cluded that at least one of any 2 cells connected by a cytoplasmic bridge must be a nurse-cell.

But we have also succeeded in finding synaptinemal complexes in several nurse-cell nuclei of somewhat more developed follicles, in which the oocyte can be distinguished from the nurse-cells by its state of differentiation and its position (Figures 2 and 3).

Older stages sometimes exhibit polycomplex-like structures in oocyte and trophocytes, which might have arisen from synaptinemal complexes after disjunction of the chromosomes[7] (Figure 4).

Discussion. Our light- and electron-microscopic findings strongly suggest that in *all* protrophocytes the homologous chromosomes pair and build up synaptinemal complexes. To this extent the situation described differs from that in *Drosophila*[6]. The differentiation between oocyte and nurse-cells after the construction of synaptinemal complexes demonstrates the germ line nature of Carabid trophocytes. The synaptinemal complexes in protrophocytes seem to have no functional significance. We believe that they may represent rudimentary organelles. This is understandable in view of the phylogenetic origin of the trophocytes from oocytes.

Not only the morphology of the young follicles suggests that the Carabids possess relatively primitive meroistic ovaries. The function of their young follicles is equally primitive. After the breakdown of chromosome pairing the oocyte enters into a lampbrush stage with extremely uncoiled chromosomes[11]. The nucleolus of the oocyte shows a considerable increase in size[12]. The rate of the RNA-synthesis is about twice as great as in nuclei of other tissues with comparable ploidy-step ($4n$)[12]. These and other observations suggest that the initial growth of the oocyte in Carabids is due to RNA which is synthesized in the oocyte nucleus. In a second previtellogenetic phase, the oocyte nucleolus disappears and – somewhat later – the chromosomes condense to form the caryosphere[11] typical of meroistic ovaries. At this point the oocyte growth is completely directed by RNA transfered from the polyploidized nurse-cells to the oocyte cytoplasm[13].

Zusammenfassung. Bei Carabiden erfolgt die Differenzierung zwischen Oocyte und Trophocyten nach der Homologenpaarung und dem Aufbau synaptischer Komplexe in beiden Zelltypen. Zusätzlich zu diesem morphologischen Aspekt deutet auch die Funktion des Oocytenkernes auf einen relativ primitiven Zustand des meroistischen Carabidenovars. In der Initialphase der Oogenese zeigt der Oocytenkern bemerkenswerte Ähnlichkeiten mit den morphologischen und funktionellen Verhältnissen des Oocytenkernes panoistischer Ovarien.

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[1] T. Günthert, Zool. Jb. Abt. Anat. *30*, 301 (1910).
[2] I. Hirschler, Biol. Zbl. *62*, 555 (1942).
[3] A. Verhein, Zool. Jb. Abt. Anat. *42*, 149 (1921).
[4] R. C. King and R. L. Devine, Growth *22*, 299 (1958).
[5] K. Bier, Accad. naz. Lincei *104*, 61 (1967).
[6] E. A. Koch, P. A. Smith and R. C. King, J. Morph. *121*, 55 (1967).
[7] T. F. Roth, Protoplasma *61*, 346 (1966).
[8] M. J. Moses, J. biophys. biochem. Cytol. *2*, 215 (1956).
[9] P. B. Moens, Chromosoma *28*, 1 (1969).
[10] G. Colombo, Rend. Accad. naz. Linzei *22*, 64 (1957).
[11] K. Bier, W. Kunz und D. Ribbert, Chromosoma *23*, 214 (1967).
[12] F. Weber, in preparation.
[13] K. Bier ,Zool. Jb. Physiol. *71*, 371 (1965).

Digestion Process of *Microsporum cookei* Spore Wall by Snail Enzyme: Scanning Electron-Microscopic Examination

Studies of the surface features of fungal wall with the scanning electron-microscope (SEM) have provided much useful information[1-5]. We have been investigating the ultrastructural and immunochemical characteristics of the cell wall of dermatophytes which cause trichophytosis in man[6,7]. Recently, observations by SEM conducted in our laboratory of the surface structure of the hyphal and spore walls of several strains of dermatophytes (*Microsporum cookei* HUT-2061; *Epidermophyton floccosum* TEF-30; *Trichophyton rubrum* T-1005; *T. megnini* 4034; *T. tonsurans* A410, *T. fluviomuniense* 3635)[8] and other non-pathogenic fungi (*Aspergillus niger* 460, *A. oryzae* 216; *Penicillium chrysogenum* 473; *Candida utilis* 553)[9], yielded some interesting results. However, there are no reports in which the surface structures of the cell walls were observed by stereoscan following treatment with mycolytic enzymes.

Materials and methods. Fungus: Cultures of *M. cookei* HUT-2061 were grown on Petri dishes containing Sabouraud's dextrose agar (4% glucose, 1% peptone, 0.5% yeast extract and 1.7% agar), for 4 weeks at 28 °C.

Preparation of spore: The compactly grown cultures, consisting almost entirely of macroconidia, were scraped off the agar medium with a spatula. The spores were freed from the accompanying mycelia by thorough washing with saline followed by centrifugation.

Digestion with snail enzyme: Snail-gut juice of *Helix pomatia*, supplied by L'Industrie Biologique Française, Seine (France), which contains the lytic enzyme complex helicase, was stored as a freeze-dried powder at −15 °C until used. The washed macroconidia were suspended in 0.05 *M* citrate-phosphate buffer (pH 5.8) containing 0.6 *M* mannitol and 0.01% cystein hydrochloride[10], to which 10 mg/ml of snail-gut juice powder was added. Digestion was then carried out by incubation at 28 °C with gentle shaking for varying periods.

[1] S. T. Williams and F. L. Davies, J. gen. Microbiol. *48*, 171 (1967).
[2] D. Jones, Trans. Br. mycol. Soc. *50*, 690 (1967).
[3] L. E. Hawker, Trans. Br. mycol. Soc. *51*, 493 (1968).
[4] H. J. Willets, J. gen. Microbiol. *52*, 271 (1968).
[5] L. E. Hawker and M. A. Goody, J. gen. Microbiol. *54*, 13 (1968).
[6] Y. Ito, T. Setoguti and Y. Nozawa, Japan. J. med. Mycol. *9*, 171 (1968).
[7] Y. Nozawa, T. Noguti, H. Uesaka, T. Hattori and Y. Ito Japan. J. med. Mycol *9*, 258 (1968).
[8] Y. Ito, Y. Nozawa, H. Suzuki and T. Setoguti, Sabouraudia, *7*, 270 (1970).
[9] Y. Ito, Y. Nozawa and T. Setoguti, Mycopath. Mycol. appl., in press.
[10] E. Burger, E. E. Bacon and J. S. D. Bacon, Biochem. J. *78*, 504 (1961).

Scanning electron microscopy: Small samples were withdrawn at intervals and were mounted on the specimen holder with an adhesive Dotite paint (Fujikura Kasei Co. Ltd., Japan). The mounts were dried in the air and coated under vacuum with gold, about 150 Å thick, in the vacuum evaporator JEE-4B[8,9]. Examination was made by a JSM-2 scanning electron-microscope at an accelerating voltage of 10 kV.

Results and discussion. Among the strains of dermatophytes tested, considerable variability in the susceptibility of the walls to digestion by helicase was noted. The spore wall of *M. cookei* HUT-2061 proved to be the most resistant to enzymatic attack (unpublished observation). This could be partly due to the exceptional thickness of the wall (2–5 μ). In addition, the spores from the young cultures (2 weeks) tended to be more susceptible to helicase attack than older ones (4 weeks).

Scanning electron-microscopy of the untreated spore wall showed a uniform outer surface with irregularly dispersed protuberances of variable size (Figure 1). The mechanism and the function of these protuberances are not clarified as yet. However, based upon the following observations of the changes caused by enzymatic attack, it can be strongly suggested that the proturberances play

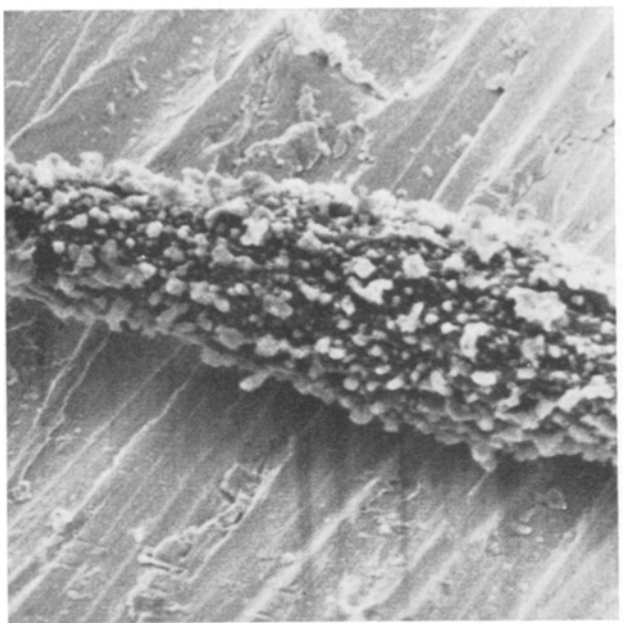

Fig. 1. Untreated spore surface. Note numerous protuberances dispersed at random. × 3000.

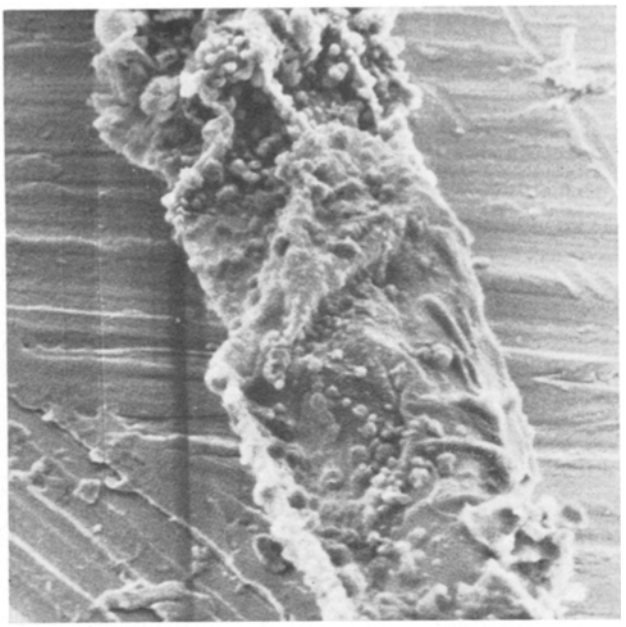

Fig. 3. Spore surface after 8 h treatment. Note reduction in protuberances and many plications. × 3000.

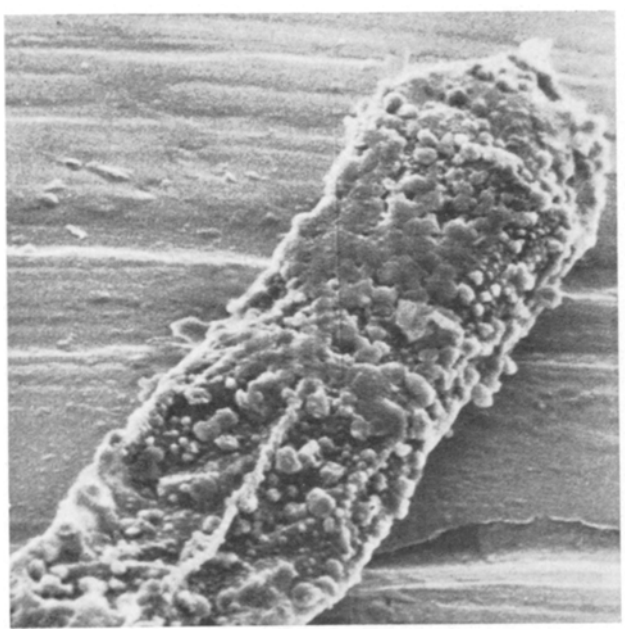

Fig. 2. Surface structure of the spore wall after 4 h treatment with snail enzyme. Note lytic effect on protuberances. × 3000.

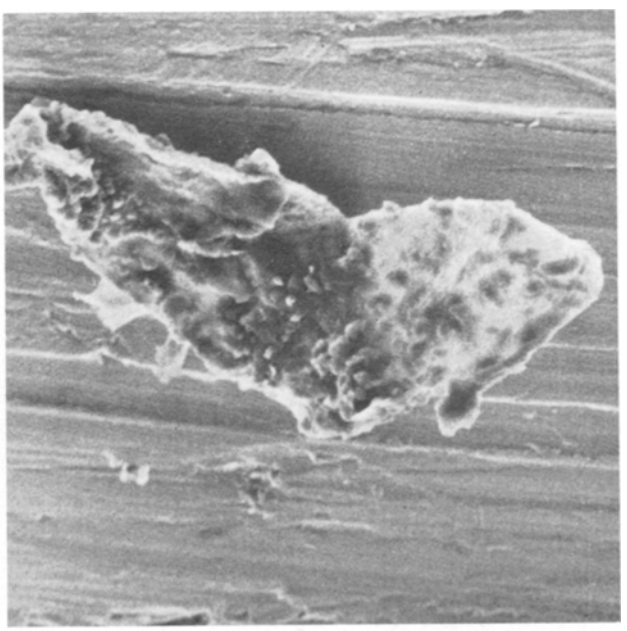

Fig. 4. 24 h treated spore. Note disappearance of protuberances and considerable shrinkage. × 3000.

a certain role in maintenance of spore wall integrity. Treatment of the walls with the snail enzyme reduced the number of these randomly oriented proturberances. Subsequently, the walls were observed to swell and expand (Figures 2 and 3). In addition, many plications appeared on the surface indicating a reduction in rigidity of the spore wall. As shown in Figure 4, further digestion by the enzyme led to the disappearance of proturberances, followed by the shrinkage of the weakened, thin-walled spore.

Observations with the scanning electron-microscope of the process of mycolytic digestion of the fungal cell and spore walls may yield important information about the structure and function of the walls. The SEM is particularly good for these types of studies because sample preparation is simple and rapid and therefore each stage of the lysis process can be observed at high magnification with good resolution. Studies of the chemical composition of the products released by the enzyme treatment are now in progress.

Résumé. Examen au microscope électronique à balayage (scanning) du processus de digestion des macroconidies mûres de *Microsporum cookei* par le suc intestinal d'*Helix pomatia*.

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Separation of Quinone Pigments from *Microsporum cookei* by Thin-Layer Chromatography

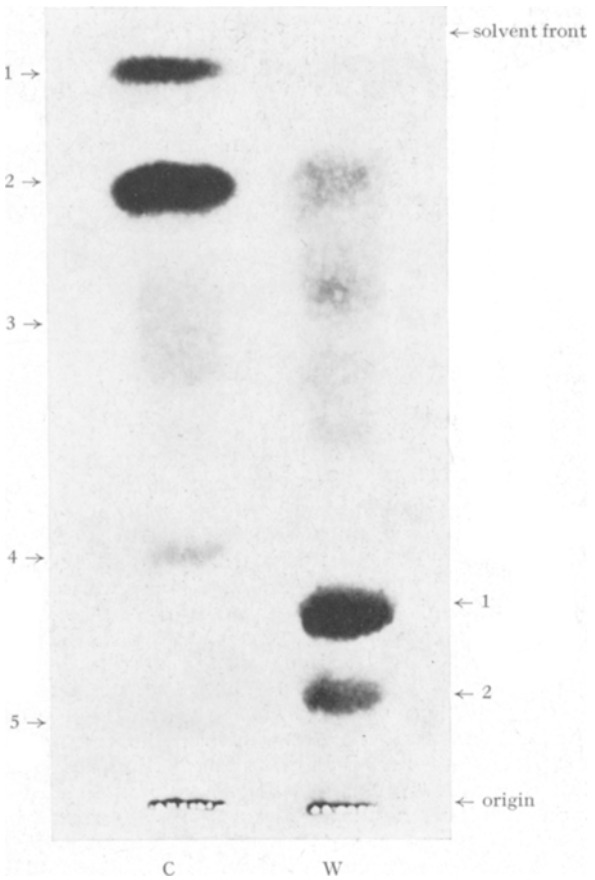
A series of extensive investigations, e.g. intracellular localization[1–4], chemical properties[5,6] and chemotaxonomy[7–10], have been conducted in our laboratory on the pigments produced in mycelia of dermatophytes, mainly *Trichophyton violaceum*, which cause trichophytosis in man. However, little attention has been paid toward the biosynthesis of the pigments, since a suitable fungal strain for this purpose has not been discovered and chromatographic separation of the individual pigments has not been satisfactory. Therefore, an attempt was made to take a preliminary step in this direction by using thin-layer chromatography to separate the pigment components of *M. cookei*. This strain was selected as the organism of choice because, of all the strains tested, it produced higher yields of a greater variety of pigments.

This paper describes thin-layer chromatographic separation of the pigments in mycelium of *M. cookei*.

Materials and methods. Organism and cultural procedure: Culture of *Microsporum cookei* HUT-2061 (from Dr. A. Hasegawa, Department of Veterinary Medicine, University of Tokyo) was grown at 28 °C in 500-ml Ehrlenmeyer flasks containing 125 ml of Sabouraud's dextrose broth (4% glucose, 1% peptone and 0.5% yeast extract). After incubation for 25 days, the mycelium was harvested by filtration, washed thoroughly to remove the adhering medium, and freeze-dried.

Pigment extraction: Freeze-dried mycelium was extracted with hot water until no color was seen. The extract was then filtered. The purple-colored filtrate was adjusted to pH 2.5–3.0 with acetic acid and then extracted with chloroform in a separatory funnel. The chloroform extract was concentrated in vacuo to a brownish purple syrup. From this, the crude pigment (W) was obtained after several washings with petroleum ether to remove contaminating lipid material. The mycelium remaining after this chloroform extraction was exhaustively extracted with chloroform in a Soxhlet apparatus and the resulting chloroform extract was evaporated to yield another crude pigment (C), reddish-violet in color. This substance also was washed with petroleum ether.

Thin-layer chromatography: A suspension of 30 g of silica gel G (Merck) in 50 ml of 0.5*N* oxalic acid solution was ground in a mortar, and 10 ml of distilled water was added. Chromatoplates were prepared by spreading this slurry onto glass plates (5 × 20 cm) to a thickness of 250 μ. After drying at room temperature, the plates were activated at 110–120 °C for 45 min and then stored in a dessicator until used. Aliquots of 2 crude pigments (C, W) were chromatographed on a chromatoplate using benzene-acetone (4:1, v/v) as a developer. The chromatographed



Photograph of thin-layer chromatogram sprayed with 2*N* sodium hydroxide to reveal the pigments extracted from dried mycelium of *M. cookei*. C, crude pigment extracted with chloroform (1 = aurosporin, 2 = xanthomegnin, 3 = violosporin, 4 = citrosporin, 5 = rubrosporin); W, water-soluble pigment extracted with hot water (1 = luteosporin, 2 = iridosporin).