REVERSIBLE AND PERMANENT EFFECTS OF
THE CARBON SOURCES AND VARIOUS ANTIBIOTICS
ON THE MORPHOLOGY AND METABOLIC PROPERTIES
OF USTILAGO CYNODONTIS CELLS

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ABSTRACT

The effects of various carbon sources and of antibiotics on the morphology of hypha cells of the fungus Ustilago cynodontis is described. Nonfermentable substrates promote readily reversible yeastlike colonies from hypha cells: all the hypha cells spread on these substrates give rise to yeastlike colonies that revert to the mycelial phenotype when transferred to glucose medium. Among the antibiotics tested, chloramphenicol (CAP) is found to promote, under certain circumstances, a long-lasting, even permanent modification on the morphology of the colonies: the colonies developed on CAP-glucose media are yeastlike, and a percentage of them give rise to colonies whose morphology remains yeastlike even on drug-free media; this effect is also obtained with cells cultivated in liquid medium. This permanent morphological modification is accompanied by a change of metabolic properties. Similar permanent effects are obtained with ethidium bromide, suggesting that mitochondrial functioning is involved in these modifications.

Ustilago cynodontis, an obligate aerobic fungus, has two morphologically different cell types: mycelial and yeastlike which, on solid 4% glucose medium, give rise to colonies that are morphologically different also (Fig. 1). Yeastlike strains are derived from mycelial cells (Nozeran and Chevalier, 1965) and it has been shown that mycelial cells of the 4001 strain (M1) are prototrophic whereas yeastlike cells (L1) which are derived from this M1 mycelial strain require lysine and arginine to grow (Talou and Tavlitzki, 1969).

In the present paper, reversible changes and long-lasting, even permanent changes in the morphology of mycelial cells and colonies of the 4001 strain are studied.

It is observed that as in many other fungi the morphology of the colonies obtained from mycelial hyphae is dependent on the nature of the carbon source in the growth medium: yeastlike development is promoted on media containing a nonfermentable substrate as the carbon source, but this state is reversible, that is, the colonies remain yeastlike as long as the nonfermentable substrate is present in the growth medium and the phenotypic change reverts to the mycelial form when the cells are transferred to a fermentable growth medium.

On the other hand, the results reported in this paper show that chloramphenicol (CAP) and ethidium bromide (EB) can initiate a long-lasting or permanent modification of the mycelial cells,
FIGURE 1  Morphology of *U. cynodontis* colonies on 4% glucose complex medium, 5 days after being spread. (a) mycelium colonies; (b) yeastlike colonies.
which then give rise to colonies whose morphology is typically yeastlike, even after repeated transfers to drugfree media. Moreover, the stable (permanent modification) yeastlike cells so obtained require lysine and arginine to grow.

The morphology of the yeastlike strain L₁ is unchanged, whatever the composition of the growth medium, and is not modified by CAP or EB.

MATERIALS AND METHODS

Cells

Mycelial and yeastlike cells of U. cynodontis 4001 strain were used throughout the experiments.

Growth Media

Experiments were performed either in 100-ml Erlenmeyer flasks containing 20 ml of a liquid complex medium or on plastic Petri dishes containing, in most cases, 30 ml of a solid complex medium. As indicated in the text, a few experiments were undertaken with a solid synthetic medium (to test the effects of the carbon sources and the auxotrophy for lysine and arginine).

The solid synthetic medium has been previously described (Talou and Tavlitzki, 1969); modifications of this medium are mentioned with the results. The solid, complex basal medium was made of 2% Difco Bacto agar and 1% Difco yeast extract (Difco Laboratories, Detroit, Mich.); the liquid, complex basal medium contained 1% Difco yeast extract; various substances were added as carbon source or as supplements. The complete medium is detailed in the text.

Growth Conditions

U. cynodontis strains were kept on 4% glucose solid medium. Cultures were grown at 28°C. Experimental liquid cultures were inoculated from a growing preculture. Aerobiosis was ensured by shaking the vials (350 rpm). The cells used to inoculate the agar surfaces were taken from an exponentially growing experimental liquid culture; the inoculum was serially diluted to give 50–100 well-isolated colonies. The spread plates were incubated at 28°C. These manipulations were all carried out under sterile conditions.

The generation time of the cells (yeastlike and mycelial) is 3 h in liquid glucose complex medium and 5 h on solid complex medium whatever the source of carbon; these generation times are increased, in the presence of antibiotics, to 5 h in liquid media, 9 h on solid media.

To transfer cells from one solid medium to another, two methods were employed: either the whole population was replicated by (velvet) replica plating, or colonies were picked from the plate, dialyzed into sterile water, and, after appropriate dilution, spread on the agar surface; this latter method will be called "transfer."

Colonies

In their colony morphology, the yeastlike strain L₁, and the yeastlike segregants from treated hypha cells are identical. Isolated cells give rise, after 23–25 generations, to colonies containing about 2–3 × 10⁷ cells.

RESULTS

Readily Reversible Changes in Morphology

INFLUENCE OF GLUCOSE CONCENTRATION: Hypha cells were spread on Petri dishes containing either 0.5% or 4% glucose medium. As expected, the colonies that developed on 4% glucose are mycelial, but on 0.5% glucose about 50% of the colonies are yeastlike. Therefore, it appears that the morphological aspect of some colonies can be altered by lowering the glucose concentration in the growth medium. In all cases this effect is reversible: after transfer to high concentration (4%) glucose medium, all the newly formed colonies developed as mycelia.

INFLUENCE OF THE NATURE OF THE CARBON SOURCE: Petri dishes containing various solid media were inoculated with hypha cells. The morphology of the surface colonies is reported in Table I.

It can be seen that yeastlike colony development is induced on lactate and glycerol, that is, on

| Carbon source | Synthetic medium | Complex medium |
|---------------|------------------|----------------|
| None          | No growth        | y              |
| Glucose (40)  | M                | M              |
| Fructose (30) | M                | M              |
| Mannose (30)  | M                | M              |
| Saccharose (30)| M               | M              |
| Lactate (30)  | Y                | Y              |
| Glycerol (30) | Y                | Y              |
| Acetate (25)  | No growth        | y              |
| Glucose (40) + acetate (10)| No growth | Y |

* Three independent experiments were performed. In each experiment, the various media were inoculated with 300–500 isolated hypha cells taken from an exponentially growing culture (4% glucose, 1% yeast extract). 5 days later (25 generations), the morphology of all the resulting colonies was: M, mycelial; Y, yeastlike; or y, yeastlike which stop rapidly growing.

† Numbers in parentheses, grams/liter.
nonfermentable substrates. Acetate, which is not utilized as a carbon source, also induces the development of yeastlike colonies from hypha cells when incorporated into 4% glucose medium at a concentration of 0.5–1%. When the yeastlike colonies obtained on those various media are transferred to low concentration (0.5%) glucose medium, about 50% of the colonies revert to mycelial form and the rest remain yeastlike; but, as soon as these modified colonies are transferred to high concentration (4%) glucose medium, they all revert to mycelial phenotype.

Thus, the morphological aspect of hypha colonies is dependent on the nature of the carbon source in the growth medium, as long as the colonies stay on this medium.

**Effect of Glucose and Chloramphenicol:** Mycelial cells were spread on 0.5% or 4% glucose medium, supplemented with CAP (400 μg/ml). This concentration is nonlethal: each cell spread on these media gives rise to a colony; however, the generation time is about 9 h instead of 5 on drug-free medium, so that the morphology of the yeastlike colonies coming from CAP-treated hypha is distinct later (9 days instead of 5 days).

The following results were obtained (Table II): (a) On high concentration (4%) glucose supplemented with CAP, about 50% of the cells give rise to yeastlike colonies, whereas 0.5% of them do when the medium is CAP-free. (b) On low concentration (0.5%) glucose medium plus CAP, all the mycelial cells give rise to yeastlike colonies whereas only about 50% of them do when the medium is CAP-free.

These morphological changes are usually readily reversible: most of the modified cells revert to mycelial form when transferred to 4% glucose medium. However, some CAP-treated cells keep the yeastlike phenotype even after successive transfers to high glucose drug-free medium. This observation led to several experiments which are described below.

**Effects of Various Other Antibiotics:** The effects of other antibiotics on mycelium development were tested: mitomycin (1 mg/ml), puromycin (1 mg/ml), cycloheximide (0.1 μg/ml), and erythromycin (2 mg/ml). These antibiotics were included in 0.5% glucose medium. The results reported in Table III were obtained in three independent experiments; in each experiment about 500 cells were spread on each medium. From these results, it can be seen that erythromycin (2 mg/ml) and cycloheximide (0.1 μg/ml), like CAP, initiate yeastlike development; higher concentrations are lethal, but at lower concentrations mycelial colonies develop. Daughter cells obtained after transfer to glucose antibiotic-free media either died or reverted to mycelial phenotype.

No stable yeastlike cells could be obtained under these conditions.

**Long-Lasting and Permanent Changes in Morphology**

**Simultaneous Action of CAP and Glucose:** As has been said above, a few CAP-treated hypha are permanently altered and remain yeastlike even after several transfers (6 at least, that is, about 140 generations) on 4% glucose and drug-free media. It thus appeared interesting to determine the percentage of CAP-treated hypha that give rise to stable yeastlike cells. Experiments

| Glucose | - CAP | + CAP (400 μg/ml)* |
|---------|-------|--------------------|
| 4%      | 12 (2,638) | 1,075 (2,314) |
| 0.5%    | 1,127 (2,275) | 2,290 (2,290) |

Results of four independent experiments. The colonies were counted after 25 generations, i.e., 5 days later on CAP-free media, 9 days later on media supplemented with CAP.

*Numbers in parentheses represent the numbers of recovered colonies on the agar surfaces.

| Antibiotic            | Morphology* |
|-----------------------|-------------|
| Mitomycin (1 mg/ml)   | M           |
| Puromycin (1 mg/ml)   | M           |
| Cycloheximide (0.1 μg/ml) | Y     |
| Erythromycin (2 mg/ml)| Y           |

*Antibiotics were added to complex glucose (0.5%) media. Three independent experiments were carried out. In each experiment, the various media were inoculated with about 500 isolated hypha cells taken from an exponentially growing culture (4% glucose, 1% yeast extract). 25 generations later, the morphology of all the colonies was either mycelial (M) or yeastlike (Y).
were then performed on low and high glucose media with and without CAP (400 μg/ml) according to the schema of Fig. 2.

(a) Using CAP-free glucose media, several quantitative experiments were undertaken in which more than 10⁴ hypha cells were spread on 0.5% and 4% glucose media: only 1% to 2% of the mycelial hypha give rise to readily reversible yeastlike cells but no stable yeastlike colonies appear. The frequency of yeastlike colonies in the mycelial population is therefore less than 10⁻⁴. These results are in agreement with those described below, obtained in experiments with liquid media.

(b) Using low concentrations of glucose and CAP (Fig. 3), 15 Petri dishes containing 0.5% glucose medium supplemented with CAP (400 μg/ml) were inoculated with 1,186 mycelial cells. All the colonies grew as yeastlike colonies. These colonies were replica plated onto 0.5% glucose medium lacking CAP. It was observed that all the resulting colonies were yeastlike. Some colonies (160) were then transferred to 2% glucose, and the experiment was continued as described in Fig. 2. Under these conditions, 0.7% of the hypha cells were transformed into stable (permanently altered) yeastlike cells. These cells required lysine and arginine to grow, as do yeastlike 4001 strain L₁ cells. Prototrophic yeastlike cells were also obtained from mycelial hypha, but, after several transfers to high glucose medium, they either reverted to mycelial phenotype or remained yeastlike and became auxotrophic for lysine and arginine.

As mentioned in Materials and Methods, the cells used to inoculate the agar surfaces were taken from an exponentially growing culture. A similar experiment was conducted with cells taken at the end of exponential growth; under these conditions, only 1% to 2% of the mycelial hypha give rise to readily reversible yeastlike cells but no stable yeastlike colonies appear. The frequency of yeastlike colonies in the mycelial population is therefore less than 10⁻⁴. These results are in agreement with those described below, obtained in experiments with liquid media.

**Figure 2** Diagram of the method employed to show up the effect of glucose and CAP on the morphology of U. cynodontis colonies.

**Figure 3** Number of transformed U. cynodontis hyphae after simultaneous action of CAP (400 μg/ml) and glucose. *Y* Yeastlike cells which require lysine and arginine to grow, prototrophic cells are not taken into consideration.
conditions, 4% of the hypha are transformed into stable yeastlike cells which require lysine and arginine. This result could mean that the number of cells whose morphology is modified by CAP is greater when the cells come from a culture in which most of them are reaching stationary phase.

(c) Using high concentration glucose and CAP, 1,115 cells were spread on 15 Petri dishes containing 4% glucose medium supplemented with CAP (400 μg/ml); 589 colonies, that is, about half of them, were yeastlike; these were replica plated onto 0.5% glucose medium lacking CAP, and the experiment was then performed as described in Fig. 2. Under these conditions, only 0.1% of the hypha cells give rise to stable yeastlike cells requiring lysine and arginine. Table IV summarizes the results obtained.

EFFECT OF CAP IN LIQUID MEDIA: In order to obtain more information on the mode of action of CAP, experiments were performed with liquid media.

Exponentially growing cultures (4% glucose) were used to inoculate experimental liquid cultures containing glucose (4%), yeast extract (1%) and various levels of CAP (400-2,000 μg/ml) at a density of 2 x 10^6 hypha cells/ml. Every 24 h, these were diluted to 2 x 10^6 cells/ml with fresh medium containing the same concentration of CAP as before, in order to maintain exponential growth. Cells were withdrawn at intervals to be examined in the microscope and plated, after appropriate dilution (10^6) in water, on a complex 4% glucose, drug-free medium, in order to determine the percentage of yeastlike colonies recovered after treatment with CAP.

(a) The examination of the resulting colonies on the agar surfaces shows that 25% yeastlike colonies deriving from hypha cells are obtained after an exposure of the cells to CAP for five generations at all concentrations of CAP (Fig. 4). This percentage can be increased to 75% when cells are exposed to the higher levels of CAP (1,500-2,000 μg/ml) for 10 generations, to 85% when exposed for 15 generations. Cells cultivated for 20 generations in the presence of CAP (2,000 μg/ml) give rise to 99% yeastlike colonies. No yeastlike colonies develop from untreated hypha.

From these results, it appears that both the number of cell generations in the presence of CAP and the level of the drug are critical factors for the number of induced yeastlike colonies.

However, among the yeastlike colonies obtained, a percentage of them reverted to the mycelial phenotype after transfers to fresh 4% glucose media; the frequency of long-lasting yeastlike colonies recovered is thus lowered (Table V); it can also be seen that this percentage differed from one set of experiments to another, although the conditions of the experiments were identical.

(b) It has been observed in our laboratory that, in liquid 4% glucose medium, the vegetative development of mycelial cells differs from that of yeastlike cells: mycelial cells elongate and a septum is formed giving rise to two isolated cells (Fig. 5 a), whereas yeastlike cells multiply by budding (Fig. 5 b) as do hypha cells in 4% glucose medium supplemented with acetate (5–10mg/ml). Microscope examination of hypha cells cultivated in the presence of CAP (1,500–2,000 μg/ml, 48 h or more) shows that most of them multiply by budding (Fig. 5 c). This observation suggests that the induced morphological modification of the cells and of the colonies reflects a modification of the mode of cell division.

EFFECT OF EB: Hypha cells were spread on 4% glucose solid medium supplemented with EB (5 μg/ml). 9 days later (about 25 generations), all the resulting colonies were yeastlike, and, after successive transfers on 4% glucose EB-free medium, about 10% of them give rise to long-lasting yeastlike colonies which require lysine and arginine to grow. Experiments were carried out in liquid media. Several levels of EB (between 0.5 and 20 μg/ml) and various times of exposure to EB (1–24 h) were tried for induction of yeastlike cells either in growing cultures or with nonproliferating cells. The conditions used in the experiments with growing cultures (4% glucose or 3% lactate liquid higher concentrations or times of exposure are lethal.

| Glucose | − CAP | + CAP (400 μg/ml) |
|---------|-------|------------------|
| %       |       |                  |
| 0.5     | <10^{-4} | 7-40 x 10^{-5} |
| 4       | <10^{-4} | 10^{-3}         |

[1] This concentration of CAP is rather high compared to the one used with solid media, but is nonlethal.

[2] Higher concentrations or times of exposure are lethal.
media) were identical to those used with CAP. The results show that, under these conditions, no yeastlike colonies develop among the mycelial population, whatever the concentration of EB is. Another experiment was undertaken with nonproliferating cells, that is, cells in potassium phosphate buffer, pH 6, 5 x 10^{-6} M at 28°C; an exponentially growing culture in 1% yeast extract and 4% glucose was used to inoculate the medium at a density of 2 x 10^6 cells/ml. Samples were withdrawn at intervals, and, after appropriate dilution (3 x 10^6) in water, spread on a solid complex glucose (4%) medium. Under these conditions, 10% of the nonproliferating hypha cells exposed to 20 μg/ml EB for at least 3 h give rise to yeastlike colonies, but after transfers to fresh drug-free media only 1%, that is, 5 among 500 treated cells, gives rise to long-lasting (75 generations) yeastlike colonies. It will be interesting to treat cells with lower concentrations of EB for a longer time.

**DISCUSSION**

**Reversible Phenotypic Changes**

**Effect of Nonfermentable Substrates:** A number of fungi exhibit a pheno-
typic duality in cell form, depending on environmental conditions (Turian, 1969). They may develop either in the form of mycelia or in the form of yeastlike cells, but these phenotypic changes usually revert to the original characters when the cells are replaced in the initial growth conditions.

Similar results are obtained with cells of *U. cynodontis*, an obligate aerobic fungus: yeastlike development from mycelial cells occurs whenever either the concentration of glucose of the growth medium is lowered or the glucose is replaced with a nonfermentable carbon source. This state is readily reversed when the modified cells are transferred to normal growth medium, that is, to 4% glucose. There are at least two hypotheses to explain these phenotypic changes and, so far, it is not yet possible to choose between them: (a) Growth on nonfermentable substrates increases respiration and synthesis of respiratory enzymes. (b) Glucose represses enzymes which are, or are not, associated with respiration.

However, in the discussion presented below, it is suggested that a modification of the respiratory mitochondrial function is involved in those changes.

**Effect of Glucose and CAP:** CAP initiates a reversible modification in the morphology of

**Table V**

| CAP  | Time exposure | After 25 generations (spread colonies) | After 50 generations (1st transfer) | After 75 generations (2nd transfer) |
|------|---------------|----------------------------------------|------------------------------------|-------------------------------------|
| 400  | 24* h         | 25 7 0                                 |                                    |                                     |
| 48*  |               | 37 24 23                               |                                    |                                     |
| 1,500| 48* h         | 71 46                                  |                                    |                                     |
| 48†  |               | 75 23 22                               |                                    |                                     |
| 72†  |               | 85 28 20                               |                                    |                                     |
| 2,000| 48* h         | 76 46                                  |                                    |                                     |
| 48†  |               | 66 5 0                                 |                                    |                                     |
| 96†  |               | 99 10 3                                |                                    |                                     |

The colonies are issued from cells treated by CAP in liquid media (cf. Fig. 4).

* One set of experiments.
† Another set of experiments.
the colonies derived from *U. cynodontis* hyphae, and the percentage of the altered colonies, on solid medium, varies with the glucose concentration in the growth medium. Similar modifications, on fungi, have been described in the literature: CAP promotes yeastlike morphology in *Mucor genevensis* (Clark-Walker, 1973); this change is accompanied by a loss of cyanide-sensitive respiration, the development of cyanide-insensitive respiration, and the modification of the mitochondrial membranes. A recent paper (Hansens et al., 1974) described similar effects of CAP on *Moniliella tomentosa*. Experiments are being undertaken in our laboratory to see whether or not morphological changes of *U. cynodontis* cells, during or after CAP treatment, are associated with such a modification of the respiratory function.

**Permanent and Long-Lasting Effects of CAP on Morphology.**

On solid growth medium as well as in liquid medium, CAP promotes the development of stable yeast-like cells, i.e. the phenotypic modification is accompanied by a true hereditary change in those cells. The glucose concentration appears to play an important role in determining the percentage of hypha cells which give rise to definitively stable yeastlike colonies on solid medium; it is seen that 0.7-4% of the hypha cells give rise to definitively stable yeastlike colonies when spread on 0.5% glucose medium with CAP, 0.1% when spread on 4% glucose medium plus CAP (Fig. 3). An effect of glucose concentration is not detectable in the experiments performed in liquid medium, but it cannot be dismissed since determination of glucose in the growth medium (results not reported here) shows that, during exponential growth, less than 0.5% glucose is consumed. This suggests that the induction of yeastlike development from hypha cells is a composite effect of CAP and glucose: CAP promotes a metabolic change which, directly or indirectly, induces the differentiation of mycelia and appears to be inhibited by glucose. Two other critical factors are revealed in liquid medium: the level of CAP and the number of generations undergone in the presence of CAP, the greatest percentages of induced yeastlike colonies being obtained after 15-20 generations; it must be noted that, on solid medium, colonies that developed on CAP medium stay in the presence of the drug for 23-25 generations before their morphology becomes distinct. Very reproducible results are obtained in liquid medium and from one set of experiments to another on solid medium as to the percentage of resulting yeastlike colonies spread on 4% glucose and CAP-free medium (Fig. 4 and Table V); as for the percentage of stable yeastlike colonies recovered after successive transfers on drug-free medium, it differs from one experiment to another. The causes of this heterogeneity are under study.

It does not seem that the effect of CAP can be explained by a selection of preexisting yeastlike mutants, for the following reasons: (a) The frequency of such mutants would be less than $10^{-4}$ among the hyphal population cultivated in absence of CAP. Now $L_1$ stable cells do have the same growth rate as $M_1$ cells in the absence or presence of CAP. Therefore the yeastlike phenotype does not seem to have any important selective advantage. (b) Even if a slight advantage did exist, one would observe an increasing number of such mutants as a function of the number of generations undergone in the presence of CAP, an expectation which is not fulfilled by the results obtained. (c) Such preexisting mutants would be immediately stable. In fact, as has been shown, among the CAP-treated cells none give rise to a population of immediately stable cells: several cell generations must pass in the absence of CAP before the appearance of definitively stable yeastlike colonies. An effect of CAP at the mitochondrial level can rather be envisaged.

CAP is known to be an inhibitor of mitochondrial protein synthesis in various eukaryotic cells (Boardman et al., 1971). It is usually reported that CAP initiates a selective decrease in or inhibition of the synthesis of the mitochondrial proteins, the synthesis of cytochrome oxidase being significantly inhibited from the beginning of the drug treatment (Howell et al., 1971; Mahler and Perlman, 1971). Moreover, cellular changes are observed during growth in the presence of CAP: the structure of the mitochondria is more or less disorganized (Kellerman et al., 1969; Lenk and Penman, 1971; Howell et al., 1971).

Therefore, our own observations on *U. cynodontis* cells treated by CAP could be explained by an effect of CAP either directly on mitochondrial protein synthesis or on other sites involved in respiration, mitochondrial membrane synthesis, or energetic metabolism.

The work of Clark-Walker (1973) is relevant to the present discussion: that author observes that CAP induces yeastlike morphology from the
FIGURE 5. Phase-contrast micrographs of U. cynodontis cells: (a) mycelial cell (M.), (b) yeastlike cell (L.) in 4% glucose complex medium, (c) mycelial cell (M.) in 4% glucose complex medium with CAP 1,500 μg/ml.
mycelial form in *M. genevensis* (a facultative anaerobe) and that the effect of CAP on this organism is reversed by 10% glucose; although CAP is shown to affect the mitochondria in this organism, that author suggests that CAP also inhibits glucose transport or glycolysis; thus, the effect of the drug on morphology cannot be precisely explained by an interference with mitochondrial function. The fact that EB is found to induce a similar modification in *U. cynodontis* hypha cells and that cytochrome oxidase is not detected (results not reported here) in hypha cells cultivated in liquid glucose medium supplemented with CAP (400 µg/ml, five generations) suggests that mitochondrial functioning is affected by drug treatment. This hypothesis could explain the fact that no induced yeastlike cells are immediately stable (a certain number of generations on CAP-free medium are necessary to obtain this state) and that most of them revert more or less rapidly to the mycelial phenotype.

The features of those reversions must be noted here: a treated cell gives rise to a typically yeastlike colony, then filamentous elements begin to appear in some colonies. Those colonies revert more or less rapidly, that is, after one or several transfers; the others revert later or give rise to stable cells. It looks as though there were a segregation during multiplication of the cells which gives rise to the mycelial type. It is, then, tempting to suppose that CAP promotes a modification of a part or of the whole mitochondrial population in the hypha cells; this modification is either accompanied or followed by secondary effects inducing the modification of the morphology and of the mode of division of the cells. These last modifications probably are consequences of an alteration in cell well metabolism. This topic is under study in the laboratory.

**Stability of Yeastlike Cells and Auxotrophy for Lysine and Arginine**

We must underline that definitively stable yeastlike cells obtained from hypha cells after CAP or EB treatment require lysine and arginine to grow as do yeastlike cells (L.) of 4001 strain, whereas hypha cells are prototrophic. These nutritional requirements are due to the absence of synthesis of arginine, lysine being synthesized but nevertheless necessary to obtain a maximal growth rate in synthetic medium (Delavier-Klutchko and Daniel, 1969).

Prototrophic yeastlike cells can also be obtained from hypha cells, following similar treatments, but those cells either revert to the mycelial form or acquire the auxotrophy for lysine and arginine and keep the yeastlike phenotype. These observations show that the auxotrophy and stability of the yeastlike cells deriving from mycelial cells (M.) of the 4001 strain are tightly linked: only the cells that become auxotrophic for lysine and arginine remain permanently modified. This suggests that the same event accounts for the appearance of the auxotrophy and of the stability. As follows from the above discussion, this event should take place at the mitochondrial level. The appearance of the auxotrophy could then result from an alteration of the mitochondrial metabolism leading to the loss of arginine synthesis if arginine is synthesized in the mitochondria, as is the case for mammalian cells (Gamble and Lehninger, 1972) and *Neurospora crassa* (Weiss and Davis, 1973). The appearance of the auxotrophy, which is not obligatorily bound to the mode of division of the cell, would be a signal that the modifications observed had become irreversible.

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