using a swimming maze as a precaution against odour cues. No evidence for information transfer has been obtained under these conditions.

Zusammenfassung. Kürzlich wurde über ein Experiment berichtet, in dem es gelungen sein soll, die spezifische räumliche Repräsentation eines Labyrinths durch Gehirnextrakte von dressierten Mäusen auf Empfängertiere zu übertragen. Wir haben das Experiment unter Ausschluss von Geruchsspuren (Schwimmlabyrinth) an Ratten wiederholt und dabei keine Informationsübertragung nachweisen können.

C. C. KUENZLE and H. ZEIER

Department of Pharmacology and Biochemistry, School of Veterinary Medicine, University of Zürich, Winterthurerstrasse 260, CH-8057 Zürich (Switzerland), and Department of Behavioural Sciences, Swiss Federal Institute of Technology, Turnerstrasse 1, CH-8006 Zürich (Switzerland), 18 March 1975.

Fine Structural Characterization of Microbodies and Woronin Bodies in *Trichophyton mentagrophytes*

Trichophyton mentagrophytes is a dermatophytic fungus of considerable medical importance. In spite of this, research regarding its ultrastructural morphology[1-4] has revealed only its general aspects, the usual techniques used to date being inadequate to bring to light the finer structural details.

An improved fixation, and new knowledge regarding the substructure of the cell wall[5], were obtained by using a prefixative containing *tris*-1-aziridinyl-phosphine oxide (TAPO), a compound recently introduced with success in biological electron microscopy[6-10].

In this study, we report that, using basically the same technique, a better preservation of the internal structures of the fungus may also be obtained. In particular, it is noted that in the hyphal cells, 2 types of organelles surrounded by a single unit membrane are present, i.e. microbodies and Woronin bodies. These terms are widely accepted and have been morphologically characterized.

Woronin bodies were already identified in foregoing ultrastructural studies in *Trichophyton* spp. and other dermatophytes, not because of their morphology which presented certain ambiguities, but on the basis of their position near the septum, and therefore also called septal or peripheral granules[1-4,11,12]. On the contrary, microbodies have not been identified in dermatophytic fungi.

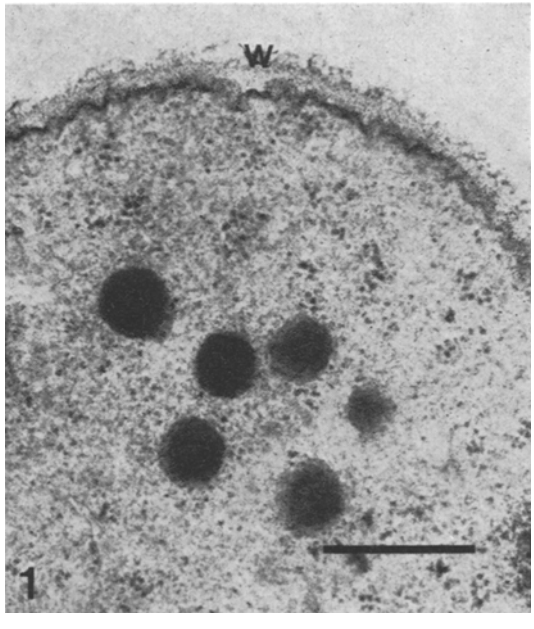

Fig. 1. Cross section through a young hyphal cell of *Trichophyton mentagrophytes* CBS 560.66 showing several Woronin bodies. M, mitochondrion; Mb, microbody; S, septum; W, hyphal wall; Wb, Woronin body. In all the electron micrographs the length of the bar corresponds to 0.5 μm.

Methods. Trichophyton mentagrophytes, strain No. 560.66 (Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands), was grown on a Sabouraud maltose agar medium, at 28 °C, on a thin sheet of cellophane, as recently described[13]. Electron microscopic observations were carried out on the youngest hyphae harvested from the outside portion of the cultures in logarithmic phase. The specimens were fixed in a mixture of 6% glutaraldehyde (Eastman Kodak Company, Rochester, N.Y.) and 1% TAPO (*tris*-1-aziridinyl-phosphine oxide; K and K Laboratories Inc., Plainview, N.Y.) in a 0.1 *M* phosphate buffer (pH 6.2), at 4 °C for 2 h. After a brief washing, the samples were postfixed in 1% OsO_4 in the same buffer for 1 h, at room temperature, dehydrated in acetone and embedded in Durcupan ACM. Ultrathin sections, mainly cut longitudinally to the hyphal strands, were obtained with a LKB Ultrotome III ultramicrotome and then stained with uranyl acetate and lead citrate and observed through a Jeol JEM-T7 at 60 Kv.

Results and discussion. The young hyphal cells of *T. mentagrophytes* contain 2 types of organelles that show the characteristic and distinctive aspects of the Woronin bodies and microbodies only when a prefixative containing a glutaraldehyde-TAPO mixture is used, followed by an osmium postfixation.

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Woronin bodies are spherical, or less often ovoid, measuring approximately 0.2 μm in diameter. They are bounded by a single unit membrane containing a homogeneous electron dense matrix (Figure 1). Because of their shape, size and electron opacity, the structure of the membrane is observed only when the organelles are sectioned medially. Although these bodies are generally observed near the septa (Figure 4), they can often be found throughout the entire cytoplasm (Figure 3). This type of organelle is very similar to the Woronin bodies described in previous structural investigations on ascomycetous fungi[14–16], and we assume that it corresponds to the structures repeatedly observed close to the septa in *Trichophyton* spp. and in other dermatophytes[1–4,11,12,17–22]. Microbodies, often of ovoid shape and ranging in size from 0.4 to 1.0 μm, consist of a fine granular matrix that is surrounded by a single membrane (Figure 2). These organelles are distributed with a certain uniformity in the hyphal cells where they are often close, in different degrees, to Woronin bodies (Figure 3). Near the septa, however, they are less frequent than elsewhere. This second type or organelle, not previously identified in dermatophytes, corresponds morphologically to the microbodies found in plant cells[23–25], including some fungi[16,26–28].

There is speculation[26,29], and one report[16], that microbodies give origin to Woronin bodies. In spite of this, in the present study, the fact that the 2 organelles often lie

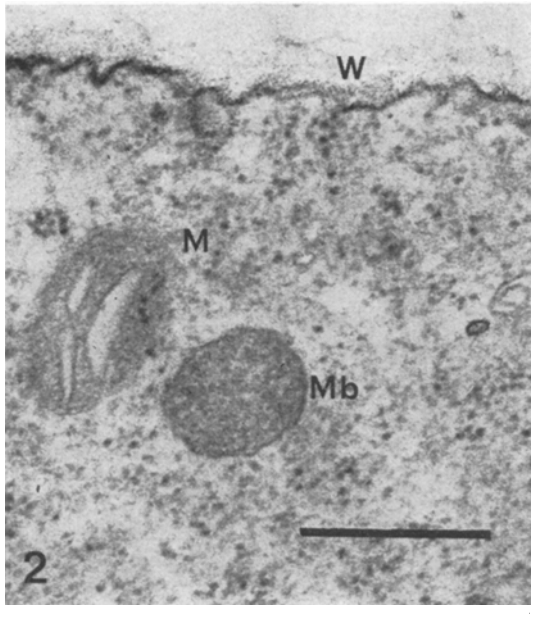

Fig. 2. Cross section showing a microbody. Note the difference from the neighbouring mitochondrion.

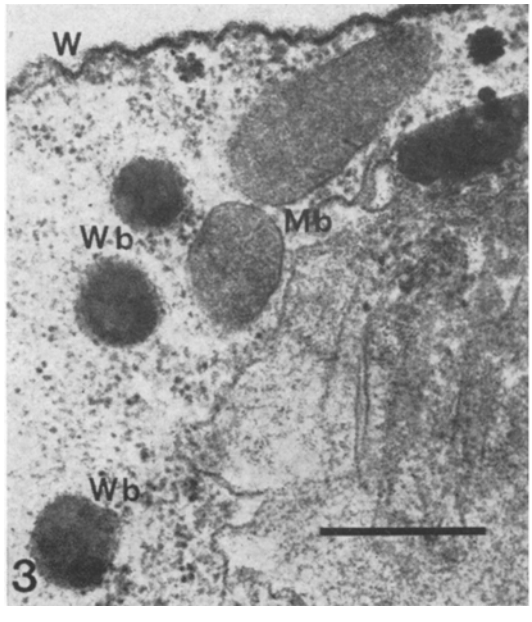

Fig. 3. Longitudinal section of a hyphal portion, at a distance from the septum, showing neighbouring microbodies and Woronin bodies. Note the different electron density of their matrix.

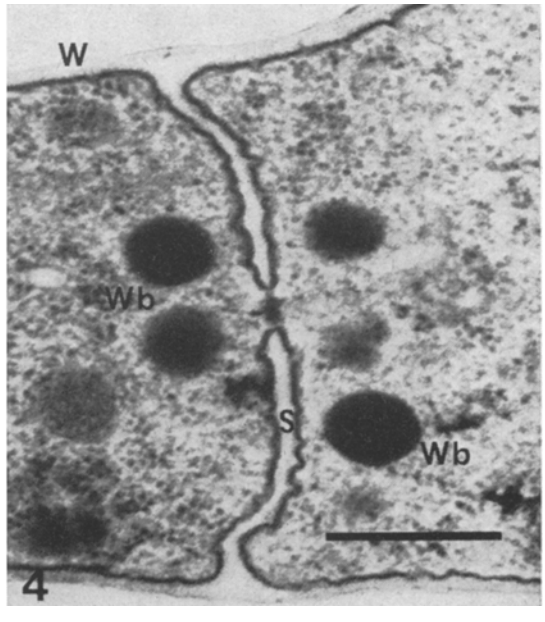

Fig. 4. Longitudinal section showing several Woronin bodies on either side of the septum.

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close to each other is not a sufficient reason to hypothesize a direct developmental relation between them.

Woronin bodies are commonly believed to act merely as plugs in the septal pore, but it is possible that they play a more complex role. In fact, because of their morphology and location, they are very similar to organelles which, based upon their content of hydrolytic enzymes, were identified as lysosomes[30]. The role of plant microbodies has instead been cleared up to a great degree[25,31]. In a heterotrophic organism such as *T. mentagrophytes*, they are very probably involved in lipid metabolism; therefore the term glyoxysomes might be more appropriate for these organelles.

Even if the biochemical function of the two organelles described above may only be verified on the basis of their respective enzymatic content, it is clear, nevertheless, that their morphology will meet the standards generally accepted for Woronin bodies and microbodies only if the cells of *T. mentagrophytes* are prefixed with TAPO. Furthermore, since the techniques used improve the fixation of the entire cell, possibly increasing the penetration speed of the osmic fixation, they can be suggested for a better vision of other internal structures of the dermatophyte which normal fixative processes do not adequately preserve.

Summary. Microbodies and Woronin bodies, organelles surrounded by a single unit membrane, were identified in the hyphal cells of *Trichophyton mentagrophytes* by employing a fixative containing TAPO. The fine structure of the organelles is described and their possible significance discussed.

G. L. Vannini and D. Mares

Istituto di Botanica dell'Università, Corso Porta Mare 2, I-44100 Ferrara (Italy),
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Acidic Nonsteroid Anti-Inflammatory Drugs Accumulating in Inflamed Tissue

A variety of acidic and non-acidic compounds are potent inhibitors of prostaglandin (PG) synthesis in vitro[1-3]. Some of them, namely the nonsteroid anti-inflammatory drugs (NSAID), exert anti-inflammatory action in vivo[1]. However, only the acidic NSAID have gained acceptance in practice[4,5]. Since inhibition of PG-synthesis is now widely believed to be the main target of NSAID in inflammation[6], the observed clinical usefulness of the acidic in contrast to the non-acidic NSAID remains unexplained. Two explanations are possible. Firstly, inhibition of PG-synthesis is not of decisive importance for the anti-inflammatory effect of NSAID, or secondly, only acidic NSAID inhibit PG-synthesis sufficiently in inflamed tissue.

To test the second hypothesis we measured the inhibition of PG-synthesis in vivo at the site of inflammation by acidic and non-acidic pyrazolone and indole derivatives. The results are given in the Table. Although the 2 pyrazolone and the 2 indole derivatives are almost equally effective in inhibiting PG-synthesis in vitro[6], approximately 10 times higher doses are required for the non-acidic compound to achieve the same effect in vivo as the acidic.

These results indicate that besides PG-synthesis inhibition, there must be an additional characteristic of the acidic NSAID which renders them especially active in inflamed tissue. This kind of selectivity might be the result of specific pharmacokinetics leading to selective accumulation and/or biological activity in certain body compartments. There have been speculations that, e.g.

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Acidic and non-acidic pyrazolone and indole derivatives: Inhibition of PG-synthesis and relative drug content in joint fluid

	Acidity	Dose i.v. (mg/kg)	PG $F_2\alpha$ in inflamed joints (% of untreated controls)	Drug content in inflamed joints (% of control joints)	
				after 3 h	after 5 h
Pyrazolone derivatives					
Antipyrine	Alkaline p.Ka 1.4	200	51 ± 26 [a]	82 ± 18	84 ± 33
Phenylbutazone	Acidic p.Ka 4.4	20	39 ± 14 [a]	429 ± 280 [a]	802 ± 198 [a]
Indole derivatives					
Indoxole	Alkaline p.Ka < 2	50	23 ± 13 [a]	102 ± 31	91 ± 28
Indomethacin	Acidic p.Ka 4.2	5	25 ± 22 [a]	350 ± 224 [a]	631 ± 225 [a]

The drugs were dissolved in DMSO and infused slowly (10 min) i.v. at zero time. 1 h later urate crystals (UC) were injected (4% w/v in saline) into the right intertarsal joint of the chicken (2 kg body wt.) the left joint receiving 0.3 ml saline as a control. 3 h later joint washes were performed, the PG $F_2\alpha$ content measured in the UC injected joints as described previously[16] and the drug dependent inhibition of PG-synthesis expressed in percent of DMSO treated controls. On other animals having received the same treatment, the drug content in the inflamed and control joints was measured 3 or 5 h after drug administration by fluorophotometric methods (antipyrine and indoxole)[14,15] or using ^{14}C-labelled drugs. Means and standard deviations of 5 and more experiments are given. [a] $p < 0.01$.