A Study of Conidiation in *Neurospora crassa*

By BARBARA WEISS* AND G. TURIAN

*Laboratoire de Microbiologie, Institut de Botanique Générale,
Université de Genève, Switzerland*

SUMMARY

Two distinct forms of growth of wild-type *Neurospora crassa* can be produced by altering the nitrogen source of the growth medium. One form completely lacks conidia and carotenoids; the other exhibits enhanced conidiation and pigmentation. Analysis of the oxidative and glycolytic metabolism of these two forms showed that the non-conidiating cultures had significantly greater glycolytic activity than the conidiating cultures, as measured by the production of ethanol, the presence of ethanol dehydrogenase and pyruvate decarboxylase activity and the response of the two culture types to glycolytic inhibitors. When glycolysis was inhibited, conidiation occurred. When oxidative metabolism in normally conidiating cultures was interfered with conidiation was suppressed. These results suggest that the relative activities of fermentative and oxidative pathways in the cell regulate both conidiation and pigment synthesis. The most probable point at which this control is exercised is at the stage of pyruvate, the common branch point of these two pathways. Conidiating cultures also were found to have very high NADP nucleotidase activity, while purely mycelial cultures were essentially devoid of this enzyme. An electron microscope examination of these two culture types revealed differences in cell-wall composition, in the distribution of osmiophilic granules within the cytoplasm and in mitochondrial structure.

INTRODUCTION

The process of conidiation in *Neurospora crassa* is poorly understood. A number of studies concerned with the identification of enzymic differences between mycelia and conidia have been made, but as yet no satisfactory picture exists of the events responsible for hyphal differentiation. Among the enzymes which have been found to have higher activities in conidia as compared with mycelia are: NAD nucleotidase (Zalokar & Cochrane, 1956), β -galactosidase (Zalokar, 1959), β -glucosidase (Eberhardt, 1961), isocitrate lyase (Turian, 1961), and trehalase (Hill & Sussman, 1964). In another direction of investigation Zalokar (1954) showed that conidiation was inhibited by preventing the formation of aerial hyphae. This was done by the addition of a surface active agent, Tween 80, to the growth medium. Zalokar determined that conidia contained also a much higher concentration of carotenoid pigments than the mycelial part of the culture. In a related study Turian (1957) found that inhibition of carotenoid synthesis in the presence of diphenylamine was accompanied by a reduction in conidiation.

The purpose of the present study was to investigate further some of the events

* Present address: Mallinkrodt Institute of Radiology, 510 South Kingshighway, Saint Louis, Missouri, U.S.A.

associated with conidiation. It concerned the analysis of two morphologically distinct forms of growth of *Neurospora* which were produced by altering the nitrogen source of the defined medium on which the organism grew (Turian, 1964). In one medium, in which ammonia was the sole nitrogen source, conidiation was totally suppressed; in the other, with nitrate as the nitrogen source, conidiation was greatly enhanced.

Essentially three methods of investigation were used. First, attempts were made to convert mycelial to conidial cultures and conidial to mycelial cultures by the addition of known metabolic inhibitors. Secondly, various enzymes related to oxidative and glycolytic metabolism and NADP-NADPH₂ metabolism were examined. Thirdly, an electron microscope study of the two growth forms (designated as C for conidial and M for mycelial) was made in the hope that the gross morphological differences between M and C cultures could be correlated with specific ultrastructural changes.

METHODS

Growth conditions

Wild-type *Neurospora crassa*, strain Lindegren +, was grown in static culture at 25° in 250 ml. Erlenmeyer flasks containing 50 ml. of liquid defined medium. Two different media designated as conidial (C) and mycelial (M) were used. These media were derived from the basic Westergaard & Mitchell medium according to the formulae devised by Turian (1964). A change in nitrogen source and the addition of citrate represent the only modifications of the Westergaard & Mitchell medium. The M medium contained 10⁻² M-diammonium citrate. The C medium contained 10⁻² M-dipotassium citrate and 2 × 10⁻² M-potassium nitrate.

Preparation of cell-free extracts

Extracts were prepared by grinding chilled mycelia with sea sand equal to twice the wet weight of the starting material. All extractions were made in the cold at about 5°. The buffer solution for extraction varied depending on the enzyme to be assayed. Sand and cellular debris were removed by centrifugation at 3640g for 10 min. A second 5 min. centrifugation was often done to eliminate cell fragments carried over from the first centrifugation.

Preparation of a particulate fraction rich in mitochondria

Extracts prepared as described above in 0.05 M-tris HCl buffer (pH 7.4), containing 0.44 M-sucrose were centrifuged in the cold at 23,630g for 20 min. The resulting pellet was resuspended in buffered sucrose.

Procedures for the measurement of respiratory quotients and cyanide sensitivity

Oxygen consumption and carbon dioxide evolution were measured in micro-Warburg vessels at 30°. To the main compartment of each flask was added 2.0 ml. of C medium or M medium. The centre well was filled with 0.2 ml. of 10% KOH for the measurement of oxygen consumption, or 0.2 ml. of distilled water for the measurement of carbon dioxide evolution. Experiments were begun by the addition of small slices of C or M mats to their respective media. Flasks were equilibrated for 10 min. and readings taken every 5 or 15 min. for 1–2 hr. At the conclusion of

each experiment the slices were recovered and dried at 60° for 24 hr in dried and pre-weighed pans. Q_{O_2} and Q_{CO_2} values were calculated as μ l. of gas consumed or evolved/hr/mg. dry weight extract.

In the cyanide inhibition studies, 0.1 ml. 0.001 M-KCN in 0.001 M-NaOH was added to the main chamber, while 0.2 ml. 4 M-KCN in 0.001 M-NaOH was added to the centre well. These concentrations were chosen from a consideration of the loss of cyanide from the medium by distillation into alkali as discussed by Riggs (1945).

Enzyme assay methods

Succinate dehydrogenase. This enzyme was assayed for in mitochondrial suspensions, at 37° in micro-Warburg vessels, with phenazine methosulphate (PMS) serving as electron acceptor (Singer & Kearney, 1951). The reaction mixture contained 60 μ moles succinate, 150 μ moles phosphate buffer (pH 7.6), 3 μ moles KCN adjusted to pH 8.0, 1.65 μ moles $CaCl_2$, enzyme, and PMS (1–4 mg). Oxygen uptake was measured at a series of dye concentrations and maximum velocities at infinite PMS concentration were determined from Lineweaver-Burk plots.

*Succinate-cytochrome *c* reductase.* Activity was measured by the spectrophotometric method of Cooperstein, Lazarow & Kurfess (1950). Each reaction mixture contained 20 μ moles succinate, 1 μ mole KCN, 10 μ moles cytochrome *c*, 0.4 μ mole $CaCl_2$, 50 μ moles phosphate buffer (pH 7.5), 3 μ moles ATP, enzyme and water to 3.0 ml. The reaction was begun by the addition of enzyme and the rate of reduction of cytochrome *c* was followed at 550 $m\mu$ for 5–10 min.

Cytochrome oxidase. The method of Cooperstein & Lazarow (1951) which measures the rate of enzymic oxidation of chemically reduced cytochrome *c* was used. Cytochrome *c* solutions were reduced by adding a few grains of powdered sodium dithionite. Excess dithionite was removed by bubbling the solution with oxygen for 2 min. Each reaction tube contained in a total volume of 3.0 ml.: 5×10^{-5} M-cytochrome *c*, 0.25 M-phosphate buffer (pH 7.5), enzyme, and water to 3.0 ml. The assay was done at room temperature. The rate of decrease in optical density at 550 $m\mu$ was followed for 3 min. Readings were taken at 30 sec. intervals.

Ethanol dehydrogenase. This enzyme was assayed by a modification of the method given by Lip Boen Khouw, Burbridge & Sutherland (1963). The reaction mixture contained 0.3 ml. of a 1% (w/v) acetaldehyde solution, 0.0015 M-NADH₂ and 0.1 M phosphate buffer (pH 7.4) to a final volume of 3.0 ml. The reaction was begun by the addition of a sample of cell-free extract. The oxidation of NADH₂ was followed at 340 $m\mu$ at 15 sec. intervals. Specific activity was expressed as the rate of change of optical density at 340 $m\mu$ /min. per mg of protein.

Pyruvate carboxylase. A modification of the manometric method of Singer (1955) was used. The assay was made with extracts prepared in 0.05 M-citrate buffer adjusted to pH 6.0 with NaOH containing 0.001 M-cysteine-HCl. The main compartment of micro-Warburg vessels contained: 0.15 M-citrate buffer (pH 6.0), 3 mM-cysteine-HCl, 3 mM-MgCl₂, enzyme, 0.05 mg thiamine pyrophosphate (TPP), and water to 3.0 ml. Reactants were added in the order given. Pyruvate (0.5 ml. of an M solution) was tipped in from the side arm after a 3 min. equilibration at 30°. The reaction was followed for 3 min. and specific activity expressed as μ l. of CO₂ produced within 3 min. after the addition of substrate/mg. protein.

*NADPH₂ cytochrome *c* reductase.* The method of Haas (1955) was used. The

increase in optical density at 550 m μ due to the reduction of cytochrome *c* was measured every 30 sec. for 3 min. The reaction mixture was the following: 0.1–0.4 mg. NADPH₂ (reduced nicotinamide-adenine dinucleotide phosphate), 8×10^{-5} M-cytochrome *c*, 0.001 M-KCN, 0.1 M-phosphate buffer (pH 7.4) to a final volume of 3.0 ml.

Glucose-6-phosphate dehydrogenase. A modification of the spectrophotometric method of De Moss (1955) was used. Homogenates were prepared in 0.1 M-tris HCl buffer (pH 7.8). The reaction mixture contained: 1.5 ml. 0.1 M-tris buffer, 0.01 M-MgCl₂, 0.4–1.2 mg. NADP, 0.01–0.4 ml. 0.0146 M-glucose-6-phosphate (barium salt), enzyme and water to 3.0 ml. The reaction was run at room temperature and readings taken every 30 sec. for 3 min. at 340 m μ . Control tubes lacking substrate were run with each series of assays. The specific activity was determined from a Lineweaver–Burk plot of $1/v$ versus $1/s$. The initial velocity was taken as the increment in optical density during the first 15–30 sec. after the addition of enzyme.

NADPH₂ nucleotidase. This enzyme was assayed according to the method of Kaplan (1955). Cell-free extracts isolated in either phosphate or tris buffer were used. The following mixture was incubated for 7 min. at 37°: 0.3 ml. 0.1 M-phosphate buffer (pH 7.4), 0.1 ml. NAD (1 mg./ml. solution), enzyme and water to 0.6 ml. The reaction was terminated by the addition of 3.0 ml. 1.0 M-KCN. The presence of a NAD cyanide complex was determined by measuring the absorbancy of this solution at 325 m μ . Extracts frozen for 1–10 days at –20° retained full nucleotidase activity.

Protein analysis. The method of Lowry, Rosebrough, Farr & Randall (1951) was used. A standard curve obtained for bovine serum albumin was run simultaneously with each protein determination.

Carotenoid analysis. Total carotenoids were extracted by repeated grinding of mats in acetone and subsequent transfer of the pigments to light petroleum (boiling point 40–60°). Quantitative estimation of carotenoid concentrations was made from measurements of optical density at 468 m μ in this last solvent ($E_{1\%}^{1\text{cm}} = 250$ according to Krzeminski & Quackenbush, 1960).

Ethanol analysis. Ethanol was titrated in glass distillates of M and C filtrates with the dichromate oxidation method of Nicloux (1931) as improved by Rochat (1946).

Electron microscope techniques

All material for microscopy was fixed in 1% osmium tetroxide solutions in Palade buffer (pH 7.3–7.5) for 2 hr and post-stained in a saturated solution of uranyl acetate in Palade buffer for 2 hr. Dehydration was carried out through a graded series of acetone + water solutions and final embedding was done in Vestopal W according to the procedure of Kellenberger & Ryter (1958). Blocks were sectioned with a Porter Blum manual ultramicrotome. Sections were mounted on cellulose acetate films and examined with an RCA EMU 3 electron microscope.

RESULTS

Description of mycelial (M) and conidial (C) cultures

Between 2 and 5 days M cultures characteristically were non-pigmented and non-conidiated. They were below the surface of the liquid medium as a thick mat and never produced aerial hyphae.

C cultures produced aerial hyphae within $1\frac{1}{2}$ –2 days and conidiated rapidly, giving rise to large quantities of pale orange conidia within 3–4 days. Their mats were usually thinner than M mats.

Total carotenoid analysis of 3-day C and M cultures indicated the extent of inhibition of pigment synthesis in M cultures. Three-day M cultures contained 0.29 mg. total carotenoids/100 g. dry weight as compared with a value of 12.0 mg./100 g. dry weight for C cultures of the same age.

Conversion studies. Fluoroacetate, sodium bisulphite, iodoacetate, fluoride and *p*-chloromercuribenzoate (PCMB) were tested for their ability to convert C to M and M to C cultures.

Fluoroacetate at concentrations of 0.025, 0.05, 0.075, and 0.1 M converted C cultures to non-pigmented, non-conidial M-type cultures. At the lowest concentration, 0.025 M, some conidiation still occurred, but it was considerably less than that of the controls. Fluoroacetate also had a general inhibitory effect on the growth of both M and C cultures, causing a decrease in growth, measured as dry weight, of 70 % for C and 83 % for M at a concentration of fluoroacetate of 0.05 M. In these experiments the inhibitor was present in the sterile medium before inoculation. It is probable that the addition of inhibitor 1 day after inoculation would have decrease the amount of inhibition.

Sodium bisulphite at a concentration of 9.6×10^{-3} M when added to C cultures after a preliminary period of growth of 24 hr transformed these cultures into M cultures.

Iodoacetate at a concentration of 10^{-2} or 10^{-3} M when added to M cultures 24 hr after inoculation, converted these cultures to C cultures. If iodoacetate was added before inoculation the cultures failed to germinate. This inhibitor caused no decrease in the rate of growth as was the case for fluoroacetate.

Sodium fluoride at a concentration of 10^{-2} M, transformed M to C cultures also. In this case again, the inhibitor was added after 24 hr.

PCMB at a concentration of 2×10^{-6} M added 24 hr after inoculation transformed M to C cultures.

Enzyme studies

Oxidative enzymes: succinate dehydrogenase, succinate cytochrome c reductase and cytochrome oxidase

It was important to study these mitochondrial enzymes because the work of Zalokar (1959), Turian (1960,) and Turian & Seydoux, (1962) indicated a large decrease in Krebs cycle activity in conidia, as compared with germinated conidia and mycelia, while the work of Weiss (1965) suggested that conidia possess an active oxidative phosphorylating system; as active, in fact, as the mycelial oxidative system. Since there was a difference in the methods used by Zalokar and Turian on the one hand, and Weiss on the other, two methods of succinate dehydrogenase analysis were used. The results of both methods of assay are given in Table 1 along with cytochrome oxidase activities.

It is clear from these results that mitochondria from both conidiating and non-conidiating cultures possess high and approximately equivalent succinate dehydrogenase, succinate-cytochrome *c* reductase and cytochrome oxidase activities.

Glycolytic enzymes: ethanol dehydrogenase and pyruvate carboxylase

It was noticed quite early in this study that M cultures produced large amounts of ethanol. Direct analysis for ethanol in filtrates of 4-day M and C cultures showed that M cultures produced 80 % more ethanol than C cultures per unit dry weight. In addition, when iodoacetate or PCMB was added to M cultures, a reduction in

Table 1. *Succinate dehydrogenase, succinate-cytochrome c reductase and cytochrome oxidase activities of mitochondria isolated from 3-day conidial (C) and mycelial (M) cultures*

Culture	Enzyme assayed*	Specific activity
C } M }	Succinate dehydrogenase	{ 109.0 91.5
C } M }	Succinate-cytochrome c reductase	{ 771 648
C } M }	Cytochrome oxidase	{ 8070 8720

* Assay methods, see p. 409.

Table 2. *Ethanol dehydrogenase, pyruvate carboxylase, NADPH₂ cytochrome c reductase, glucose-6-phosphate dehydrogenase and NADP nucleotidase activities of 3-day conidial (C) and mycelial (M) cell-free extracts*

Culture	Enzyme assayed*	Specific activity
C } M }	Ethanol dehydrogenase	{ 125 1,659
C } M }	Pyruvate carboxylase	{ 7.0 13.7
C } M }	NADPH ₂ -cytochrome c reductase	{ 743 205
C } M }	Glucose-6-phosphate dehydrogenase	{ 605 760
C } M }	NADP nucleotidase	{ 12,618 309

* Assay methods, see pp. 409–10.

ethanol concentration in distilled filtrates of 90–96 % accompanied the transition to C type growth. Since the assay method for ethanol was not as precise as desired, ethanol dehydrogenase was assayed for in extracts of C and M cultures. In addition the activity of pyruvate carboxylase was measured. The results of these experiments are given in Table 2.

There is a fivefold to tenfold increase in ethanol dehydrogenase activity and a twofold increase in carboxylase activity in M as compared with C cultures.

Respiratory quotients and cyanide sensitivity

A third test for oxidative and glycolytic activity involved the determination of Q_{O_2} and Q_{CO_2} values for small segments of C and M cultures. Since these values varied greatly from one experiment to another, while the respiratory quotients, Q_{CO_2}/Q_{O_2} , remained relatively constant, Table 3 lists only the respiratory quotients. Also included in this table are the values for the amount of inhibition of oxygen

consumption in the presence of cyanide. These data indicate that M cultures produced 3 to 5 times more carbon dioxide than C cultures. Cyanide produced approximately the same amount of inhibition of oxygen consumption in conidial and mycelial cultures.

Table 3. *Respiratory quotients and cyanide sensitivity of whole cells from conidial (C) and mycelial (M) cultures, 3-days old*

Culture	R.Q.	% inhibition of O ₂ uptake
C	1.2	63.2
	1.4	94.8
M	4.2	68.6
	7.5	—

Oxygen consumption, carbon dioxide evolution and cyanide inhibition were measured in micro-Warburg flasks at 30° according to methods described on pp. 408–9.

NADPH₂ cytochrome c reductase activity

This enzyme was measured in order to obtain an estimate, admittedly crude, of the activity of an NADPH₂-requiring reaction in C and M cultures. The results given in Table 2 show that this reductase was approximately three times more active in C than in M extracts.

Glucose-6-phosphate dehydrogenase activity

This reaction provides a measure of the activity of the pentose phosphate pathway of glucose metabolism. It is one of the major NADPH₂-producing reactions in the cell.

One problem which arose during the measurement of this activity was the destruction of the NADP added by an NADP nucleotidase. C cultures have very high nucleotidase activity (see Table 2). Because of this enzymic destruction of NADP it was not possible to obtain accurate measurements of dehydrogenase activity in C cultures. Within 1 min. after the addition of enzyme, the reaction velocity approached zero. The measurements recorded in Table 2 were obtained from 1/*v* versus 1/*s* plots, where *v* represented the velocity of the reaction between 15 and 30 sec. after the addition of enzyme. Thus although M extracts show greater activity than C extracts, full activity for C may not have been measured due to rapid NADP destruction.

Electron microscopy

The general characteristics of conidia and hyphae of wild-type *Neurospora crassa* have already been described (Shatkin & Tatum, 1959; Zalokar, 1961; Weiss, 1965). The only features that will be pointed out here are those that seem to be specifically related to the C–M transition.

For this work 5-day C cultures and 2- to 3-day M cultures were used. These different ages were chosen because each culture had reached a high state of differentiation at these times. The M cultures had a well-developed submerged mycelial growth which was not excessively thick and therefore was believed to be more homogeneous than in older cultures. The C cultures had large numbers of pigmented

conidia and conidia associated with hyphae which were more easily located in the electron microscope than in younger C cultures with fewer conidia.

Three major differences were observed between M and C cultures. These differences were found in the outermost layer of the cell wall, in the mitochondria, and in the cytoplasm.

The major layer of the cell wall of M and C cultures was completely electron transparent. An inner layer which was in contact with the cell membrane (eventually a retraction zone of the membrane, but also present in sectioned conidia doubly fixed in formalin (phosphate buffer, pH 7.0 + OsO₄ 1 %) appeared as a grey region in photographs (see Pl. 1, and 2). It varied in thickness and inner contour following the variation of the protoplast membrane. An outer, thin, membrane-like layer was found only in C cultures. It was invariably partially detached from the rest of the cell and at some distance from it (Pl. 2). Where a mature conidium maintained contact with a hypha, two of these membranes were adjacent to each other (Pl. 1.) Mycelia of M cultures occasionally had an osmiophilic substance adhering to the cell wall. However, it was granular and not membranous as in conidiating cultures.

Both M and C cultures contained mitochondria similar to those described by Zalokar (1961), Luck (1963) and Weiss (1965). However, length and width distribution curves revealed that C mitochondria were considerably more swollen (also in formalin-OsO₄ preparations) than M mitochondria. The modal width value for C mitochondria was between 0.25 and 0.45 μ . For M mitochondria this value fell between 0.05 and 0.25 μ . The length distributions for the two cultures were approximately the same, 0.65 μ representing the modal value for both. Besides this difference in width, mycelial mitochondria contained long arrays of cristae filling most of a dense matrix region while conidial mitochondria contained short cristae and clear matrix (Pl. 1, and Pl. 4.).

With regard to the cytoplasm, a difference existed between M and C cultures which was difficult to quantify. The cytoplasm of C cultures had a homogeneous appearance, with the ribosomes lying freely dispersed in it. In M cultures the ribosomes (osmiophilic granules, Pl. 3) seemed to be clustered forming large granular islands in the cytoplasm. The significance of this clustering is not known.

DISCUSSION

In the mycelial, M state, the hyphae are vegetative and submerged growth prevails (not a necessary condition, however, as shown by maintenance of vegetative growth on the surface of solid C medium maintained in a low oxygen atmosphere; unpublished observations with Dr M. Kobr, 1965). In the C condition, the hyphae break the liquid surface and the aerial hyphae formed soon (1½–2 days) differentiate asexual reproductive structures, the macroconidia. The main objective in our investigation has been a biochemical and ultrastructural comparison between cultures of *Neurospora crassa* maintained in a vegetative non-conidiating condition (ammonium nutrition favouring anaerobic type of metabolism) and cultures provided the possibility of producing aerial conidiogenous hyphae (nitrate assimilation favouring an aerobic type of metabolism).

The studies of cyanide inhibition of oxygen consumption in whole cells and the

enzymic studies of mitochondrial enzymes suggest that M and C cultures have the same aerobic oxidative systems at the same level of activity. The fact, however, that conidiation was suppressed when fluoroacetate (a Krebs cycle inhibitor; Peters, 1957) or sodium bisulphite was added to C cultures implies that the process of conidiation is very sensitive to changes in Krebs cycle activity (entrance steps at least). Since the deep orange pigmentation of C cultures is also lost by the addition of fluoroacetate, the synthesis of certain unsaturated carotenoids must also depend on acetate metabolism.

The conversion of M to C cultures grown in the presence of either iodoacetate or PCMB was accompanied by up to 96% decrease in ethanol production. The glycolytic block probably occurred at the stage of 3-phosphoglyceraldehyde dehydrogenase. This enzyme is known to require free sulphydryl groups for activity and to be inhibited *in vitro* by both iodoacetate and PCMB (Koeppel, Boyer & Stulberg 1956). Sodium fluoride which produces the same conversion may be an inhibitor of glycolysis at the stage of enolase (Fruton & Simmonds, 1958). If these are the major sites of action of these three inhibitors, then blocking the glycolytic metabolism permits aerial hyphae and conidia to develop.

The results suggest that *in vivo* a balance must be maintained between the metabolism of glucose through the glycolytic route and through the TCA cycle. The addition of iodoacetate, PCMB or fluoride to an M medium alters this balance by interfering with the completion of glycolysis in such a way as to favour conidiation. On the other hand, fluoroacetate by partially blocking the TCA cycle favours glycolysis and completely arrests conidiation and decreases pigment synthesis. Sodium bisulphite may also be acting at this stage by blocking the utilization of either acetaldehyde or pyruvate through the TCA cycle.

The *in vitro* data for ethanol dehydrogenase and pyruvate carboxylase support this idea by verifying that M cultures which have a high sensitivity to glycolytic inhibitors have very active glycolytic enzymes, while C cultures which are unaffected by iodoacetate or fluoride have much lower glycolytic activity.

The fermentative and oxidative pathways of glucose metabolism branch at the stage of pyruvate. Holzer (1961) presented evidence supporting the hypothesis that the steady-state concentration of pyruvate controls the rate of utilization of this substrate by pyruvate decarboxylase or pyruvate oxidase. Thus the maximum rate of decarboxylation of pyruvate to form free acetaldehyde occurs at much higher concentrations of pyruvate than are required to achieve maximum utilization through the TCA cycle. This means that at low concentrations of pyruvate, a situation produced by inhibition of 3-phosphoglyceraldehyde dehydrogenase or enolase, the rate of utilization of pyruvate fermentatively is greatly decreased, while its rate of oxidation via the TCA cycle remains unchanged. The concentration of pyruvate therefore may be the factor controlling its metabolism in *Neurospora* and, in consequence, controlling conidiation.

Besides the relative activity of fermentative and oxidative pathways in the cell which undoubtedly serves as a regulator of both conidiation and pigment synthesis another important factor may be the relative concentrations of oxidized and reduced forms of the nicotinamide-adenine dinucleotide phosphates. In this connexion one must consider both the strikingly high NADP nucleotidase activity in conidiating cultures as well as the high activity of NADP-producing reactions. In

this regard it is assumed from the work of Kinsky & McElroy (1958) and Sorger (1963) that nitrate reductase is also very high in C and low in M cultures. The fact, however, that NADP-requiring glucose-6-phosphate dehydrogenase activity is found in C cultures implies that there is sufficient NADP *in vivo* that is not affected by the nucleotidase. The problem of localization of the nucleotidase and therefore the control of its activity in the cell may be closely related to the role of NADP in conidiation.

As has been recently pointed out by Nickerson & Bartnicki-Garcia (1964), the changing composition of the cell wall can influence cell morphology. It is possible that in the C/M system the changes produced by a change in nitrogen source influence production or physical state of some cell-wall component which is essential for the hyphae to break through the liquid surface and give rise to conidia. The electron microscope results suggest that a difference does exist between the composition of the outermost layer of the cell wall of M and C cultures. In addition, the observation that conidia are hydrophobic while mycelia are easily submerged in water solution, also points to a chemical alteration in the cell wall.

The other differences observed between M and C cultures in the electron microscope are not easily related to the biochemical data. The differences in mitochondrial structure are not the result of differences in either succinic dehydrogenase or cytochrome oxidase concentrations in the particles. Either other enzymes not studied in this investigation or environmental factors external to the mitochondria themselves must be involved. The cause of the more generalized difference in the cytoplasm of C and M cultures also remains to be explained and may be related to products that have accumulated in the cytoplasm and are not detectable with the microscope method used.

We would like to thank Miss Nina Matikian for her excellent technical assistance and Dr E. Kellenberger for permitting us to use his electron microscope facilities. This work was supported in part by a National Institute of Health Postdoctoral fellowship to one of the authors (B.W.); grant number 1-F2-GM-24,061-01 A1.

REFERENCES

- COOPERSTEIN, S. J. & LAZAROW, A. (1951). Cytochrome oxidase assay: a microspectrophotometric method for the determination of cytochrome *c* oxidase. *J. biol. Chem.* **189**, 665.
- COOPERSTEIN, S. J., LAZAROW, A. & KURFESS, N. J. (1950). A microspectrophotometric method for the determination of succinic dehydrogenase. *J. biol. Chem.* **186**, 129.
- DEMOSS, R. D. (1955). Glucose-6-phosphate dehydrogenase. *Meth. Enzym.* **1**, 328.
- EBERHARDT, B. M. (1961). Exogenous enzymes of *Neurospora* conidia and mycelia. *J. Cellular comp. Physiol.* **58**, 11.
- FRUTON, J. S. & SIMMONDS, S. (1958). *General Biochemistry*, 2nd ed. p. 472. New York: J. Wiley and Sons, Inc.
- HAAS, E. (1955). TPNH cytochrome *c* reductase. *Meth. Enzym.* **2**, 699.
- HILL, E. P. & SUSSMAN, A. S. (1964). Development of trehalase and invertase activity in *Neurospora*. *J. Bact.* **88**, 1556.
- HOLZER, H. (1961). Regulation of carbohydrate metabolism by enzyme competition. Cellular regulatory mechanisms. *Cold Spring Harb. Symp. quant. Biology*, **26**, 277.
- KAPLAN, N. O. (1955). *Neurospora* DPNase. *Meth. Enzym.* **2**, 660.

- KELLENBERGER, E. & RYTER, A. (1958). L'inclusion à polyester pour l'ultramicrotomie. *J. Ultrastruct. Res.* **2**, 200.
- KINSKY, S. & McELROY, W. D. (1958). Nitrate reductase: the role of phosphate, flavine and cytochrome *c* reductase. *Arch. Biochem. Biophys.* **73**, 466.
- KOEPPE, O. J., BOYER, P. D. & STULBERG, M. P. (1956). On the occurrence, equilibria, and site of acyl-enzyme formation of glyceraldehyde-3-phosphate dehydrogenase. *J. biol. Chem.* **219**, 569.
- KRZEMINSKI, L. F. & QUACKENBUSCH, F. W. (1960). Stimulation of carotene synthesis in submerged cultures of *Neurospora crassa* by surface active agents and ammonium nitrate. *Arch. Biochem. Biophys.* **88**, 64.
- LIP BOEN KHOUW, BURBRIDGE, T. N. & SUTHERLAND, V. C. (1963). The inhibition of alcohol dehydrogenase. I. Kinetic studies. *Biochim. biophys. Acta* **73**, 173.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265.
- LUCK, D. J. L. (1963). Formation of mitochondria in *Neurospora crassa*. Quantitative radioautographic study. *J. Cell Biol.* **16**, 483.
- NICKERSON, W. J. & BARTNICKI-GARCIA, S. (1964). Biochemical aspects of morphogenesis in algae and fungi. *A. Rev. Plant. Physiol.* **15**, 327.
- NICLOUX, M. (1931). Recherche sur l'alcool éthylique. Microdosage. *Bull. Soc. Chim. biol.* **13**, 857.
- PETERS, R. A. (1957). Mechanism of the toxicity of the active constituent of *Dichapetalum cymosum* and related compounds. *Advanc. Enzymol.* **18**, 113.
- RIGGS, B. C. (1945). Cyanide loss from media in studies of tissue metabolism *in vitro*. *J. biol. Chem.* **161**, 381.
- ROCHAT, J. (1946). Le dosage de l'alcool éthylique sanguin: une modification de la méthode de Nicloux. *Helv. Chim. Acta* **4**, 819.
- SHATKIN, A. J. & TATUM, E. (1959). Electron microscopy of *Neurospora crassa* mycelia. *J. biophys. biochem. Cytol.* **6**, 423.
- SINGER, T. P. (1955). Plant carboxylases. *Meth. Enzym.* **1**, 460.
- SINGER, T. P., & KEARNEY, E. B. (1951). Determination of succinic dehydrogenase activity. *Meth. biochem. Analysis.* **4**, 307.
- SORGER, G. J. (1963). TPNH-cytochrome *c* reductase and nitrate reductase in mutant and wild type *Neurospora* and *Aspergillus*. *Biochem biophys. Res. Commun.* **12**, 395.
- TURIAN, G. (1957). Recherches sur l'action anticaroténogène de la diphénylamine et ses conséquences sur la morphogenèse reproductive chez *Allomyces* et *Neurospora*. *Physiol. Plant.* **10**, 667.
- TURIAN, G. (1960). Déficiences du métabolisme oxydatif et différenciation sexuelle chez *Allomyces* et *Neurospora*. *Path. Microbiol.* **23**, 687.
- TURIAN, G. (1961). L'acétate et son double effet d'induction isocitratasique et de différenciation conidienne chez les *Neurospora*. *C.r. hebd. Séanc. Acad. Sci., Paris* **252**, 1374.
- TURIAN, G. (1964). Synthetic conidiogenous media for *Neurospora crassa*, *Nature, Lond.* **202**, 1240.
- TURIAN, G. & SEYDOUX, J. (1962). Déficience d'activité de la déshydrogénase succinique dans les mitochondries isolées du *Neurospora* en condition d'induction isocitratasique. *C.r. hebd. Séanc. Acad. Sci., Paris* **255**, 755.
- WEISS, B. (1965). An electron microscope and biochemical study of *Neurospora crassa* during development. *J. gen. Microbiol.* **39**, 85.
- ZALOKAR, M. (1954). Studies on the biosynthesis of carotenoids in *Neurospora crassa*. *Arch. Biochem. Biophys.* **50**, 71.
- ZALOKAR, M. (1959). Enzyme activity and cell differentiation in *Neurospora*. *Am. J. Bot.* **46**, 555.
- ZALOKAR, M. (1961). Electron microscopy of centrifuged hyphae of *Neurospora*. *J. biophys. biochem. Cytol.* **9**, 609.
- ZALOKAR, M. & COCHRANE, V. W. (1956). Diphosphopyridine nucleotidase in the life cycle of *Neurospora crassa*. *Am. J. Bot.* **43**, 107.

EXPLANATION OF PLATES

PLATE 1

A section through a portion of a conidial (c) culture of *Neurospora crassa* showing a conidium attached to a hypha. The cell wall consists of an inner grey region, a middle electron transparent, non-stainable layer and an outer partially dislodged osmiophilic membrane. A single nucleus dominates the cytoplasm and is surrounded by swollen mitochondria, containing few cristae and a clear matrix. The osmiophilic granules filling the rest of the cytoplasm are evenly distributed. $\times 41,000$.

PLATE 2

This is a section through the tip of a conidia-bearing hypha. The complete conidium is separate from the hypha by a thick double-layered wall. The three layers surrounding the rest of the protoplasm are similar to those described in Pl. 1 and in the text. A rudimentary reticulum is evident both in the hypha and the conidium. The mitochondria are large and contain characteristically few cristae and matrix granules. $\times 28,000$.

PLATE 3

A section through a hypha of a mycelial (M) 2-day culture of *N. crassa*. The mitochondria contain numerous long parallel arrays of cristae, stain very deeply and are for the most part narrow and irregular in contour. The cytoplasm contains many osmiophilic granules which are concentrated in dense clusters, and irregular lacunae above the two nuclei. The cell-wall structure is restricted to the major, electron transparent layer. $\times 40,700$.

PLATE 4

A section through a small portion of a mycelial (M) culture clearly showing the long, parallel cristae in the mitochondria and the dense islands of ribosomes characteristic of these nonconidiating hyphae. $\times 44,400$.

B. WEISS AND G. TURIAN

(Facing p. 418)

B. WEISS AND G. TURIAN

B. WEISS AND G. TURIAN