THE CHEMICAL COMPOSITION OF THE CELL WALLS OF DERMATOPHYTES

by

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INTRODUCTION

The dermatophytes are pathogenic fungi which, in their parasitic phase, live in the dead and keratinized layers of the skin, hair, and nails. This particular habitat makes it attractive to investigate the chemistry of their cell walls and to determine whether the framework of their membranes is made of chitin, cellulose, or another high polymeric substance synthesized from keratin.

MATERIAL AND METHODS

a. *Preparation of the cell walls*

Fifteen species of human and animal origin of the four genera Ctenomyces, Sabouraudites, Trichophyton, and Epidermophyton[3] were cultured in Petri dishes or Erlenmeyer flasks on a modified Sabouraud medium containing 4% crude maltose, 2% Neopeptone "Difco", 0.1% yeast extract, 10% horse serum, and 2% agar[1]. The cultures were killed by autoclaving at 121° C for one hour. The whole mycelium was taken from the warm liquid medium and washed several times with hot water to remove the adhering media, then put on a Buchner funnel, washed further with water, dried at 105–110° C, and ground in a mortar.

The mycelium was then treated as described by SCHOLL[6], by boiling in a twenty-fold volume of water, then filtering through a fritted glass filter. This procedure was repeated several times until the filtrate became colourless. Since most of our material contained non-water-soluble pigments the mycelia were further extracted by boiling them in a ten-fold volume of a 10% aqueous solution of potassium hydroxide for one hour. After boiling the mass was washed with distilled water on a fritted glass filter. This extraction was repeated four times. Afterwards, they were alternately boiled for one hour in a 10% aqueous solution of potassium hydroxide and distilled water; between each boiling the material was washed on a fritted glass filter. This process was repeated four or five times. The residual extracted material was then covered by a 1% aqueous solution of potassium permanganate until the permanganate was completely converted to manganese dioxide. The brownish material was then filtered off and gently warmed with hydrochloric acid (1:40) to dissolve the manganese dioxide. By this procedure, the mass became white or yellowish-white and was washed free of acid on a fritted glass filter. The material was then refluxed in 96% alcohol three times, in ether twice, and dried in vacuo. The material obtained from most of the dermatophytes was white or yellowish-white, loose and fluffy; that from Sabouraudites canis, Trichophyton discoides, T. Schönleini, T. tonsurans, and T. violaceum was of the same colour, but compact, consisting of small round or rectangular particles.

Placed under the microscope, the treated material still showed all the morphological features as before extraction and oxidation.

For further purification[7], the material was dissolved in 40% hydrochloric acid, filtered through asbestos and precipitated by ice-cold water. The gelatine-like precipitate was washed with 25% acetic acid and water. After dissolving the material in concentrated hydrochloric acid, it was precipitated by 20% alcohol, washed with 96% alcohol, then ether, and dried in vacuo.

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b. *Debye-Scherrer diagrams*

Debye-Scherrer diagrams of the material obtained from Ctenomyces farinulentus, Sabouraudites audouini, Trichophyton ferrugineum, and Epidermophyton floccosum were prepared by Dr W. EPPRECHT, Eidgenössische Materialprüfungs- und Versuchsanstalt, Abteilung Röntgenographie, Zürich, Switzerland. The material was put in small acetylcellulose tubes and irradiated by Cu–K-radiation, filtered through a nickel filter to avoid β-rays, for two hours. The films, covered by a thin leaf of aluminium, were used with a camera having a diameter of 114.4 mm.

c. *Nitrogen determination*

The nitrogen content of the material was determined by the Micro-Kjeldahl method using concentrated, nitrogen-free sulphuric acid and a mixture of seven parts potassium sulphate and one part selenium for the digestion of the material. The apparatus designed by PARNAS AND WAGNER[4] was employed for distillation using 30% sodium hydroxide to release the ammonia. The excess $N/70$ hydrochloric acid was titrated with $N/70$ sodium hydroxide using methyl red as the indicator.

RESULTS

a. *Debye-Scherrer diagrams*

Trichophyton ferrugineum (see Fig. 1).

Fig. 1. DEBYE-SCHERRER diagram of *Trichophyton ferrugineum*

Spacings in A-units	*Intensity*
4.66	very strong
3.76	medium strong
3.40	strong
3.07	weak
2.78	very weak
2.57	weak
2.33	weak
2.10	very weak
1.92	very weak

Spacings and intensities of the diagrams of Ctenomyces farinulentus, Sabouraudites audouini, and Epidermophyton floccosum were practically identical with those obtained from Trichophyton ferrugineum.

All four diagrams showing the typical sequence of lines for chitin were identical with diagrams obtained for chitin of cell walls from other fungi as described by FREY[2].

b. *Nitrogen content of the cell walls*

The results of the nitrogen determinations of the extracted and oxidized material as obtained by the SCHOLL method are as follows (Table I).

Since most of the extracted material contained a certain amount of ash and probably other impurities we used the method of ZECHMEISTER AND TÓTH[7] for further purification. Owing to the scarcity of material only the cell membranes of eight species could be purified. The nitrogen determinations gave the following values (Table II).

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TABLE I

NITROGEN CONTENT OF THE CELL WALLS OF DERMATOPHYTES

Ctenomyces farinulentus	5.76%
Ctenomyces granulosus	5.27%
Ctenomyces interdigitalis	5.23%
Sabouraudites audouini	5.46%
Sabouraudites canis	5.76%
Sabouraudites gypseus	6.01%
Trichophyton discoides	4.83%
Trichophyton ferrugineum	5.43%
Trichophyton Megnini	5.67%
Trichophyton rubrum	5.71%
Trichophyton Schönleini	5.53%
Trichophyton soudanense	6.60%
Trichophyton tonsurans	5.76%
Trichophyton violaceum	5.49%
Epidermophyton floccosum (non-pleomorphic)	5.83%
Epidermophyton floccosum (pleomorphic)	6.21%
Boletus edulis[6]	5.98%
Agaricus campestris[5]	6.15%
pure chitin[8]	6.9 %

TABLE II

NITROGEN CONTENT OF THE CELL WALLS OF DERMATOPHYTES AFTER FURTHER PURIFICATION[7]

Ctenomyces interdigitalis	6.63%
Sabouraudites audouini	6.83%
Sabouraudites canis	6.81%
Sabouraudites gypseus	6.69%
Trichophyton discoides	6.62%
Trichophyton Megnini	6.73%
Trichophyton rubrum	6.51%
Trichophyton Schönleini	6.63%
pure chitin[8]	6.9 %

CONCLUSIONS

We have thus proved a) *qualitatively* by the Debye-Scherrer diagrams that the cell walls of the dermatophytes investigated contain chitin. The results of the nitrogen determinations corroborate these findings and show the presence of chitin in the membranes of all the species examined.

The values obtained for the amount of nitrogen in the cell walls using the SCHOLL method indicate the presence of chitin in almost pure state (see Table I); they thus prove b) *quantitatively*, that the cell walls, thus prepared, consist of chitin alone, especially since the material after application of the SCHOLL method was found to have the same microscopic features as before extraction and oxidation.

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The dermatophytes, therefore, in their parasitic phase, use the nitrogen-containing keratin for the synthesis of a nitrogen-containing polysaccharide, chitin, as skeletal material of their cell walls.

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SUMMARY

The mycelia of fifteen species of the genera Ctenomyces, Sabouraudites, Trichophyton, and Epidermophyton were extracted and oxidized as described by SCHOLL in 1908. DEBYE-SCHERRER diagrams of the prepared cell walls show the presence of chitin and give no evidence of the presence of cellulose or another high polymeric substance in the membranes. Nitrogen determinations of the same material corroborate these findings; they prove quantitatively that the framework of the cell walls of the dermatophytes is made of chitin alone.

RÉSUMÉ

Les mycéliums de quinze espèces des genres Ctenomyces, Sabouraudites, Trichophyton et Epidermophyton ont été extraits et oxydés d'après la méthode décrite par SCHOLL en 1908. Les diagrammes DEBYE-SCHERRER des parois cellulaires préparées montrent la présence de chitine et ne donnent aucune indication de la présence de cellulose ou d'un autre polymère. Les déterminations d'azote dans le même matériel confirment ces résultats: elles prouvent quantitativement que la matière de soutien des parois cellulaires des dermatophytes est constituée de chitine seulement.

ZUSAMMENFASSUNG

Die Myzele von 15 Arten der Gattungen Ctenomyces, Sabouraudites, Trichophyton und Epidermophyton wurden extrahiert und oxydiert nach der im Jahre 1908 von SCHOLL beschriebenen Methode. DEBYE-SCHERRER-Diagramme der präparierten Zellwände zeigen, dass Chitin in der Zellwand vorkommt; für das Vorhandensein von Zellulose oder einer anderen hochpolymeren Substanz sind keine Anzeichen zu finden. Diese Befunde werden durch Stickstoffbestimmungen im gleichen Material bestätigt; sie beweisen quantitativ, dass das Gerüst der Zellwand bei den Dermatophyten allein aus Chitin besteht.

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