Growth of *Trichophyton mentagrophytes* and *Trichophyton rubrum* in Increased Carbon Dioxide Tensions

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SUMMARY: The formation of macrospores in normally mycelial strains of *Trichophyton mentagrophytes* and *T. rubrum* was induced by growing the cultures in a gas phase containing air + 12-24 % (v/v) CO₂. When conidial transfers were made from strains which had thus been induced to form macrospores and were incubated in normal air, the subsequent growth was mycelial and downy. One strain of each fungus, normally conidial and granular, formed arthrospores under increased CO₂.

The bases for genus and species differentiation of the superficial dermatophytes are shape and size of macroconidia and gross cultural characteristics (Emmons, 1934). Identification of these *Fungi imperfecti* is complicated by loss of the ability to form macroconidia and variation in cultural morphology; most of the dermatophytes exist in two morphological types, conidial and mycelial (Wilhelm, 1947). When these fungi are first isolated from lesions on to Sabouraud's glucose agar, they usually grow as the conidial, macroconidia-forming, type. Upon repeated transfer, however, they often lose the ability to sporulate and continue growth as the mycelial type. Reversion of the mycelial to the conidial form is very uncommon. Investigations in this laboratory have shown that non-sporulating strains of *Trichophyton mentagrophytes* and *T. rubrum* can be induced to form macroconidia, thus reverting to the conidial type, by incubation in a gas phase containing concentrations of carbon dioxide above that of normal air (c. 0.03 %, v/v).

METHODS

Cultures. The cultures employed in this study included 5 strains of *Trichophyton mentagrophytes* (408, My-874-55, A-538, X-15, X-10) and 6 strains of *T. rubrum* (T-3, X-14, My-38-56, X-2, My-274-56, My-640-55). Of these, only strains X-10 and X-14 were the conidial type and formed typical macroconidia and microconidia on Sabouraud's glucose agar when incubated in normal air; the other strains formed microconidia but no macroconidia under these conditions. Strain 408 formed vegetative mycelium without differentiated structures. All cultures were maintained as stocks on slopes of Sabouraud's glucose agar (Difco). After inoculation, slope cultures were incubated at 30° for 1 week then sealed with tinfoil and wax stored at 5° until used.

Inoculum. The inoculum was prepared by dispersing the growth from a stock slope with 10 ml. sterile water in a Waring blender for 1 min. One drop of this suspension was inoculated on to the centre of each of a series of

Sabouraud's glucose agar plates. Eight or more of these plates were incubated in each concentration of carbon dioxide.

CO₂ concentration. Carbon dioxide concentration was adjusted, after a set of plates had been sealed in a Brewer's anaerobic jar, by drawing a vacuum of approximately 30 mm. Hg in excess of the desired concentration of CO₂. The desired amount of CO₂ was run in from a cylinder and was measured by noting the fall in negative pressure on a manometer, then the valve of the jar was momentarily opened to return the internal pressure of the jar to atmospheric. Control plates were incubated in laboratory air.

All cultures were incubated at 30° and the CO₂ concentrations were re-adjusted daily. During the period of incubation, 2 plates from each concentration were removed at 2- to 3-day intervals for microscopic examination of the mycelium. At the end of 7 or more days of incubation, all the remaining plates were removed and examined macroscopically and microscopically.

RESULTS

Macroconidia formation by 3 out of 4 mycelial strains of *Trichophyton mentagrophytes* and 5 out of 5 mycelial strains of *T. rubrum* was induced by incubation at increased concentrations of CO₂; Table 1 indicates the response to CO₂ of all the cultures of definite history that were tested. However, this response to CO₂ was discovered with a stock strain of *T. mentagrophytes* and 2 similar strains of *T. rubrum* which are not included in this discussion because of their unknown backgrounds. Pl. 1, fig. 1, shows typical mycelial growth of *T. rubrum* T-3; Pl. 1, fig. 2, shows the conidial growth of the same strain under 16% (v/v) CO₂; this profuse formation of macroconidia is typical of most of the other strains when incubated under CO₂. *T. mentagrophytes* 408, which did not respond to increased CO₂, was not typical of other downy strains; it was completely vegetative at all times, producing neither macro- nor microconidia. The conidial strains *T. rubrum* X-14 and *T. mentagrophytes* X-10 formed arthrospores exclusively at CO₂ concentrations of 16% or more; Pl. 1, fig. 3, shows arthrospore formation by *T. rubrum* X-14. To determine that the increased concentration of CO₂ and not the decreased concentration of oxygen was inducing macroconidia formation, *T. mentagrophytes* strain A-538 was incubated in an atmosphere containing 20% (v/v) more nitrogen than normal. Under these conditions, macroconidia were not produced and no changes in morphology were apparent.

As macroconidia formation increased with increasing CO₂ concentrations, the macroscopic appearance of mycelial strains changed gradually from downy to granular; Pl. 1, fig. 4, shows the increased granulation and pigmentation of *Trichophyton rubrum* T-3 as the CO₂ concentration was increased. As the concentration of carbon dioxide in the atmosphere was increased, the diameters of the colonies decreased somewhat (Table 2).

At the concentrations of CO₂ shown in Table 1 the dermatophytes fell into four groups with regard to pigment formation: (i) the intensity of pigment increased stepwise as CO₂ concentration was increased from 0 to 24%

Table 1. *The effect of increased concentrations of carbon dioxide on macroconidia formation by strains of Trichophyton mentagrophytes and T. rubrum.*

Three plates were examined for each concentration.

Organisms	Increased CO ₂ in gas phase (% v/v)					Period of incubation (days)
	0	12	16	20	24	
	Effect on growth					
<i>T. mentagrophytes</i>						
408	—	—	—	—	—	7
My-874-55	—	—	+	++	++	7
A-538	—	nr	+++	+++	+++	7
X-15	—	+++	+++	+++	+++	7
X-10	++	nr	arth.	arth.	arth.	7
<i>T. rubrum</i>						
T-3	—	+++	+++	nr	nr	11
My-38-56	—	—	—	++	++	9
X-2	—	—	—	++	+++	9
My-274-56	—	++	+++	+++	+++	7
My-640-55	—	—	++	+++	+++	8
X-14	+++	+	arth.	arth.	arth.	9

— = no macroconidia; + = occasional macroconidia; ++ = abundant macroconidia; +++ = profuse macroconidia. arth. = arthrospore formation only. nr = not run at this concentration.

Table 2. *Effect of increased carbon dioxide concentrations on the colony diameter of Trichophyton mentagrophytes and T. rubrum*

Three plates were averaged for each concentration.

Organisms	CO ₂ in gas phase (% v/v)					Period of incubation (days)
	0	12	16	20	24	
	Colony diameter (mm.)					
<i>T. mentagrophytes</i>						
408	54	50	49	47	46	7
My-874-55	52	48	45	39	36	7
A-538	40	nr	30	26	25	7
X-15	47	27	26	27	25	7
X-10	47	nr	34	32	30	7
<i>T. rubrum</i>						
X-14	25	21	20	18	18	9
My-38-56	34	28	25	23	21	9
X-2	35	33	30	26	24	9
My-274-56	25	23	18	17	16	7

nr = not run at this concentration.

(strains T-3, My-874-55, A-538); (ii) the intensity of pigment decreased stepwise as CO₂ concentration was increased from 0 to 24% (strains 408, My-38-56, X-10, X-14, My-640-55); (iii) the intensity of pigment increased from 0 to 12% CO₂, then decreased from 12 to 24% CO₂ (strain X-15); (iv) the intensity of pigment decreased from 0 to 12% CO₂, then increased from 12 to 24% CO₂ (strains My-274-56, X-2). There seemed to be no consistent relationship

between the two species or strains within a species regarding the relation of pigmentation and CO₂ concentration.

Conidial subcultures from mycelial strains which were induced to form macroconidia under increased CO₂ were made at the end of the incubation periods. After incubation for a week in air at 30°, these subcultures again had the typical downy gross morphology and no macroconidia were found upon microscopic examination.

DISCUSSION

In this laboratory the terms 'downy' and 'granular' are used for macroscopic descriptions, and 'conidial' and 'mycelial' are used for microscopic descriptions. The difference between granular and downy is not clear-cut; one strain may appear more downy or granular than another and intermediates exist between both extremes. Conidial strains (X-10, X-14) form macroconidia on slopes of Sabouraud's glucose agar in normal atmospheric conditions of incubation; mycelial strains (My-874-55, A-538, My-38-56, X-2, My-274-56, My-640-55, X-15, T-3) do not, both conidial and mycelial strains form microconidia. A granular strain is usually of the conidial type and a downy strain is usually in the mycelial type. However, strains which appear downy may form a few macroconidia and strains which appear granular because of intensive microconidia formation may form no macroconidia. Because of this colonial variation, any cultural condition resulting in more consistent macroscopic and microscopic morphology is noteworthy.

The reason for the change from the conidial type to the mycelial type in the dermatophytes is not known, although Wilhelm (1947) and Robbins & McVeigh (1949) suggested that a mutation at some point in the life cycle of the fungus was involved. The observations made here, namely, the rapid change from the mycelial to the conidial type by increased concentrations of carbon dioxide and the immediate return to the mycelial type upon incubation in laboratory air, seems to argue against mutation.

Whether carbon dioxide acts as a physical or chemical agent in producing these morphological changes is not at present known. Macrospore formation under increased CO₂ concentration may be associated with the metabolism of purines and pyrimidines or tricarboxylic cycle components since CO₂ is readily fixed into these compounds by many micro-organisms. However, it is not apparent why some strains form macroconidia readily on Sabouraud's glucose agar in normal atmospheric conditions, while others must be induced by increased concentrations of carbon dioxide to form these spores and lose the ability immediately upon transfer to normal atmosphere.

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EXPLANATION OF PLATE

- Fig. 1. Mycelium of *Trichophyton rubrum* T-3 incubated in air; a few microconidia can be seen ($\times 280$).
- Fig. 2. Mycelium of *T. rubrum* T-3 showing profuse macroconidia formation after incubation for 11 days in air + 16 % (v/v) CO₂ ($\times 280$).
- Fig. 3. Arthrospore formation by granular *T. mentagrophytes* X-10 incubated in air + 16 % CO₂ ($\times 280$).
- Fig. 4. Giant colonies of *T. rubrum* T-3 incubated 9 days at different concentrations of CO₂. Increased granulation and pigment formation is apparent.

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B. CHIN & S. G. KNIGHT--CARBON DIOXIDE AND THE GROWTH OF DERMATOPHYTES.
PLATE 1

(Facing p. 646)