Table II. Effects of glucose concentration of enzyme levels and glucose utilization in *Neurospora*

	Initial growth conditions					
	2% Glucose			0.5% Glucose		
Time (h)	Cytochrome oxidase (a)	MDH (b)	Glucose level (c)	Cytochrome oxidase (a)	MDH (b)	Glucose level (c)
3	1.5	0,835	100	3.1	2,473	30.0
5	6.0	1,860	94	8.5	4,420	29.2
7	25.0	2,360	86	31.3	6,942	28.5
9	22.0	4,900	80	24.7	9,050	28.0
10	19.0	6,520	78	21.7	9,270	27.5

Experimental conditions: the enzymatic activities were determined as indicated in the Methods and they are expressed as: a) K, mg prot^{-1}; b) units, mg prot^{-1}. The level of glucose in the culture media is given as c) μmoles of glucose/ml of culture.
After 10 h the yield of cells in the 2 conditions were: 0.28 mg dry weight/ml of culture in 2% glucose and 0.31 mg dry weight/ml of culture in 0.5% glucose. The constant of exponential growth, determined as dry weight[3] was 0.37 h^{-1} for both cultures.

together. The results reported in Table II exclude the occurence of an inhibitor because the recovered activity was the sum of the two activities measured independently. The increase of specific activity of cytochrome oxidase occurs in cells growing in glucose as well in those growing in acetate. Besides, both types of cells have been shown to have fairly similar mitochondrial structures[7]. Therefore it is unlikely that a release from catabolite repression plays a major role in enhancing the level of cytochrome oxidase. To gain more information on this point, the level of cytochrome oxidase was measured in cells growing in 2 different initial concentrations of glucose: 2% and 0.5%. As shown in Table II, it was found only slightly higher in cells growing with a lower initial availability of glucose. Also the level of malate dehydrogenase has been found to increase during the early exponential growth, although not as dramatically as cytochrome oxidase (Table II). The lower availability of glucose modifies the way the cells utilize glucose. In fact, although the yield at the 10th h of growth is the same for the culture started in 2% glucose or in 0,5% glucose (see legend of Table II), in the 3 to 10 h period the cells with more glucose available utilize 22 μmoles of glucose/ml, while the cells with less glucose available utilize 2.5 μmoles/ml.

The results presented here indicate that the level of a key respiratory enzyme may vary extensively during exponential growth in *Neurospora*, when conidia germinate into coenocytic hyphae, initiating a defined progression of events whose order cannot easily be changed, thus differing from the exponential phase of growth in bacteria. Indirect support of our results is offered by a recent study on respiratory and energy metabolism during exponential growth in *Neurospora*[8]. The oxygen uptake per unit of cell mass has been found to increase at the beginning of the exponential growth; then it slowly declines. The AMP and ADP levels increase as soon as the germination of conidia starts, reach a maximum at mid-exponential phase, then decline. The ATP level remains at the conidia level during early exponential growth, then it raises reaching a maximum during late exponential growth[8]. The pattern of change of the cytochrome oxidase level is very similar both for cells grown in glucose or in acetate, confirming the indication given by previous findings[7] that in *Neurospora* catabolite repression plays only a marginal role in controlling the synthesis of mitochondrial enzymes and structures. As for the question which this paper aimed to answer, it is clear that *Neurospora* cells collected during a given exponential phase of growth may differ widely in their enzymatic set-up and metabolic activities, although they appear identical when gross parameters such as RNA and proteins levels are considered. Several patterns of cytodifferentiation may be evoked in a temporal sequence by a given nutritional condition and all of them seem compatible with the same rate of exponential growth. This suggests the possibility that nutrients control the rate of cellular growth independently of the regulation of the synthesis and activity of metabolic enzymes.

Summary. In *Neurospora* cells growing in various media, the specific activity of cytochrome oxidase increases very markedly during early exponential growth, reaching a maximum after 4–5 duplication times, then it slowly declines.

Riassunto. L'attività specifica della citocromo ossidasi è stata determinata in miceli di *Neurospora crassa* (ceppo selvatico) in crescita esponenziale bilanciata in diversi mezzi liquidi di cultura. Indipendentemente dalla qualità della fonte di carbonio usata l'attività specifica è molto bassa durante la fase esponenziale precoce, raggiunge un massimo dopo 4–5 tempi di duplicazione, quindi decresce. Il valore massimo dell'attività specifica dipende dalle condizioni nutrizionali, basso in glucosio minimo più elevato in acetato minimo e in glucosio più casaminoacidi.

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Surface Features and Histochemistry of the Pollinial Wall of *Calotropis gigantea*

Angiosperm microspores normally develop to form granular pollen, but occasionally they adhere to form dyads or tetrads (Ericaceae) or even polyads of 8–16 spores (Mimosoideae). A greater degree of pollen aggregation occurs in some members of Asclepiadaceae and Orchidaceae, where the pollen of the entire sac are agglutinated to form a body of definite shape called pollinium. The ontogeny of the pollinium and the structure of its wall have not received much attention so far. Some aspects of the morphology and chemistry of the pollinial wall of *Calotropis gigantea* R & Br. (Asclepiadaceae) are examined in this report.

Pollinia from two varieties of *Calotropis gigantea* were used in this study. Freshly collected mature pollinia were

grown in the medium of BREWBAKER and KWACK[1]. The procedure recommended by ERDTMAN[2] was followed in acetolysis. The chemical constitution of the pollinial wall was tested by the histochemical methods described by JENSEN[3] and SOUTHWORTH[4].

The pollinia of *Calotropis* are flat structures with wide rounded bases and narrow apexes. Of the two longer edges, one is almost straight and the other is slightly convex. Under the microscope, the pollinium appears to have a cellular flat surface bordered with thick homogenous edges. At the time of germination, pollen tubes emerge from the distal half of the flat edges of the pollinia. As suggested by ZERONI and GAIL[5], the pollinial wall was cut open at various places to test the polarity of the growing pollen tubes. The direction of tube growth was not markedly affected even with the new outlets so opened.

Asymmetrical germination of pollinia has already been reported in *Asclepias*[5]. In 3 other members of Asclepiadaceae also we found that the pollen tubes emanate only through particular regions of the pollinia which appear to be genus-specific. Thus, the position of emergence is on the straight edge of the pollinium near the base in *Calotropis*, near its apex in *Dregea* and near the base on the convex edge in *Daemia*. Since this region has not been marked by any visible characteristics, histochemical methods were employed to study the nature of the pollinial wall with particular reference to the area of emergence of the tubes. These tests were confined to the pollinia of *Calotropis*.

Previous reports ascribe a waxy or cuticular[5,6] nature to the pollinium. However, no disintegrating effects on the wall were seen when the pollinia were placed in closed vials containing lipid solvents such as acetone, benzene or chloroform for as long as 3 days. Of the 3 solvents, benzene alone appeared to have dissolved some of the deposits on the corpusculum and connectives of the pollinia although the pollinial wall remained intact at the end of the treatment. It is therefore reasonably certain that 'waxy substances' are not the chief constituents of the pollinial wall. The suspected cuticular nature of the wall was also tested. Cutin is saponified by an alcoholic solution of KOH, but the pollinial wall is found to be insoluble in this reagent. In contrast, the wall is saponified in the sporopollenin solvent of fused KOH. It may be noted here that the reaction of the sporopollenin of the pollen exines to the above tests is also identical to that of pollinial walls. Lignin shares the solubility properties of sporopollenin[4], but the usual staining techniques, specific for this constituent, have not revealed its presence in the pollinia.

The sporopollenin composition of pollinial wall is further emphasized by acetolysis. Although it had no effect on the bag-like nature of the pollinia, acetolysis leaves an open area on the pollinial wall corresponding to the germinating region. The slit so revealed has well defined edges followed by clear cellular outlines all around. In the pollinia of *Calotropis* which we examined, this slit measured about 350–400 μm in length. Since the length of the opening is much greater than its breadth, it may be defined as a furrow, following the terminology suggested by FAGERI and IVERSEN[7].

Our study reveals some of the similarities between pollen and pollinial wall. The furrow on the pollinial wall and the germ pore on the pollen exine are really differentiated wall regions for the emanation of pollen tubes. Again, as in the case of the germ pore in pollen wall, the position and morphology of the pollinial furrow appear to have some diagnostic significance. Besides, the chemical composition of the two walls shows resemblances. Both are resistant to lipid solvents, alcoholic KOH and acetolysis. At the same time, acetolysis clears the regions of the pore and the furrow. The various tests mentioned above indicate that the pollinial wall, like that of the pollen exine, is composed of sporopollenin[8].

Zusammenfassung. Nachweis, dass das Pollinarium von *Calotropis gigantea* reichlich Sporopollenin enthält und dass die Pollenschläuche das Pollinarium durch eine präformierte Furche verlassen.

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A Combination of Sister Chromatid Differential Staining and Giemsa Banding

When a cell is grown in a medium containing 5-bromodeoxyuridine (BrdUrd or the older abbreviation BUdR which is used throughout this paper) for two cell cycles, the two sister chromatids of each chromosome are different: one with bromouracil (BU) substituting thymine in both of the DNA strands and the other with only one BU-substituted DNA strand. LATT[1] demonstrated that when such cells are stained with the fluorochrome 33258 Hoechst and observed with UV optics, the chromatid with bifiliary substitution would show dull fluorescence or no fluorescence whereas the chromatid with unifiliary substitution would show bright fluorescence. In the preparations showing sister chromatid differential staining (SCD), sister chromatid exchanges (SCE) can be detected with excellent resolution.

The fluorescence technique was later modified for Giemsa staining[2–4]. In these preparations, the bifiliary substituted chromatid stains lightly and the unifiliary substituted chromatid stains deeply. Again SCE are extremely clear.

Since these procedures already have important applications[5,6] and more uses are expected in the future, it

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