CHEMICAL AND IMMUNOLOGICAL STUDIES ON DERMATOPHYTE CELL WALL POLYSACCHARIDES*

EARL G. McNALL, PH.D., THOMAS H. STERNBERG, M.D., VICTOR D. NEWCOMER, M.D. AND LLOYD J. SORENSEN, PH.D.

Investigations on dermatophyte infections in man and experimental animals have provided considerable evidence relating to the various stages of such infections. (1–6) The allergy and associated immunity which has been reported in these infections have been extensively studied in a wide variety in dermatophyte infections in several species of experimental animals. (7–9) Some investigators propose that the resistance which occurs following an initial dermatophyte infection is local, residing only in the vicinity of the cured lesion, while others state that there is no such localization under these conditions. (7–9)

The onset of hypersensitivity to the fungus and/or products liberated by the fungus in the parasitized skin usually occurs prior to the clearing of the infection. (9–10) This hypersensitivity may take place not only during the course of the infection but in response to the injection of live fungus or after injection of dead fungus or material extracted from the fungus. (11–14) Partial immunity to dermatophyte fungi have been reported in some cases after prior treatment of animals or man with these preparations. (13–14)

This paper reports the isolation, chemical characterization and immunological studies on the high molecular weight polysaccharide derived from the outer wall of *Trichophyton mentagrophytes*.

METHOD

PREPARATION OF *T. Mentagrophytes* AND *T. Rubrum* P I X POLYSACCHARIDES

Mycelia of *Trichophyton mentagrophytes* and *T. rubrum* were grown in mycophil broth on a rotary shaker at 37° C. under highly aerobic conditions and repeatedly transferred to achieve rapid growth. Mycelia were harvested during the log phase of growth, heat killed and washed with distilled water. Ten grams wet weight of the mycelia were suspended in 200 ml of normal sodium hydroxide and autoclaved at twenty pounds pressure for twenty minutes, centrifuged, and washed with distilled water until the supernatant no longer contained detectable quantities of amino acids or protein. The mycelia were again suspended in sodium hydroxide, the entire sequence repeated for three cycles, and the precipitate was then treated with 200 ml of 5% trisodium phosphate and 0.1% ethylene diamine tetra-acetic acid to remove residual metal ions and nucleotides. Following extensive washing with distilled water, the mycelia were refluxed successively in two 500 ml quantities each of 95% ethanol, ethanol-acetone (50-50), and petroleum ether to remove residual flavins and lipid and dried from absolute ethanol. These polysaccharide fractions were dried in a desiccator over P_2O_5.

IMMUNIZATION AND DETERMINATION OF ANTIBODY

Twenty randomly bred hybrid mature chinchillas weighing approximately 500 grams were injected with 0.5 mg/kg of body weight of *T. mentagrophytes* P I X polysaccharide in 0.25 ml of Freund's complete adjuvant using the intramuscular route. Blood samples were obtained at 0, 10, and 24 days following injection, allowed to clot for six to eight hours at 5° C., centrifuged at approximately 1,500 × g and the clear non-hemolyzed serum was removed and frozen in a mechanical deep freeze at minus 20° C. Quantitative precipitin determinations were performed on each sample.

CHEMICAL CHARACTERIZATION OF *T. Mentagrophytes* P I X POLYSACCHARIDE

The molecular weight of *T. mentagrophytes* P I X polysaccharide was determined by assaying

* From the Division of Dermatology, Department of Medicine, University of California Medical Center, Los Angeles 24, California.

This investigation was supported in part by the Research and Development Division, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-694 and USPHS Grant, #E-1478.

Presented at the Twenty-first Annual Meeting of The Society for Investigative Dermatology, Inc., Miami Beach, Florida, June 15, 1960.

TABLE I

Chromatographic Identification of T. Mentagrophytes *Residues*

Solvent System	Rf Sample	Rf Authentic Compound	Compound
Butanol-ethanol	0.42	0.42	Glucose
H_2O 5:1:4	0.38	0.39	Glucosamine
Pyridine-Butanol	0.61	0.61	Glucose
H_2O 4:6:3	0.56	0.56	Glucosamine
H_2O Saturated	0.67	0.68	Glucose
Collidine	0.54	0.54	Glucosamine

TABLE II

A Summary of the Chemical Characteristics of T. Mentagrophytes *P I X Polysaccharide*

Glucosamine	0.97 ± 0.040 residues
Glucose	2.02 ± 0.033 residues
Nitrogen (as glucosamine)	332 mg/gram
Molecular wt. (reducing ends)	1/253 residues
Ash	0.37%
Non Reducing Ends	1/8 residues

the number of reducing ends utilizing the ferricyanide reduction assay. Chromatography of the acid hydrolyzed polysaccharide was performed utilizing circular chromatography technic using a variety of solvent systems. Hydrolysis of the polysaccharide was carried out by pretreatment with 80% sulphuric acid at 0° C. for ten to twelve hours followed by rapid dilution in the cold to a final concentration of six normal after which the polysaccharide was hydrolyzed at 100° C. until the optical rotation was constant. The acid was neutralized by adding solid barium carbonate until a pH of 6 to 7 was attained. The glucosamine in the acid hydrolysate was determined by a modification of the method of Elson and Morgan. (15) The non-reducing ends were assayed by determining the quantity of formic acid liberated following treatment of the polysaccharide with periodate. Total nitrogen was determined by the Kjeldahl method.

RESULTS

Glucosamine and glucose were identified as the constituent sugar residues comprising polymer P I X (Table I). The molecular weight, ash content, total nitrogen analysis, the number of

TABLE III

Antibody Production Following Immunization Injection

Time (Days)	Antibody (Mg)/Ml Serum		
0	>0.05	>0.05	>0.05
10	2.05	2.22	2.81
24	3.71	3.50	3.62

TABLE IV

Quantitative Precipitin Reaction

Polysaccharide Added	Antibody Precipitated (*T. Mentagrophytes* Polysaccharide)	Antibody Precipitated (*T. Rubrum* Polysaccharide)
(mg)	(mg)	
0.04	1.95	0.8
0.08	3.22	1.11
0.16	3.65	1.20
0.20	3.60	1.22
0.24	3.50	1.22

non-reducing ends and the ratio of glucosamine to glucose in *T. mentagrophytes* P I X polymer as shown on Table II. The amount of glucosamine present accounts for the quantity of nitrogen obtained by Kjeldahl analysis. The ratio of glucose to glucosamine approximates two to one. Since one in eight sugar residues in the polymer were detected as nonreducing ends, this polysaccharide must be highly branched.

The time course of antibody production following the immunization injection of polysaccharide is shown on Table III. In normal, non-injected chinchillas no antibody was detected, however by the tenth day post immunization, appreciable quantities of precipitin antibody could be demonstrated. By the 24th day maximal antibody titers on the order of 3.6 mg/ml of serum were observed. (Table III) Antibody determinations utilizing the quantitative precipitin reaction are shown on Table IV. The zone of large antigen excess did not appreciably decrease the total amount of antibody precipitated at the highest quantity of polysaccharide used in the assay. In order to determine whether cross reactions occur with the polysaccharide derived from *T. rubrum*, analogous quantitative precipitin tests were performed and it was apparent from the results that approximately ⅓ of the antibody produced against *T. mentagrophytes* polysaccharide cross reacted with *T. rubrum* P I X polysaccharide.