato agar)		Cysteine (10^{-3} M)		Cysteine (10^{-2} M)	
	Y → M	Growth	Y → M	Growth	Y → M	Growth
C. albicans	+++	+++	++	+++	--	++++
C. tropicalis	+++	+++	+++	+++	--	+++
Trich. capitatum	++	+++	+++	+++	++	+++
H. anomala	+	+++	--	+++	--	+++

TABLE II

EFFECT OF ASCORBIC ACID ON Y → M CONVERSIONS

Organism	Control		Ascorbic acid (10^{-3} M)		Ascorbic acid (10^{-2} M)	
	Y → M	Growth	Y → M	Growth	Y → M	Growth
C. albicans	+++	+++	+++	+++	+++	+++
C. tropicalis	+++	+++	+++	+++	++++	+++
Trichosporon sp.	+++	+++	+++	+++	+++	+++
H. anomala	++	+++	+++	+++	++	+++

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EFFECT OF DIFFERENT —

Organism	Control		Sodium thioglycollate (10^{-2} M)		Sodium thioglycollate (10^{-3} M)	
	Y → M	Growth	Y → M	Growth	Y → M	Growth
C. albicans	+++	+++	+++	+++	+	+++
C. tropicalis	+++	+++	+++	+++	+++	+++
Trich. capitatum	+++	+++	+++	+++	+++	+++
H. anomala	++	+++	+++	+++	+	+++

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conversions in *C. albicans*, can be seen to indicate that readily metabolizable carbon sources (*e.g.*, glucose) inhibit Y → M, while carbon substrates metabolized slowly, or hardly at all, favour Y → M. It is clear that in the latter case the sulfhydryl ⇌ disulfide equilibrium will lie towards the right. There are also the well known observations of LANGERON AND GUERRA[17] on the failure of M development on the inner sides of *closely-spaced* parallel streaks of *C. albicans*, with abundant Y → M conversions developed on the outer sides of the same streaks. Diffusion of Y → M inhibitors between adjacent streaks was advanced as an explanation of the observed phenomenon[17]. Recently, MAGNI[18] has confirmed these observations; he found, furthermore, that removal of the agar from one side of an isolated streak (leaving up to 8 mm of agar adjacent to the streak) prevented the appearance of M on the side with a narrow agar boundary, but did not interfere with abundant M production on the opposite side of the streak. The observations of LANGERON AND GUERRA and of MAGNI would seem to be readily accounted for in terms of the metabolic production of a higher concentration of diffusible reducing substance on one side of a streak (sufficiently high on one side to inhibit Y → M).

THE UNCOUPLING OF CELL DIVISION FROM GROWTH

Promotion of Y → M

As already pointed out[9] one of the very first effects of penicillin on bacteria that is observable is the selective inhibition of cell division, without the simultaneous inhibition of other metabolic processes resulting, in many instances, in the growth of bacteria into elongated filaments. From Tables IV and V it will be seen that Y → M was promoted by a concentration of penicillin which was without visible effect on the amount of growth of the test organisms used under our conditions. Addition of cysteine completely antagonized the penicillin effect (we have not yet investigated the minimum concen-

TABLE IV

EFFECT OF PENICILLIN ON THE UNCOUPLING OF CELL DIVISION FROM GROWTH OF YEASTS, AND THE ANTAGONISM OF PENICILLIN BY CYSTEINE

Organism	Penicillin (11 U/ml)		Penicillin + Cysteine (10^{-2} M)		Control	
	Y → M	Growth	Y → M	Growth	Y → M	Growth
C. albicans . .	++++	+++	—/+	+++	+++	+++
C. tropicalis . .	++++	+++	—/+	+++	+++	+++
H. anomala . .	+++	+++	—	+++	++	+++

MPOUNDS ON Y → M CONVERSIONS

Potassium dithiooxalate (10^{-3} M)		Glutathione ($3.6 \cdot 10^{-3}$ M)		Cysteine (10^{-2} M)	
Y → M	Growth	Y → M	Growth	Y → M	Growth
++	+++	+	++++	—	+++
+	+++	++	++++	—	++++
+	+	+++	+++	—/+	+++
—	+++	++	+++	—	+++

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TABLE V

EFFECT OF PENICILLIN AND OF CYSTEINE ON B → F IN *Bacillus cereus*

Penicillin (11 U/m)		Penicillin + Cysteine (10^{-2} M)		Control	
B → F	Growth	B → F	Growth	B → F	Growth
+ + + +	+ +	-	+ +	-	+ +

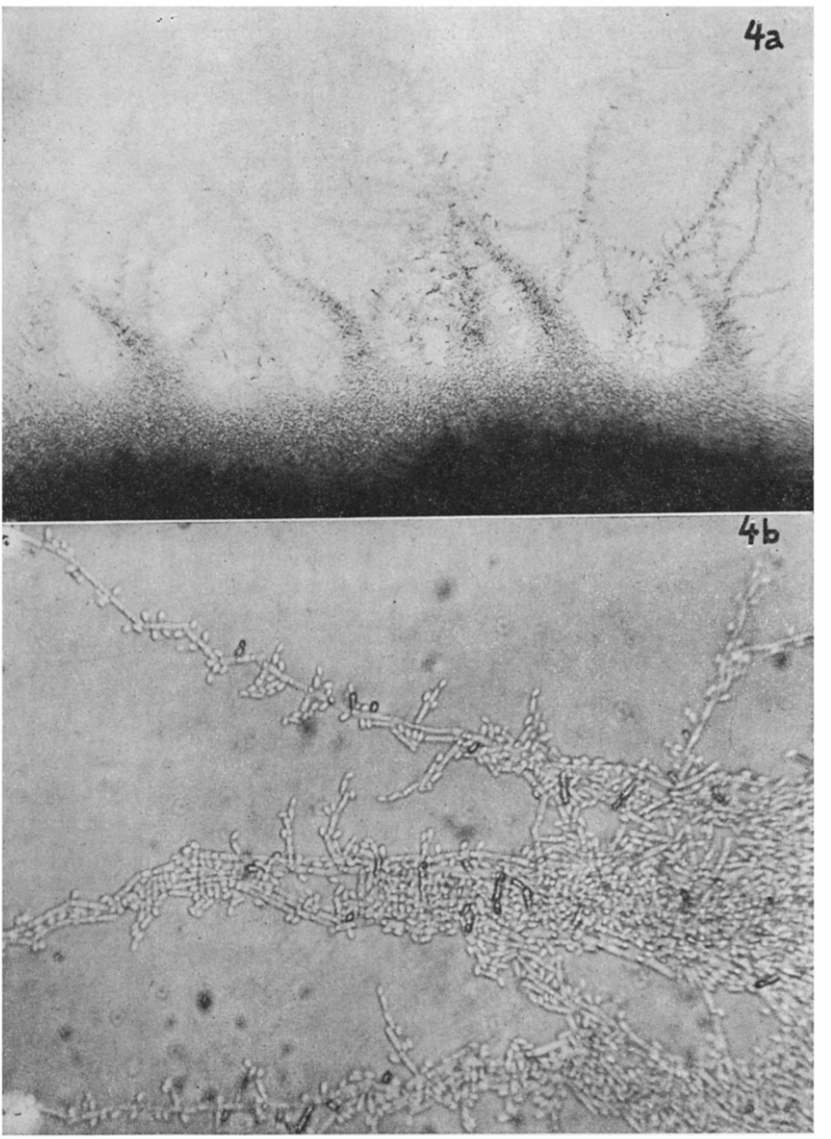

Fig. 4. a) *Hansenula anomala*, from slide culture treated with M/100 ascorbate; filamentous tendencies pronounced; 100 ×; b) same, 400 ×

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tration of cysteine necessary for penicillin antagonism under these conditions). With *Bacillus cereus* (Delft strain E XI.1.11.4) as test organism there was a most remarkable increase in cell length with penicillin treatment; while cysteine completely suppressed B → F tendencies. The *B. cereus* slide cultures with cysteine contained a very high percentage of sporulating cells.

Although we have not observed mycelial production with cells of *Saccharomyces cerevisiae* exposed to penicillin (or to other treatment), a definite change in cell morphology has been regularly observed. As shown in Fig. 5a the cell shape in penicillin treated slide cultures is considerably more elongate than that of the normal-appearing cells from penicillin plus cysteine treatment (Fig. 5b).

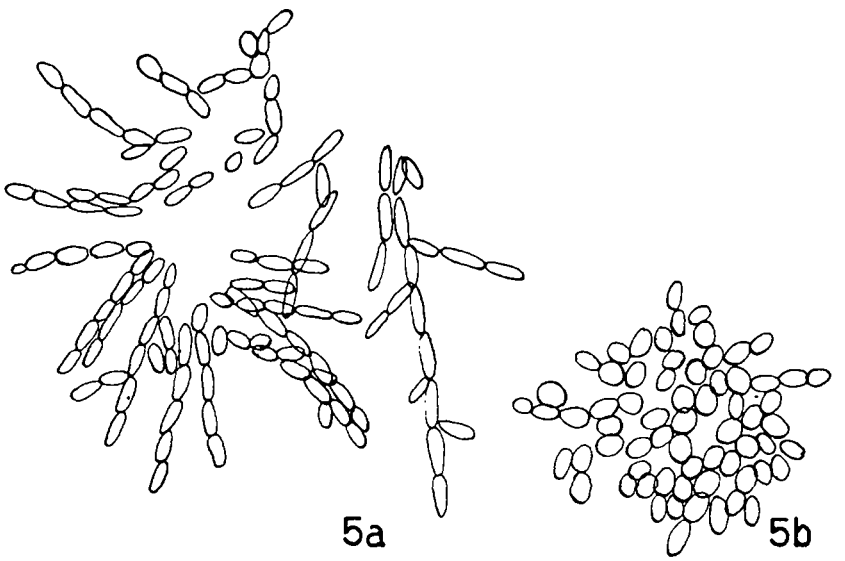

Fig. 5. a) *Saccharomyces cerevisiae*, camera lucida drawing of cells from slide agar culture treated with penicillin (see text); cells elongated, 440 ×; b) same, from agar culture with penicillin plus M/100 cysteine, 440 ×

In some earlier investigations, in which -SH groups were associated with cell division of microorganisms, emphasis was placed on the inhibitory action of heavy metals on cell division processes. The Cu^{++} ion was found by VOEGTLIN AND CHALKLEY[19] to be especially toxic for the cell division of *Ameba proteus*. Cobalt enhancement of the action of penicillin both *in vitro* and *in vivo*[10] has already been mentioned. We have studied the effect of certain heavy metals on Y → M processes in several yeasts. Table VI

TABLE VI

EFFECT OF COBALT ON THE PROMOTION OF Y → M

Organism	Control		$CoAc_2$ ($4 \cdot 10^{-5}$ M)		$CoAc_2$ (10^{-3} M)	
	Y → M	Growth	Y → M	Growth	Y → M	Growth
C. albicans	+++	+++	+++	+++	+++++	+++
C. tropicalis	+++	+++	++++	+++	++++	+++
Trich. capitatum . . .	+++	+++	+++	+++	++++	+++
H. anomala	++	+++	+++	+++	+++	+++

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EFFECT OF COBALT AND OTHER HEAVY METALS ON Y → M

Organism	Control		$CuSO_4$ (10^{-3} M)		$K_2B_4O_7$ (10^{-3} M)	
	Y → M	Growth	Y → M	Growth	Y → M	Grow
C. pulcherrima	—	+++				
T. colliculosa	—	+++				
T. Molischiana						
T. utilis	—					
H. anomala	++				—	

indicates the effect of low concentrations of cobaltous acetate on certain yeasts that readily produced M structures on the potato agar control slide cultures. At 10^{-3} M Co^{++} there was a markedly more abundant production of M, with all the species tested, than was observed in the controls. In Table VII there is registered the effect of several heavy metals on some strains of yeasts that did not produce M under our control conditions.

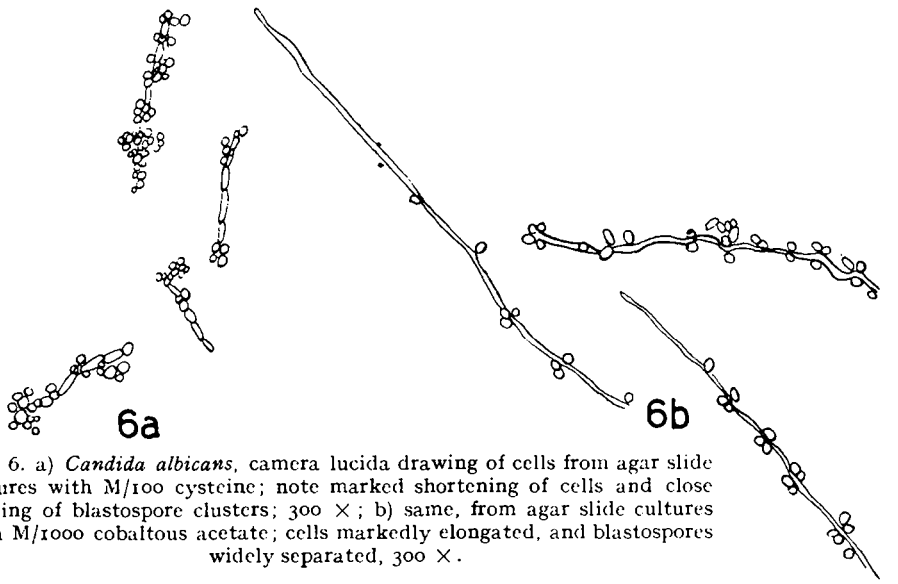

Fig. 6. a) *Candida albicans*, camera lucida drawing of cells from agar slide cultures with M/100 cysteine; note marked shortening of cells and close spacing of blastospore clusters; 300 ×; b) same, from agar slide cultures with M/1000 cobaltous acetate; cells markedly elongated, and blastospores widely separated, 300 ×.

A marked effect of 10^{-3} M Co^{++} is to be noticed. Aside from cobalt, only boron produced a significant effect on Y → M, though this effect was much less pronounced than that of cobalt. In Fig. 6 the shapes of cells of *C. albicans* from a 10^{-3} M Co^{++} slide culture are compared with those from a 10^{-2} M cysteine slide culture; the cobalt-treated cells form mycelial elements — no cross walls were visible in the segments drawn. In contrast, the cysteine-treated cells, supporting blastospore structures, are extremely short.

Effect of Cobalt and Oxine on Yeasts on Solid and in Liquid Media

Recent publications by ALBERT *et al.*[20] have shown that cobalt is by far the most effective substance for reversing the growth inhibitory effect of oxine (8-hydroxy quinoline) on Gram + bacteria. We tried the effect of oxine on Y → M using the slide culture technique (Table VIII) but, at the concentrations employed, the most noticeable effect of oxine was on total growth. Experiments were then made on the effect of oxine

IE YEASTS THAT DO NOT USUALLY PRODUCE M STRUCTURES

$MnCl_2$ (10^{-3} M)		Na_2HAsO_3 (10^{-3} M)		Na_3AsO_4 (10^{-3} M)		$CoAc_2$ (10^{-3} M)	
Y → M	Growth	Y → M	Growth	Y → M	Growth	Y → M	Growth
—	+++	—	+	—	—/+	+	+++
—	+++	—	+	—	++	+/—	+++
—	+++	—	+	—	—/+	—	+++
—	+++	—	+++	—	+	++	+++
++	+++	—	+	—	—/+	+++	+++

on the growth of *Candida albicans* (Gram+, as are all yeasts) in liquid media. A wort medium was prepared with additions as noted in Table IX; only at M/10000 oxine was an effect on growth detectable (ALBERT *et al.* reported M/80000 oxine prevented the growth of Gram+bacteria; they, too, tested in a "natural" medium). Higher concentrations of oxine were therefore prepared (Table X) and inoculated; except for a moderate effect on growth when examined at 24 hours, oxine was not inhibitory at M/2500 concentration.

While it is clear that oxine is without appreciable effect itself, under our conditions, on Y → M or on total visible growth of yeasts in liquid media, it is also clear that it prevents the appearance of cobalt effects. Compare Y → M enhancement by cobalt, as shown in Tables VI and VII, with Table VIII. Cobalt has, moreover, been shown[11] to completely inhibit the growth of yeasts (*S. cerevisiae* and *C. albicans*) when added to natural media (beer wort) in concentrations greater than M/10000. Thus, oxine antagonizes both the growth-inhibitory and the Y → M promoting, effects of cobalt.

TABLE VIII

EFFECT OF 8-HYDROXY QUINOLINE (OXINE) AND COBALT + OXINE ON Y → M

Organism	Control		Oxine (10^{-4} M)		Oxine (10^{-4} M) + $CoAc_2$ ($2 \cdot 10^{-4}$ M)	
	Y → M	Growth	Y → M	Growth	Y → M	Growth
C. albicans	+++	+++	+++	++	+/—	+++
C. tropicalis . . .	+++	+++	++++	+/++	++++	++
Trich. capitatum .	+++	+++	—	+/—	++	++
H. anomala . . .	++	+++	+++	+++	++	++++

TABLE IX

EFFECT OF 8-HYDROXY QUINOLINE (OXINE), COBALT, AND CYSTEINE ON THE GROWTH OF *Candida albicans* IN WORT; 22 HOURS INCUBATION AT 25° C

Addition / Concentration	Oxine	Oxine (M = 1/x) + $CoAc_2$ (M = 2/x)	Oxine (M = 1/x) + cysteine (M/5000)	Oxine (M = 1/x) + $CoAc_2$ (M = 2/x) + cysteine (M/5000)
M/10000	+	++	+	+
M/25000	+++	+++	+++	+++
M/50000	+++	+++	+++	+++
M/80000	+++	+++	+++	+++

Blank (plain wort) = +++; Wort + M/5000 cysteine = +++

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TABLE X

EFFECT OF 8-HYDROXY QUINOLINE ON THE GROWTH OF *Candida albicans* IN WORT AT 25° C

Concentration \ Addition	Oxine		Oxine (M = 1/x) + $CoAc_2$ (M = 2/x)		Oxine (M = 1/x) + $CoAc_2$ (M = 2/x) + cysteine (M/2500)	
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
M/2500	+	+ + +	+ +	+ + +	+ +	+ + +
M/5000	+ +	+ + +	+ + +	+ + +	+ +	+ + +
M/10000	+ +	+ + +	+ + +	+ + +	+ + +	+ + +

Blank (plain wort) = + + + at 24 hours; Wort + M/2500 cysteine = + — + at 24 hours

Inhibition of Cell Division without Simultaneous Inhibition of Nuclear Division

The processes involved in nuclear division may in many instances be regarded as precursors, in point of time, of the processes leading to the division of one cell into two. During Y → M conversions, however, it has been found that long M elements may exhibit several nuclei[3], or only one or a few nuclei (Figs 7 and 8) along the length of an extensive filament. Evidently then, cell division may be inhibited under conditions that permit nuclear division to continue*. LEVAN[21] described much the same situation

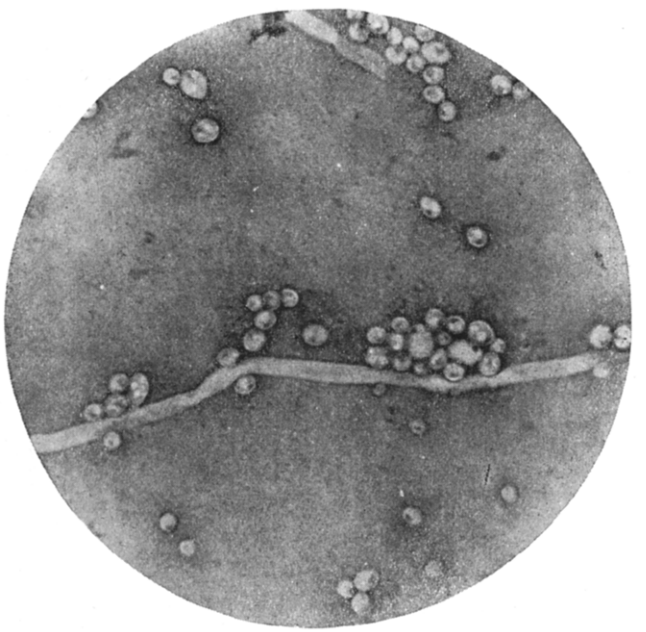

Fig. 7. *Candida albicans*, cells from control slide agar cultures stained with 1% aqueous toluidine blue (see text); 1000 ×, from 2 day culture.

* Blastospores, which are produced from the M elements of *C. albicans*, *C. tropicalis*, *Trichosporum capitatum*, and many other yeasts, are seen (Figs 7–8) to be uninucleate Y entities. The morphogenetic phenomena underlying the intensive nuclear division which gives rise to a cluster of blastospores, localized at "nodes" spaced more or less regularly along an M filament, are worthy of serious study but are outside the scope of this paper. Blastospore production was rarely completely absent in these yeasts, even under the most pronounced Y → M stimulation, (Table VI, Fig. 6b); it should be noted, however, that at least the first blastospore produced at a given node must have derived its nucleus from the division of an M-nucleus.

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from his study on the induction of aberrant forms in *S. cerevisiae* by treatment with camphor; nuclear division was found to proceed in the absence of cell division.

The appearance of the structure designated as a nucleus by us (Fig. 8) is fairly uniform from cell to cell. The cytological picture observed by us provides confirmation of the concept of the yeast nucleus presented by NAGEL[22]. Additional confirmation, by a completely different technique, has been supplied by the nuclear localization of yeast pyrophosphatase[23].

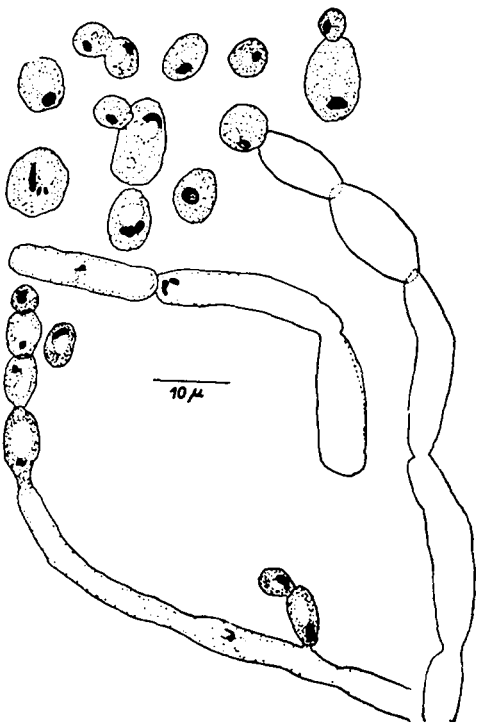

Fig. 8. As Fig. 7, from 5 day old culture

The very low level of cytoplasmic basophily in the yeasts grown on the potato agar medium seems worthy of note. Several of the species studied were grown in a wort medium and subsequently stained with toluidine blue as described; cells from such growths invariably showed such a dense overall staining that no internal detail could be discerned. As WIAME[24] and BRACHET[25] have pointed out, the affinity of a cell for toluidine blue is probably the result of its content of the components of a nucleic acid-metaphosphate complex. Metaphosphate is probably responsible for the metachromatic staining of yeasts as WIAME AND MICHAELIS[26], have shown. By reason of the fairly firm cytochemical basis for the toluidine blue staining, we may infer that the cells shown in Figs 7–8 have a very low cytoplasmic content of ribonucleic acid and metaphosphate. Such an interpretation is in line with the findings[3] that Y → M is accompanied by a loss of Gram staining positivity and by a very greatly reduced affinity for pyronin from the Unna-Pappenheim stain. It is also important to note that PRATT AND DUFRENOY[10] have shown that B → F, induced in Gram+ bacteria by penicillin, is accompanied by a loss of Gram positivity. Some implications of these cytochemical findings for the relationship between nucleic acids and the protein synthesis accompanying growth in the absence of cell division have already been indicated[3]. In contrast to these findings (based on the use of staining reactions) which indicate a lowered nucleic acid content in cells that have undergone elongation, HENRY *et al.*[27] found, by analytical determination, that no change was produced in the total amount of nucleic acid, or in the ratio of the two types of nucleic acid, in cells of *Clostridium Welchii* in which elongation had been induced by sub-bacteriostatic concentrations of penicillin. On the other hand, they obtained[27] certain of the protein constituents of the cytoskeleton in unusual fibrous form. Their findings are not necessarily in contradiction to the results achieved with staining procedures, which probably depend upon the physical state of nucleate-protein linkages (through –SH protein bonds) in the case of the Gram stain[28], and probably upon the degree of polymerization of ribonucleic acid in the case of the basophilic stains. The parallel between prevention of fibrous protein formation and the

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maintenance of cell division has already been discussed[3], see also[7]. In this connection it is of interest to note that LWOFF AND JONESCO[37], with *Moraxella Lwoffi*, have obtained extensive elongation of the nuclear apparatus accompanying filament formation, induced by cultivation of the bacterium in the absence of potassium.

DISCUSSION

The importance of sulfhydryl groups in the cell division processes of microorganisms, as demonstrated in this paper, is, we believe, the first demonstration of the importance of such groups in the cell *division* processes, as distinguished from the total growth processes involved in cell *multiplication*. The importance of –SH groups for cell multiplication processes in yeasts has been known since RAPKINE[8] inhibited multiplication in *Schizosaccharomyces pombe* with dilute iodoacetate and overcame the inhibition with glutathione; fermentation was also inhibited by iodoacetate at the concentrations inhibiting multiplication. In our experiments energy yielding processes, sufficient to support growth, are not inhibited when the cell division mechanism is affected. While a great many experiments, on a variety of biological forms, have demonstrated the undeniable importance of sulfhydryl group-substances in cell multiplication, it must be emphasized that most experiments have not distinguished between effects on cell division *per se* and effects on growth (defined as an irreversible increase in volume). With the relatively favourable material at our disposal we may say that these initial experiments indicate very strongly that *cell division, as a process distinct from growth,* depends upon the maintenance of functional intracellular sulfhydryl groups.

The relationship of the phenomenon we designate as Y → M to the "camphor-reaction" of yeasts as reported by BAUCH[29, 30], LEVAN[21, 31], and SKOVSTED[33] requires consideration. Camphor, and numerous other water-insoluble substances, have been shown to induce the development of rather characteristic morphological irregularities during the growth of *Saccharomyces cerevisiae*. Elongated cells with undulating contours, and a marked tendency to remain attached in chains, have been induced in yeasts by the action of camphor. LEVAN[21] has made an extensive study of chemical structure *vs* "camphor-reaction" activity with *S. cerevisiae*; over 50 compounds, chiefly known narcotizing agents, were found to be effective. A pronounced parallel, within an homologous series, could be noted between activity and increasing insolubility in water; activity was believed to reside in relatively non-specific surface-acting properties of the compounds. Such action is in contrast to the relatively specific action of the compounds we have studied which are effective in high dilution and involve sulfhydryl groups. It seems entirely possible, however, judging from the similarities in the results effected by the two classes of agents, that we are witnessing two modes of attack on the cell division mechanism: (1) the water soluble, –SH specific class (exemplified by penicillin) directly affecting the cell division enzyme mechanism, and (2) the water insoluble, non-specific, surface active narcotics (exemplified by camphor) affecting the formation of new, discontinuous cellular phase boundaries by interference at the "product end" of the division mechanism. The discussion by VELDSTRA[34] of the mode of action of non-polar ergons is pertinent to our consideration of the existence of two modes of chemical attack on the cell division mechanism. From the facts amassed by VELDSTRA it seems clear that we may expect a relative non-specificity among the lipophilic substances studied by LEVAN, and for which we may also expect an effect on protoplasmic boundary

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systems (apparently interference with protoplasmic boundary formation in the present instance).

In his work on camphor-treated yeasts BAUCH observed a primary reaction, essentially as outlined above, and a secondary permanent cell modification which he regarded as a colchicine-type, chemically-induced polyploidy because of the permanently increased cell volume. SKOVSTED has reviewed the literature on the induction of polyploidy in yeasts by treatment in this manner, and concluded the question is not settled. It is clear from SKOVSTED's work, however, that camphor treatment leads to an increased rate of mutation in yeasts, as well as to temporary morphological modification. It is becoming clear that substances inhibiting cell division in microorganisms and the mitotic poisons of higher plants and animals (colchicine-type and "radiomimetic") have some interesting common aspects. As DUSTIN[35] points out, "while mitosis may be affected in several ways, the one important thing is that we have many substances that specifically affect dividing cells". He has suggested enzyme inhibition as a mode of action of mitotic poisons, including radiation effects as well as chemical mitotic poisons[36]. The sulfhydryl-enzyme-inhibiting properties of X-rays and of many of the mitotic poisons are well known. In comparison, our data permit the conclusion that maintenance of intracellular –SH groups is a necessity for cell division processes, and that their temporary inhibition by –SH enzyme-specific substances leads to temporary morphological modification as a result of cell division inhibition.

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SUMMARY

A concept is developed regarding the occurrence of yeast to mycelia conversions (Y → M), resulting in the appearance of filaments from single cell yeasts, as the differential inhibition of cell division processes without the simultaneous inhibition of growth processes (defining growth as an irreversible increase in volume).

Sulfhydryl group substances, such as cysteine and glutathione, are shown to promote cell division and to inhibit the appearance of Y → M in organisms that regularly produce M structures. Ascorbic acid is without such effect. It is suggested that the metabolically controlled intracellular sulfhydryl ⇌ disulfide equilibria may reasonably account for many of the observations in the literature concerning the influence of the growth medium on the dimorphism (Y → M) of *Candida albicans* (and other species of the *Mycotoruloideae*).

Inhibition of cell division (and stimulation of Y → M) is shown to result from the exposure of different species of yeasts to penicillin and to cobaltous ions. With the exception of boron (slight effect), other heavy metals examined were without such a differential effect. The effect of penicillin and cobalt was antagonized by cysteine.

Preliminary cytological studies (employing toluidine blue and cells with a greatly reduced cytoplasmic basophily) indicate that nuclear division may proceed even though cell division stops. This results in the occurrence of multinucleate M elements, as a result of Y → M.

The findings are considered in relation to some general aspects of cell division, including comparisons with the effect of camphor on yeasts and the action of mitotic poisons.

RÉSUMÉ

Une hypothèse est discutée concernant la transformation de la forme levure en forme mycelium (Y → M), cette transformation étant due à l'inhibition spécifique des processus de division cellulaire sans l'inhibition simultanée des processus de croissance (la croissance étant définie comme une augmentation irréversible de volume).

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Les substances à fonction SH telles que la cystéine et le glutathion provoquent la division cellulaire et inhibent le passage Y → M chez les organismes qui produisent régulièrement la forme M. L'acide ascorbique n'a aucune action. On imagine que l'équilibre intracellulaire sulfhydryle ⇌ disulfure peut raisonnablement rendre compte de la plupart des observations concernant l'influence du milieu de croissance sur le dimorphisme (Y → M) de *Candida albicans* (et d'autres espèces de *Mycotoruloideae*).

L'inhibition de la division cellulaire (et la stimulation de Y → M) est provoquée par le traitement de différentes espèces de levures par la pénicilline et par les ions cobalteux. Sauf en ce qui concerne le bore (effet léger) les autres métaux lourds étudiés ne présentent pas d'action. L'effet de la pénicilline et du cobalt est empêché par la cystéine.

Des études cytologiques préliminaires (à l'aide de bleu de toluidine) montrent que la division du noyau peut avoir lieu même lorsque la division cellulaire est bloquée. Ceci provoque l'apparition d'éléments M multinucléés comme on les trouve dans Y → M.

Ces observations sont discutées en relation avec quelques aspects généraux de la division cellulaire, en particulier l'action du camphre sur les levures et l'action des poisons de la mitose.

ZUSAMMENFASSUNG

Eine Hypothese über die Verwandlung der Hefezellen in ein Hefemycelium (Y → M) wird aufgestellt. Diese Verwandlung ist auf eine spezifische Hemmung der Zellteilung ohne gleichzeitige Hemmung des Wachstums zurückzuführen, wenn man das Wachstum als eine irreversibele Zunahme des Volumens definiert.

Verbindungen die wie Cystein oder Glutathion SH-Gruppen enthalten, rufen Zellteilungen hervor und hemmen die Verwandlung Y → M bei Organismen die regelmässig Mycelien bilden. Ascorbinsäure hat keine derartige Wirkung. Die Verfasser nehmen an, dass das in der Zelle herrschende Gleichgewicht Sulfhydryl ⇌ Disulfid die meisten Beobachtungen über den Einfluss des Nährbodens auf den Dimorphismus (Y → M) von *Candida albicans* (und anderer Mycotoluloideae-Arten) erklären kann.

Es wird gezeigt dass Hemmung der Zellteilung (und von Y → M) durch Behandlung verschiedener Hefearten mit Penicillin und mit Co^{++} bewirkt wird. Mit Ausnahme von Bor (das eine schwache Wirkung hat) zeigen die anderen untersuchten Schwermetalle keine Wirkung. Die Wirkung von Penicillin und Co^{++} wird durch Cystein gehemmt.

Cytologische Vorversuche (mit Toluidinblau) lehren, dass die Kernteilung vor sich gehen kann, auch wenn die Zellteilung aufhört. Das Ergebnis sind Mycelien mit mehreren Kernen, wie man sie in Y → M antrifft.

Diese Ergebnisse werden im Hinblick auf einige allgemeine Aspekte der Zellteilung insbesondere den Effekt von Kampfer auf Hefe und die Wirkung der Mitosegifte erörtert.

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