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

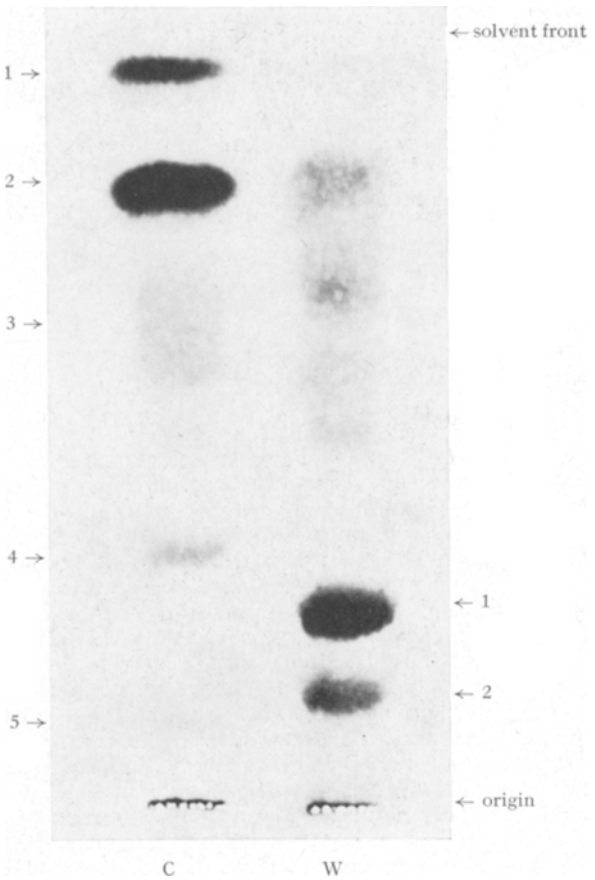
A series of extensive investigations, e.g. intracellular localization[1–4], chemical properties[5,6] and chemotaxonomy[7–10], have been conducted in our laboratory on the pigments produced in mycelia of dermatophytes, mainly *Trichophyton violaceum*, which cause trichophytosis in man. However, little 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).

plate was then sprayed with $2N$ sodium hydroxide solution to define spots more sharply.

Results and discussion. Thin-layer chromatogram of the 2 crude pigments after alkali treatment are shown in the Figure. The crude pigment (C) could be separated into 5 principal components: aurosporin, xanthomegnin, violosporin, citrosporin and rubrosporin. The water-soluble crude pigment (W) yielded luteosporin and iridosporin, with lower Rf values. Rf values and colors of the spots before alkali treatment are summarized in the Table. Upon spraying $2N$ sodium hydroxide on the plate, all of these spots turned to purple in varying intensities and were more sharply defined.

A major component, xanthomegnin, had been first isolated from *Trichophyton megnini* by BLANK et al.[11], and was identified as (–) 3,3′-*bis*[2-methoxy-5-hydroxy-7-(2-hydroxypropyl)-8-carboxy-1,4-naphthoquinone lactone] by JUST et al.[12]. We are performing studies on chemical structures of the other pigments. From their UV- and IR-spectra, it can be proposed that these pigments are quinones, and are analogs of the 1,4-naphthoquinone compound, xanthomegnin.

Rf values and colors of the individual spots before alkali treatment

Pigment	Rf values	Colors of spots
Aurosporin	0.91	pale yellow
Xanthomegnin	0.76	bright yellow
Violosporin	0.58	violet
Citrosporin	0.32	orange
Rubosporin	0.10	reddish orange
Luteosporin	0.23	yellowish orange
Iridosporin	0.14	reddish orange

Such good separation by thin-layer chromatography of the 7 principal pigment components can provide a useful tool for the biosynthetic study of the pigments of *M. cookei* as radioactivity from ^{14}C-glucose incorporated into each pigment can be measured.

Résumé. Le *Microsporum cookei* HUT-2061 qui a une intense pigmentation diffuse s'est montré satisfaisant pour l'étude biosynthétique des pigments quinoniques. Une bonne séparation des composés fut exécutée par chromatographie en couche mince sur gel de silice G.

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Enzymatic Degradation of Human Lipoproteins by Mycoplasmas

The presence of mammalian blood sera is a prerequisite in the growth medium of many mycoplasmas (Pleuropneumonia-like organisms or PPLO). SMITH et al.[1] have isolated the growth-promoting factor present in the sera and characterized it as a lipoprotein. The different moieties comprising this lipoprotein have been found to be required for the promotion of mycoplasmal growth[2,3]. The exact function of lipoprotein in the growth of mycoplasmas is unknown at present, although these organisms possess the peculiar capacity of incorporating the sterol portion into the cell membrane[4]. SMITH and MORTON[5] suggested that 'since the PPLO lack the ability to degrade this protein it is possible that the lipoprotein may be incorporated into the cell intact'. The results presented in this communication constitute an examination of the capacity of various mycoplasma strains to degrade the α_1- and β-lipoprotein fractions of the human serum by the microimmunoelectrophoresis method.

Material and methods. The mycoplasma strains used in this study are listed in the Table. The media and techniques used in the maintenance and propagation of mycoplasmas were essentially the same as suggested by CHANOCK et al.[6] and HAYFLICK[7] and have also been described in detail in an earlier report[8].

SCHEIDEGGER's[9] micro-modification of the immunoelectrophoresis technique originally described by GRABAR et al.[10] has been used to examine the degradation of human lipoproteins by the various mycoplasma strains. The material which was separated electrophoretically on agar consisted of mycoplasmas grown on 'standard PPLO agar' containing 15% unheated human serum instead of unheated horse serum. The human serum used lacked the specific mycoplasma antibody to the human mycoplasmas

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