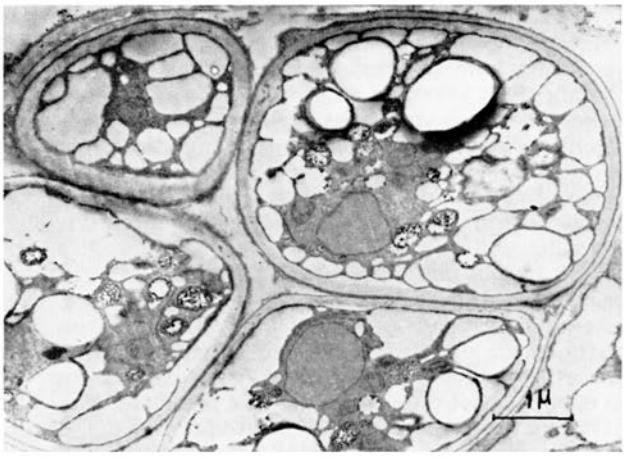

Fig. 2. With glucose. Great lipid drops have replaced the chloroplasts, the cellular mass has been reduced. Fixation as in Figure 1.

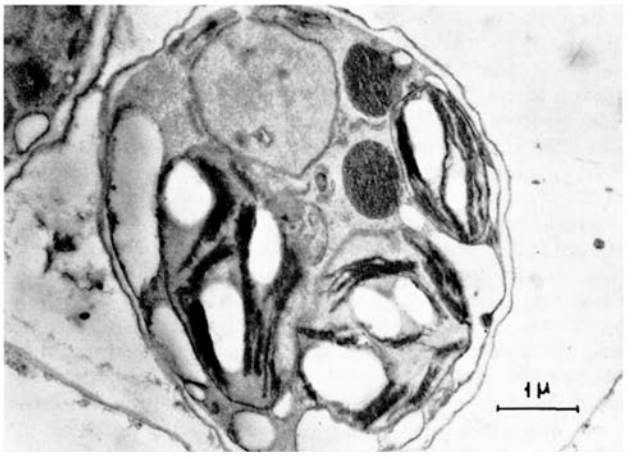

Fig. 3. With galactose. Chloroplasts with many compact bundles of lamellae, lipid and starch. Fixation as in Figure 1.

in those cultures which contain fructose or galactose. It disappeared almost completely in cultures with lactose, while arachic acid was found; in cultures with saccharose, linolenic acid was accompanied by an unidentified fatty acid with 20 carbon-atoms and 4 double bonds.

In all the cultures studied, there is a direct proportionality between carbohydrate uptake and carotenoid synthesis and an inversed relationship between carbohydrate uptake and chlorophyll synthesis (Tables I and II).

The electron microscope photographs confirm the results obtained, demonstrating that the blushing effect is the visible feature of chloroplasts deformation (Figures 1–3)[6].

Riassunto. È studiato l'effetto di arrossamento provocato dall'aggiunta di alcuni zuccheri a colture sommerse dell'alga cloroficea *D. cinnabarinus*. Questo effetto è dovuto alla formazione di cheto-carotenoidi, alla diminuzione delle clorofille ed alla degradazione dei cloroplasti. Le osservazioni al microscopio elettronico mettono in evidenza le variazioni della struttura dei cloroplasti.

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STUDIORUM PROGRESSUS

Induced Macroconidia Formation in *Neurospora crassa*

Asexual development in *Neurospora* includes the differentiation of laterally growing vegetative hyphae into aerial hyphae and these, in turn, into numerous conidiophores which form vegetative spores called macroconidia. Previous investigations reveal an impressive array of fundamental biochemical differences between vegetative hyphae and the conidia they may eventually yield[1–3] and that the process is under genetic control[4]. However, the first requirement for experimental analyses of macroconidiation is that it be brought under stringent and precise control. A method which improves upon previous attempts to do so[5,6] is given here together with observations which make some contribution to the biology of *N. crassa*.

Both of the earlier methods took advantage of the fact that conidia are never formed on submerged hyphae[7] or in the presence of the wetting agent Tween 80[8]. Strauss[5] reports conditions under which conidiation will begin after about 12 h and continue for a period of 4–5 h. Stine and Clark[6] find that aerial development requires 8 h and the differentiation of conidia continues for another 8 h. Our basic method reduces the durations of both aerial formation and conidiation without decreasing the final conidial yield. In addition, the method can be modified in a number of ways permitting the emergence of new and fruitful experimental situations.

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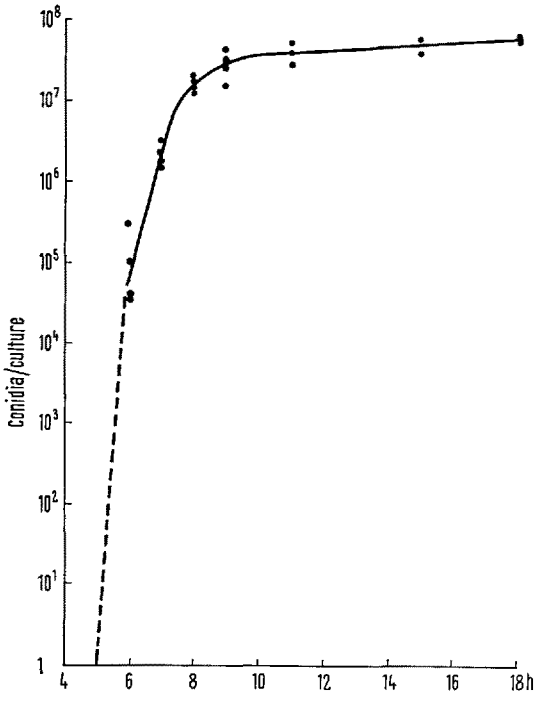
Mycelial pads are obtained by transferring suspensions of wild-type conidia (strain 74-A) from 5-day-old slant cultures to 250 ml. Erlenmeyer flasks containing 50 ml BEADLE and TATUM's minimal medium[9] together with 1.5% sucrose, 0.23% sodium acetate and 0.1 ml Tween 80/l[6]. The flask cultures are incubated for 66–68 h in a stationary light-free moist chamber at 30 °C. Using sterile techniques, each mycelial pad, now about 7.5 cm diameter and 1 mm thick, is gently poured into a Buchner funnel containing 200 ml 0.1 *M* potassium phosphate buffer (pH 6.1) and a thin plastic disc, 9 cm diameter with nine 1 mm holes and a fixed stem or handle, supporting a 9 cm disc of Whatman No. 1 filter paper. As the pad is transferred to the Buchner funnel its orientation is retained so that the uppermost surface of the pad, as it grew in the flask, is kept uppermost in all subsequent operations. By gently manipulating the plastic disc, the pad is centered wrinkle-free on the filter paper and the buffer-wash is slowly drained through the funnel. The pad is washed again in 200 ml buffer and then transferred to a buffer-soaked filter paper with the aid of the plastic disc. The fact that there is no net protein synthesis following the second wash attests to the efficacy of the procedure. Nor were the final populations of conidia produced by pads given 2, 4 or 6 such washes found to differ significantly. Next, the center of the pad is cut out and discarded leaving a mycelial 'ring' 0.5 cm wide to be used for further conidiation studies. (The basis for this procedure lies in the fact that conidiation occurs first within the outermost 0.5 cm of a pad and, demonstrably later, towards the center.) The mycelial ring on its ring of filter paper is placed on a 10 × 10 cm wire screen supported by the bottom of a 100 mm Petri dish. This dish is then suspended 2.5 cm over 60 g $CaCl_2$ contained in the Petri dish top and the preparation is sealed in an 8-inch glass-covered finger bowl. Finally the culture is exposed to 300 f.c. artificial daylight at 25 °C. With experience, a pad can be transferred from the growth flask to the glass chamber in 5–6 min.



The number of macroconidia per culture versus time since induction. Mycelial pads were grown for 66–68 h, washed, then induced to yield conidia by transfer to 25 °C, 300 f.c. light, and reduced humidity. Each point represents 1 culture. All cultures assayed at 5 h and earlier gave counts lower than the resolution of the hemocytometer (1×10^3).

For conidial assays, the mycelial ring is removed from the filter paper, the conidia are freed from the hyphae by gentle mincing in an omnimixer, and are counted with a hemocytometer. In order to make reliable counts on small populations of conidia, the preparation is concentrated by centrifugation.

The data in the Figure attest to the reliability of the basic method. No conidia were found among 10 cultures assayed 5 h (or less) after induction. In developing the method certain of its features were systematically varied; since these observations bring to light particular aspects of the biology of conidiation they are presented below.

The results given in Table I relate the duration of the period of vegetative hyphal growth to quantitative aspects of conidiation. The closest approach to the desired synchronous 'burst' of conidiation was found among pads incubated for 66–72 h prior to induction. The fact that aged ('stationary phase') hyphae go on to form conidia but do so reluctantly is noteworthy but unexplained.

The foregoing observations together with ZALOKAR's[2] report that the hyphae uppermost in the proliferating mycelial mass are the youngest and those at the bottom are the oldest, suggested that pads kept young side up during induction should produce conidia more efficiently than pads turned about so that the youngest hyphae rest on the filter paper. The data in Table II establish this point. However, a decisive test of the notion that hyphal age per se influences conidiation has yet to be carried out. In addition, the experiment led to the discovery that conidia may form from both of the surfaces of a pad although in any case the original upper surface, where the youngest hyphae are located, gives rise to the greatest numbers of conidia.

The effects of the drying agent $CaCl_2$ are shown in Table III. To collect the data in the first 3 rows, mycelia were placed in moist chambers at various distances from 60 g of $CaCl_2$. The data in the last row of the Table were collected from pads which formed conidia in absence of $CaCl_2$ and on continuously buffer-soaked filter papers. The results suggest the following relationships: first, the dura-

Table I. The effect of various growth periods on subsequent conidial yield

Growth period (h)	Mass dry weight (mg)	Conidia/pad at time (h) since induction	
		9	18
48	17.83	2.75×10^6	3.70×10^6
54	22.67	4.20×10^6	3.78×10^7
60	26.50	1.35×10^7	3.18×10^7
66	32.00	3.33×10^7	3.60×10^7
72	35.33	2.63×10^7	4.05×10^7
96	44.16	1.03×10^7	3.30×10^7
120	43.50	1.55×10^6	3.63×10^7
144	46.67	5.50×10^4	7.75×10^6

Mycelial pads were grown for the indicated periods, washed and induced under standard procedures. At the time of induction, 1 group of pads was set aside for weight determination.

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Table II. The effect of pad orientation on conidia production

Orientation of pad during conidiation	Pad surface	Conidia/pad at time (h) since induction 5	6	7	8	9	11
Normal	Upside[a]	0	3.0×10^5	2.25×10^6	1.67×10^7	2.51×10^7	2.64×10^7
	Downside[b]	0	0	0	0	0	0
Reversed	Upside[b]	0	0	5.62×10^3	2.03×10^4	1.35×10^5	3.03×10^5
	Downside[a]	0	8×10^3	1.32×10^4	5.6×10^4	3.0×10^5	2.25×10^6
	Total	0	8×10^3	1.88×10^4	7.63×10^4	4.35×10^5	2.55×10^6

Mycelial pads grown for 66–68 h were divided into 2 groups. Group 1. Normal orientation: the pads were washed so that the upper surface during growth was the upper surface during induction. Group 2. Reversed orientation: the pads were inverted during washing so that the upper surface during growth was the surface in contact with the filter paper during induction and faced downward. At each hour indicated, the conidia on a single pad from each group were assayed. The upside counts are the total number of conidia above the mycelial pad. The downside counts are the total number of conidia formed on aerials which grew down through the supporting filter paper.
[a] This is the top of the pad as it grew in the flask. [b] This is the bottom of the pad as it grew in the flask.

Table III. The effect of varying humidity on conidia production

Conditions $CaCl_2$	Distance	Conidia/pad at time (h) since induction 5	6	7	8	9	11	24	48
+	5 cm	0	0	0	1.25×10^5	1.0×10^6	1.0×10^6	1.25×10^6	–
+	8.6 cm	0	0	8.75×10^5	7.75×10^6	3.5×10^6	1.78×10^7	1.1×10^7	–
+	11 cm	0	0	1.5×10^6	1.38×10^7	1.40×10^7	3.35×10^7	2.98×10^7	–
–	–	1.91×10^3	5×10^5	2.5×10^6	4.83×10^7	4.68×10^7	8.53×10^7	1.2×10^8	1.24×10^8

Mycelial pads were grown for 66–68 h, washed and induced in the presence or absence of $CaCl_2$. Conidia from sections of the pads 5.0, 8.6 and 11.0 cm distant from the $CaCl_2$ were counted and the counts normalized to total yield of conidia/whole pad. Conidia induced in the absence of $CaCl_2$ were counted in the normal way. Each count represents 1 culture.

tion of development prior to the appearance of the first conidia is inversely related to the humidity; second, the duration of conidiation is directly related to humidity; and third, the number of conidia formed is directly related to the humidity.

Cultures induced in darkness versus light (300 f.c. and 1200 f.c. illumination) differ in 3 important respects. First, the orange pigment, a carotenoid, characteristic of both hyphae and conidia is formed only in light[8]. Second, the duration of conidiation is shortened by at least an hour if cultures are continuously exposed to light. Third, when a culture is induced in light, the formation of aerial hyphae and conidia is restricted to the upper (lighted) surface of the mycelial pad. But in darkness hyphae and conidia are formed from both top and bottom surfaces of the mycelial mass.

The basic method outlined above provides an opportunity to investigate biochemical events which may accompany particular morphological changes. For example, our unpublished observations show that the enzyme NADase – previously reported as lacking in vegetative hyphae and present in large amounts in conidia[1] – to be rapidly synthesized during aerial development and then accumulated in the conidia. The method has also encouraged what now appears to be a profitable search for macroconidial mutants; thus in some strains we note that the time course for the differentiation of conidia from aerials is upset and in others the morphology of aerials or conidia or both is atypical[10]. By modifying the basic method for inducing conidiogenesis experimentally useful situations appear. Under conditions of high humidity conidia formation is preceded by the elaborate growth and development of aerial hyphae, while relative dryness causes the formation of extremely short, comparatively simple, less extensively branching aerials. Again, the fact that aerials will grow through filter paper suggests at once that the aerials can be separated from the underlying mycelial pad by merely removing the paper which supports them; thus one can ask whether such detached aerials contain the materials necessary for full conidia formation. Indeed they do! And finally, it is now possible to trace the syntheses of particular molecules during development, because mycelial pads will differentiate while in continuous contact with phosphate buffer to which inhibitors and labels can be added.

In conclusion, the process of conidiation may provide an especially attractive example of morphogenesis because it is relatively simple, non-essential, and occurs in a eucaryote well-suited for sophisticated biochemistry and genetics[11].

Zusammenfassung. Es wird eine Methode zur Kontrolle diverser Umwandlungsstadien bei Neurosporen beschrieben: Umbildung wachsender, vegetativer Hyphen in luftige Hyphen, von diesen in Conidienträger und Macronidien. Während der Produktionsperiode für luftige Hyphen (5–6 h) bildet jede Kultur ca. 3×10^7 Conidien (32 mg Trockengewicht/3 h). Die Methode ist besonders brauchbar zum Studium morphogenetischer und biochemischer Begleitphänomene bei Wildtypen und Mutanten.

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[10] R. E. Nelson, unpublished.

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