

Immunology of Dermatophytes and Dermatophytosis

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INTRODUCTION	222
IMMUNITY IN DERMATOPHYTOSIS	223
Acquired Resistance	223
Animals	223
Humans	224
Hypersensitivity ("Trichophytin" Reaction)	225
Dermatophytid Eruptions	227
Antibodies	228
Animals	229
Humans	230
Nonspecific Resistance	231
IMMUNOCHEMISTRY OF DERMATOPHYTES	232
Chemical Composition	232
Antigenic Composition	233
Differentiation of species	233
Hypersensitivity factors ("trichophytin")	234
Purified Antigenic Components	234
Isolation of antigens	234
Glycopeptides	234
Polysaccharides	235
Keratinases	235
Chemistry of antigens	235
Glycopeptides	235
Polysaccharides	236
Keratinases	237
Immunological reactivity	237
Relation to structure	237
Taxonomic importance	239
IMMUNOPROPHYLAXIS AND THERAPY	240
Animals	240
Humans	240
SUMMARY	241

INTRODUCTION

The dermatophytes include species of the genera *Epidermophyton*, *Microsporum*, and *Trichophyton*. These fungi cause superficial infections known as dermatophytosis (ringworm) in humans and animals. Dermatophytes usually invade and parasitize only the keratinized layers of skin, nail, and hair. This highly developed host-parasite relationship is responsible for a multitude of clinical manifestations of dermatophytosis. However, the disease process is greatly influenced by the host response to the dermatophyte infection.

Sabouraud's (198) fundamental studies on the structure and taxonomy of the dermatophytes and their relationships to clinical manifestations of dermatophytosis laid the basis for subsequent studies on changes induced in

the infected host. These investigations began with the work of Plato and Neisser in 1902 (173) on "trichophytin" sensitivity, which was followed by the work of J. Jadassohn and B. Bloch on the manifold aspects of the immune response in man and experimental animals. The early work on the immune response of the host was summarized by B. Bloch in 1928 in German (29). Since then, Götze (75) has reviewed the literature in detail in German, and Lepper (144) has published a concise review in English.

Other reviews which have covered immunological aspects of dermatophytosis are those by Sulzberger (217), Kligman and DeLamater (132), Huppert (93), and Salvin (202).

This review includes selected references from both the earlier and more recent literature in order to illustrate the variety of immune phe-

nomena which occur in dermatophytosis, the antigenic composition of the dermatophyte group, and the status of immunoprophylaxis and therapy for dermatophytosis.

No attempt has been made to change the nomenclature of the dermatophytes as used by the authors.

Synonyms are listed below with perfect states in parentheses (Table 1).

IMMUNITY IN DERMATOPHYTOSIS

Acquired Resistance

A state of relative acquired resistance to reinfection with the same or another species of dermatophyte may result from cutaneous infection. This resistance may vary both in degree and duration, depending upon several factors, including the species or strain of dermatophyte (zoophilic or anthropophilic), the host (animal or human), and the site of infection (smooth skin, hair, or nails).

In general, the zoophilic species cause more inflammatory infections which may heal spontaneously and result in relative resistance to reinfection. The anthropophilic species usually cause more chronic, less circumscribed infections which result in less resistance to reinfection.

Animals. The characteristics of the primary infection in experimental infections of several animal species by a variety of dermatophyte species have been reviewed by DeLamater and Benham (52).

Bloch (28) was the first to show that cutane-

ous inoculation of guinea pigs with the zoophilic dermatophytes *Achorion quinckeanum*, *Trichophyton gypseum*, or *Microsporum lanosum* produced infections which healed spontaneously and resulted in a relative resistance to subsequent reinfection. The phenomenon was later called "le phénomène de la réaction accélérée" or "le phénomène de Bruno Bloch" (193). The resistance resulting from the first infection was manifested by an accelerated inflammatory response and course of disease in which fungi were not always demonstrable. Bloch claimed that this immunity, although not usually complete, was generalized and not confined to the site of the primary infection. The immunity was apparent 7 to 9 days postinoculation and persisted for 18 months. It was not species-specific. Inoculation of the host by subcutaneous or intraperitoneal injection of the fungus also altered the susceptibility of the skin to cutaneous infection, but to a lesser degree.

This initial report was later confirmed, modified, and expanded by Bloch and Massini (31), Thardshimanjanz (225), and several other investigators. Hanawa (88) claimed that an acceleration of the disease process in reinfected animals was detectable as soon as 2 to 3 days after the first infection and was reflected in the histopathological changes seen in biopsies of the site. A longer time interval between the primary inoculation and the reinfection resulted in a greater modification of the second infection.

Sutter (222), Greenbaum (87), and Kogoj (134) claimed that a local immunity was produced in guinea pigs after a cutaneous infection

TABLE 1. Nomenclature of the dermatophytes

Name	Synonym
<i>Achorion gypseum</i>	<i>Microsporum gypseum</i> (<i>Nannizzia incurvata</i> and <i>N. gypsea</i>)
<i>Achorion quinckeanum</i>	<i>Trichophyton</i> or <i>Microsporum quinckeanum</i>
<i>Achorion schoenleinii</i>	<i>Trichophyton schoenleinii</i> or <i>Trichophyton schönleinii</i>
<i>Epidermophyton Kaufmann-Wolf</i>	<i>Trichophyton mentagrophytes</i> var. <i>interdigitale</i> (<i>Arthroderma benhamiae</i> ?)
<i>Microsporum lanosum</i>	<i>Microsporum canis</i>
<i>Microsporum praecox</i>	<i>Sabouraudites praecox</i>
<i>Trichophyton asteroides</i>	<i>Trichophyton mentagrophytes</i> var. <i>granulosum</i> (<i>Arthroderma benhamiae</i>)
<i>Trichophyton faviforme</i>	<i>Trichophyton verrucosum</i>
<i>Trichophyton ferrugineum</i>	<i>Microsporum ferrugineum</i>
<i>Trichophyton granulosum</i>	<i>Trichophyton mentagrophytes</i> var. <i>granulosum</i> (<i>Arthroderma benhamiae</i>)
<i>Trichophyton gypseum</i>	<i>Trichophyton mentagrophytes</i> var. <i>granulosum</i> (<i>Arthroderma benhamiae</i>)
<i>Trichophyton interdigitale</i>	<i>Trichophyton mentagrophytes</i> var. <i>interdigitale</i> (<i>Arthroderma benhamiae</i> ?)
<i>Trichophyton purpureum</i>	<i>Trichophyton rubrum</i>
<i>Trichophyton sabouraudii</i>	<i>Trichophyton tonsurans</i> var. <i>sabouraudii</i>
<i>Trichophyton sulfureum</i>	<i>Trichophyton tonsurans</i> var. <i>sulfureum</i>

with *A. quinckeanum*, *T. gypseum*, or *M. lanosum* which was complete only at the site of the spontaneously healed infection, but not in other areas, where a second infection resembled the first one.

Rivalier (193) avoided trauma to the skin prior to cutaneous inoculation of guinea pigs. He used *Trichophyton granulosum* and showed that an autoclaved spore suspension applied 3 weeks after the first infection could induce the same cutaneous reactions, although less pronounced, as a second infection with live spores. Thus, the altered host response, rather than the proliferation of the fungus, appeared to be responsible for the accelerated cutaneous reactions seen in a second infection.

DeLamater and Benham (53) studied the dermatophytic infection in guinea pigs extensively and characterized the acquired immunity to several strains of dermatophytes. These investigators concluded that in the guinea pig: (i) a cutaneous infection by a virulent strain, whether infection occurred via the skin or the blood stream, altered the animal's subsequent response to infection; (ii) the lesion caused by a second infection resembled the first one, but developed and healed more rapidly and rarely contained demonstrable fungi; and (iii) the resistance was greatest at the previously infected site.

These observations in guinea pigs were confirmed by Bonk et al. (33) who also showed that virulent strains of both zoophilic and anthropophilic forms of *Trichophyton mentagrophytes* induced resistance which was greatest at the previously infected site.

DeLamater and Benham (53) showed that the resistance reached a peak 2 to 3 weeks after healing of a first infection and corresponded to the development of delayed cutaneous hypersensitivity which also peaked at that time. By repeated inoculations of guinea pigs, Catanei (39) showed that the maximal resistance to reinfection was obtained after a second infection.

Several investigators (53, 155, 182) infected pregnant guinea pigs with dermatophytes and found that the acquired resistance to infection was not passed on to the offspring.

In rabbits and cats, the course of the second infection resembled that of the first one. No evidence was obtained for immunity or cutaneous sensitization after one infection. Resistance and cutaneous sensitization were apparent after more than one infection in rabbits (53). Reiss and Leonard (186) reported that reinfection of dogs, cats, and rabbits resulted in a shorter

course of infection as compared with the first infection. No uniform cutaneous sensitization was associated. Kligman (131) found no evidence of acquired resistance or sensitization to dermatophytes in mice and rats.

Kielstein (123) and Lepper (145) studied the development of resistance in cattle by experimental infections with *Trichophyton verrucosum*. The primary infection healed spontaneously, and cattle became resistant to cutaneous reinfection both on previously infected sites and noninfected areas. The resistance persisted for up to 1 year or more after recovery. Lepper (145) observed that although the infection rate was the same, the inflammatory response and the resolution of lesions were more rapid in older animals. Elimination of the infection was associated with a marked, delayed hypersensitivity reaction.

Humans. Bloch (28) and Bloch and Massini (31) were the first to show that a dermatophytic infection in humans results in a relative resistance to subsequent infection. They infected smooth skin with a highly virulent strain of *A. quinckeanum*, a zoophilic species, which caused inflammation, kerion-like infiltration, and scutula formation. The regional lymph nodes became enlarged, and the infection healed spontaneously in 3 months. The infection, even with this zoophilic species, was less circumscribed and more chronic than in guinea pigs. Reinfection resulted in a highly accelerated, less severe course of disease. No fungi were isolated, and there was no scutula formation. These investigators claimed that the acquired resistance was not confined to the site of previous infection, but was generalized. The resistance was not species-specific.

Subsequent investigations have shown that, in humans, increased resistance usually follows the severe inflammatory forms of infection such as kerion formation, usually caused by zoophilic species, but does not always follow the more chronic infections caused by anthropophilic species (12, 194). Barlow and Chattaway (12) pointed out that fungi which do not invade the hair follicle do not seem to give rise to an equivalent immunity when growing in the horny layer of the smooth skin.

Kligman (130) infected the scalp of children by using hairs infected with *Microsporum audouinii*. Upon reinfection, only two of seven children developed lesions. These lesions were of a trivial nature and of shorter duration.

Resistance to reinfection was also demonstrated in natural infections. Although children with tinea capitis returned to heavily infected

environments after cure, they rarely became reinfected (66, 232).

In contrast, Desai et al. (54) could not demonstrate such acquired immunity in experimental *Trichophyton rubrum* infection of smooth skin. The second infection resembled the first one both in extent and duration.

Although Bloch's early work indicated that acquired resistance is generalized, other investigators disagreed and demonstrated a strong local immunity only at the previously infected site and not in skin areas distant from it (87, 222). They concluded that experimental infection of the smooth skin by the zoophilic species, *A. quinckeanum*, *M. lanosum*, and *T. gypseum*, produced only a local tissue immunity in humans. The immunity was accompanied by cutaneous sensitization. Epstein and Grünmandel (60) also demonstrated a localized immunity in experimental superficial infections. The immunity extended to a distance of up to 20 mm surrounding the focus and was manifested by failure of the reinfection to take. Resistance was demonstrated in almost healed or cured foci only and lasted 3 months.

Hypersensitivity ("Trichophytin" Reaction)

The "trichophytin" reaction is the term used for cutaneous hypersensitivity to dermatophyte antigens injected intradermally in humans or experimental animals. Both immediate- and delayed-type reactions occur, but the latter is most often associated with infection.

Studies on hypersensitivity in dermatophytosis started in 1902 when Neisser published the work of his late assistant Plato on the preparation and usefulness of "trichophytins" (173). Plato grew *Trichophyton* species isolated from patients with deep-seated trichophytosis in a liquid medium consisting of beef extract, peptone, and maltose. After 2 to 3 months at room temperature, the growth was ground and filtered. Plato called the filtrate "trichophytin."

Plato was unable to infect rabbits with dermatophytes. Injection of "trichophytin" into noninfected rabbits did not cause any irritation at the site of injection, nor did he observe a rise in temperature. However, subcutaneous injection of mycelium into the rabbit's ear, followed 4 days later by "trichophytin" injection, resulted in an elevated temperature.

In patients with deep-seated trichophytosis, Plato (173) found that parenteral injection of "trichophytin" caused signs and symptoms analogous to those induced in tuberculous patients by injection of tuberculin: general toxic reactions including elevated temperature, per-

spiration, loss of appetite, headache, and pain in the joints. There was inflammation, formation of pustules, and burning at the injection site. "Trichophytin" did not give a tuberculin-like reaction in tuberculous patients, and patients with superficial dermatophytosis did not react to tuberculin. Plato, therefore, regarded the "trichophytin" reaction as specific.

Bloch and Massini (31) showed that experimental trichophytosis induced hypersensitivity to "trichophytin" in both humans and in guinea pigs. In humans, the cutaneous reactivity to "trichophytin" became apparent 7 to 10 days after infection. After healing, the reaction remained positive for up to 3 years. The induced hypersensitivity was specific for dermatophytes, but was not species-specific. An infection due to *T. gypseum* caused hypersensitivity not only to "trichophytin" prepared from this dermatophyte, but also to favin, prepared from *A. schoenleinii*, and to microsporin, prepared from *M. lanosum*.

Sutter (222) showed that the level of cutaneous sensitivity which developed in guinea pigs was much lower than in humans. Subcutaneous injection of infected patients with "trichophytin" regularly caused a general reaction including fever and leukocytosis. However, large doses injected into sensitized guinea pigs did not cause a rise in temperature or other toxic reactions. The reaction was as specific as in humans and characterized by the absence of general toxicity. The guinea pigs reacted only if "trichophytin" was injected intradermally (225). The "trichophytin" reaction was characterized by a lentil-shaped, large, inflamed papule with an erythematous halo at 24 h, after which it decreased. Bloch (29) pointed out that the intensity of the reaction varied considerably. He considered the erythematous halo around the papule very specific. Thardshimanjan (225) and Sutter (222) found that the "trichophytin" reaction became positive 7 to 9 days after infection, corresponding to the height of infection. It reached its maximum when the infection subsided.

In humans, repeated inoculation with the same dermatophyte led to a state of hyposensitization or anergy. Both dermatophyte infections and "trichophytin" injections produced decreased inflammatory reactions at previously infected sites (222).

The development of delayed cutaneous hypersensitivity during the course of cutaneous infection with *T. gypseum* was later studied in guinea pigs by DeLamater (50, 51). The "trichophytin" reaction peaked at 15 days after in-

oculation and then started to regress. It increased again 2 to 3 weeks after clearing of the infection, then decreased sharply and remained at a low level for several months. The site of previous infection was relatively anergic as manifested by a reduced inflammatory reaction to "trichophytin." The ability to react to "trichophytin" increased with age, being greatest in adult and least in newborn guinea pigs. There was considerable variation in degree of sensitivity in the adult group. The younger animals were more susceptible to progressive infection.

Cruickshank et al. (47) showed that the delayed hypersensitivity reaction to "trichophytin" could be transferred to noninfected guinea pigs with peritoneal exudate cells of sensitized donors.

Immediate forms of hypersensitivity have also been demonstrated in experimental animals. W. Jadassohn and associates (108, 109) reported anapylactic and smooth muscle sensitization to "trichophytin" in infected guinea pigs. Cruickshank et al. (47) detected immediate cutaneous reactions to "trichophytin" in guinea pigs infected with *T. mentagrophytes* by using Evan's Blue dye. Ito and Nishitani (99) showed that repeated intravenous injection of rabbits with *T. mentagrophytes* var. *asteroides* caused a systemic vascular reaction comparable to allergic vasculitis in humans.

According to Jaksch (111) and Kielstein (121), naturally infected horses and cattle also develop cutaneous hypersensitivity. Lepper (145) studied the development of cutaneous delayed hypersensitivity in cattle experimentally infected with *T. verrucosum*. The ability to eliminate infection was associated with the development of a marked delayed hypersensitivity response in four of six cattle 14 days after inoculation. A mild delayed-type reaction developed on all reinoculated skin sites within 48 h. The intravenous injection of 10^4 viable units of *T. verrucosum* resulted in an immediate skin reaction at the original site of infection. The animals also had elevated temperatures. These reactions disappeared 24 h after injection.

Neves (175) found mainly delayed-type cutaneous hypersensitivity to "trichophytin" in human volunteers infected with different species of dermatophytes. The degree of sensitization was greater in individuals in whom lesions became more inflammatory. He did not find a high incidence of immediate-type reactivity, even in cases of chronic *T. rubrum* infection.

The development of cutaneous hypersensitivity in an experimental *T. rubrum* infection in

humans was reported by Ito (96). The delayed cutaneous reaction developed by 14 days after inoculation. It disappeared after 35 days, at which time an immediate-type urticarial reaction developed.

Patients with chronic *T. rubrum* infections frequently have a negative delayed-type or a retarded delayed-type "trichophytin" reaction. Immediate-type "trichophytin" reactions and circulating antibodies are found more often in these patients than in those infected with other dermatophytes (55, 149, 217, 244). The retarded delayed-type reaction was often found associated with dermatophytid eruptions (46, 244).

Immediate-type reactions to "trichophytin" have been found to be exceptionally prevalent in patients infected with *T. rubrum*. Desai et al. (55) found these reactions in as many as 85% of infected adults. However, this immediate-type urticarial reaction occurred both in patients infected by other dermatophytes and in noninfected individuals with a similar lower frequency (244). It has also been observed in atopic individuals (154), in individuals with recurrent erysipelas-like eruptions (219), in individuals with recurrent lymphangitis (115), and in patients under treatment with "trichophytin" (220).

Sulzberger and Wise (220) and later Jillson and Huppert (115) showed that the delayed-type, but not the urticarial reaction, was suppressed by repeated intradermal injections of "trichophytin." If the urticarial reaction was absent prior to treatment with "trichophytin," the immediate reaction appeared at the time the delayed reaction disappeared. A constant supply of antigen over a long period of time was considered to be important for the induction of the immediate-type reaction to "trichophytin."

Sulzberger and Kerr (218) showed that the immediate-type reaction to "trichophytin" in humans could be transferred by serum, indicating a circulating antibody reaction. It might also be mentioned that Sulzberger (216) observed that injection of "trichophytin" into sensitive subjects often provoked allergic reactions such as asthma and rhinitis, also associated with circulating reaginic antibodies.

Lewis and Hopper (147) compared "trichophytin" reactions in patients with *T. rubrum* and *T. mentagrophytes* infections. Of patients with *T. rubrum* infections, 53% had immediate-type reaction only, 10% delayed-type reaction only, 32% both reactions, and 5% no reactions; of patients with *T. mentagrophytes* infections, 1.5% had immediate-type reaction, 72.9% de-

layed-type reaction, 1.5% both reactions, and 10.6% no reactions. *T. rubrum* infections, in which the immediate-type reaction predominates, are more recalcitrant and resistant to treatment than *T. mentagrophytes* infections in which the delayed-type reaction predominates.

By using a "trichophytin" purified from *T. mentagrophytes*, Jones et al. (116) found no specific association between immediate-type reactions and *T. rubrum* infection. However, they did find a strong correlation between immediate-type reactivity and chronic infection. Although 7.0% of individuals with only delayed-type hypersensitivity had recurrent infections, 75% of those with both immediate- and delayed-type hypersensitivity or only immediate-type hypersensitivity had recurrent infections.

The appearance of the delayed reaction hypersensitivity is closely associated with the accelerated response to reinfection in both animals and humans (183).

Balogh et al. (6) reported a correlation between the results of "in vitro" lymphocyte transformation tests and the spread of the disease and sensitization of patients with dermatophytosis.

The lack of either standardized or pure, homogeneous "trichophytins" for skin testing has severely limited the usefulness of the "trichophytin" reaction for diagnostic purposes. Wilson (242) also pointed out that the "trichophytin" reaction cannot establish or exclude the presence of dermatophytosis because it may never develop or may persist long after the infection is cured. Commercial "trichophytins" are composed mainly of pooled, heat-treated, concentrated culture filtrates of from 1 to 15 species. There are more than 10 "trichophytins" available which vary in their manufacturing process (208). These crude preparations usually contain antigens common to other microorganisms as well as dermatophytes.

Although the delayed reaction may be most reliably associated with dermatophytosis, some investigators have reported reactions in individuals with other sensitivities, including penicillin and tuberculin (181, 206). In addition, W. Jadassohn (105) reported cross-reactions in as high as 90% of noninfected individuals.

Dermatophytid Eruptions

A dermatophytic infection in humans may result in the production of secondary skin eruptions. They occur in a large variety of clinical forms and are known as dermatophytids, epidermophytids, favids, microsporids, trichophy-

tids, or "ids." The lesions occur at a distance from the focus of infection. Fungal elements are not usually demonstrable. The "trichophytin" test is always positive. The secondary eruptions disappear after successful treatment of the primary infection.

J. Jadassohn (102) was the first to recognize these lesions as allergic reactions of the skin sensitized by the process of dermatophytosis. He chose this term in analogy to Darier's tuberculid after he had originally called this allergic manifestation "lichen trichophyticus."

Williams (241) reviewed the early work of many investigators who demonstrated the occurrence of fungi in the blood of individuals with dermatophytid eruptions. Mycelia were very rarely found in dermatophytid eruptions, but could be demonstrated in fresh lesions less than 1 or 2 days old.

The "id" lesions are believed to occur as a result of the hematogenous spread of fungi or their allergenic products from the primary focus of infection. The reaction between circulating antigens and skin-sensitizing antibodies is believed to be responsible for the skin manifestations.

According to Bloch (29), the dermatophytid reaction appears at the height of infection or shortly thereafter due to a massive liberation of antigens. The eruptions may last from 5 to 20 days. Dermatophytids often occur after X-ray treatment, "trichophytin" skin tests, or local irritations.

Bloch did not believe that lymphatic spread of the antigens was of great importance. Transport of the antigens by the blood stream would explain (i) the sudden appearance of eruptions at different parts of the body or the whole integument including mucosa, (ii) the symmetrical distribution of the dermatophytids, (iii) the episodic eruption, (iv) fever, (v) changes in the blood picture, (vi) the occurrence of subcutaneous dermatophytids, and (vii) the analogy with tuberculids and syphilids.

The fungistatic components of blood and the body temperature, not optimal for the growth of dermatophytes, may explain why fungal elements are only very rarely found in dermatophytid lesions (75). However, the time at which the presence of fungal elements is investigated is of utmost importance. Experiments with guinea pigs showed that spores penetrate the blood stream from 1 to 48 h after cutaneous infection (103). Sulzberger (215) observed a second phase of penetration of fungal elements into the blood at the height of infection 9 to 13 days after cutaneous inoculation of guinea pigs.

Dermatophytid reactions are most often seen in humans at the height of infection.

The histopathological changes seen in biopsies of dermatophytids resemble those observed in tuberculids and have been described in detail by Gans and Steigleder (71) and by Montgomery (170). Sulzberger (217) grouped these polymorphous skin manifestations into 17 different forms, and Götz (75) classified them into epidermal, cutaneous, subcutaneous, and vascular forms.

J. Jadassohn (102) observed dermatophytids mainly in children with kerion Celsi and less frequently in adults with sycosis barbae. The dermatophytids were located mainly on the trunk and more rarely on the extremities and face. They consisted of small, pale to intensely red, follicular nodules which were disseminated or arranged in groups. Very fine, spiny projections or pustules sometimes appeared on the nodules. In other cases, plaques resembling seborrheic dermatitis were observed as well as reddening of the oral mucosa. Scarlatinoid eruptions were seen at the onset of dermatophytids. More rarely, nodules resembling erythema nodosum were found on the lower extremities. The general health of the patients was also affected. High fever, changes in the blood picture (leukocytosis and lymphocytosis), lymphadenopathy, splenomegaly, and involvement of the joints accompanied the acute onset of the dermatophytids. Even cases of anaphylaxis have been reported (75).

Although Jadassohn's cases were mainly due to zoophilic dermatophytes, subsequent reports also implicated anthropophilic species. Bloch (29) believed that these anthropophilic species were abnormally virulent. The majority of dermatophytids had occurred in children whose bodies, according to Bloch, became sensitized more easily. However, dermatophytosis, at that time, occurred more frequently in children, tinea pedis (athlete's foot) not being as widespread as it is now. Dostrovsky et al. (57) found "id" reactions in only 0.2% of 6,390 cases of tinea capitis, a disease of childhood. These usually occurred following X-ray epilation.

In a group of patients with dermatophytosis seen at the Skin and Cancer Hospital of Philadelphia, 4.2% of children and 4.6% of adult patients had dermatophytids. The ratio of male-female was 1:1. The infections were located on the scalp in children and on the smooth skin, usually the feet, in adults. The causative organisms were *M. audouinii*, *Microsporum canis*, *Trichophyton sulfureum*, *T. mentagrophytes* var. *granulosum*, *Epidermo-*

phyton floccosum, *T. rubrum*, and *T. mentagrophytes* var. *interdigitale*. Most of these organisms did not cause deep-seated infections (F. Blank et al., unpublished data). Bloch (29) gave a male-female ratio of 3:1. He pointed out that boys were more often afflicted with ringworm than girls.

Williams (240) and later Jadassohn and Peck (106) reported that many dermatophytids on the hands are the sequel of infections of the feet (athlete's foot). This is probably the most common location of dermatophytids seen presently in patients.

Successful treatment of the primary focus of infection results in the disappearance of the "id" lesion. However, corticosteroids will hasten the involution of the "id" reaction and aid in hyposensitization.

Dermatophytids do not usually occur in infections of animals; however, Kuroda (141, 142) and Inaba (95) observed such lesions on the ears of rabbits after experimental cutaneous reinfection with *T. mentagrophytes* var. *asteroides*. Their appearance corresponded to a high level of complement-fixing antibodies and cutaneous hypersensitivity. Dermatophytids could also be induced experimentally by intravenous injection of large amounts of fungi after a primary infection. Ito and Kuroda (98) studied the histopathological changes in these trichophytids and found them comparable to those seen in some human dermatophytids.

Trichophytids have also been produced experimentally in guinea pigs. Bloch (29) showed that intracardial reinoculation caused tuberculid-like skin papules. Later Henrici (90) found that a trichophytid could be produced in the guinea pig by a single, cutaneous infection with *T. mentagrophytes* followed after several weeks by parenteral injection of a massive dose of spores, cell extract, or crude polysaccharide antigen. The "ids" were localized in the areas of the healed infection or extended beyond and occurred on hairless areas, usually paws and ears. The reaction consisted of a generalized erythema followed by scaling and peeling. No organism was cultured from the lesions.

Antibodies

In spite of the superficial nature of most infections, circulating antibodies have been demonstrated in sera from both animals and humans with either natural or experimental dermatophytosis.

Disagreement has arisen among some investigators as to their presence or the specificity of these antibodies for the infecting organism. The

differences in experimental results may be accounted for by (i) the variety of methods used for analyses, including Prausnitz-Küstner, agglutination, precipitation, complement-fixation, immunofluorescence, and "in vitro" growth inhibition tests; (ii) the different test antigens used, including concentrated culture filtrates, commercial "trichophytins," mycelial extracts and crude polysaccharides, or other fractions; (iii) the time during the course of infection at which blood was drawn from the host; and (iv) the nature of the infection (chronic versus acute).

Animals. Citron (41) was the first to report the presence of circulating antibodies to dermatophytes in sera of experimental animals. He injected rabbits with suspensions of live *T. gypseum* and detected precipitating antibodies to extracts prepared from this species. The antibodies also reacted with extracts of *Acho- rion schoenleinii* and *A. quinckeanum*.

His studies were followed by several unsuccessful attempts to demonstrate antibodies in sera of animals with dermatophytosis (44, 143, 222).

Verotti (234) was the first to demonstrate circulating antibodies in serum from an animal with a natural cutaneous infection. He detected complement-fixing antibodies to *M. lanosum* in the serum of a dog. These antibodies also reacted with other species of dermatophytes.

Brocq-Rousseau et al. (34) infected guinea pigs with *Trichophyton equinum*, *T. granulosum*, and *A. gypseum*. They found that commercial "trichophytin" was not a good test antigen for serological reactivity, but by using homologous spore suspensions as test antigens, they detected complement-fixing antibodies in sera of 85% of the infected guinea pigs. Injections with "trichophytin" increased the circulating antibody level in the infected guinea pigs, but did not produce detectable levels in controls. They also detected complement-fixing antibodies in one of nine horses with natural *Microsporum equinum* infections. Serological reactions were negative when heterologous dermatophyte species were used as test antigens.

Martenstein (155, 157) described a factor (antibody) which could be found in serum or extracted from epithelial cells of guinea pigs infected with *A. quinckeanum*. The factor was thermostabile and when mixed with live spores "in vitro" at 37 C, a "toxic substance" was formed. Intradermal injection of the incubated mixture into normal guinea pigs resulted in an inflammatory infiltration without producing dermatophytosis.

Several investigators have demonstrated the production of agglutinins, precipitins, and complement-fixing antibodies by immunization of animals, especially rabbits, with killed dermatophyte cells. The earliest reports were those of Kolmer and Strickler (136), Fuké (67-69), and Sharp (210).

Later, Wharton et al. (238) detected precipitins in sera of rabbits experimentally infected with *Trichophyton purpureum* or immunized with killed suspensions of this species. They found that resistance to reinfection decreased with disappearance of precipitins.

Kuroda (139) detected agglutinins in both sera of rabbits cutaneously infected and sera of rabbits immunized with killed *T. mentagrophytes* var. *asteroides*. He used an antigen obtained by mechanical disruption of the mycelium. By using a similar antigen, Tomomatsu and Inaba (229) demonstrated precipitins and agglutinins in sera of cutaneously infected rabbits.

Ito and Kashima (97) also found agglutinins in sera of rabbits infected with *T. purpureum* which reacted most strongly to an antigen prepared from the homologous species. These studies were extended and reviewed by Ito (96).

Cox and Moore (45) infected rabbits with *T. verrucosum*. The experimental rabbit infection ran a short course as compared with the more chronic natural infection of cattle. Precipitins were frequently detected, appearing after 24 days following infection and persisting up to 13 weeks. Low complement-fixing antibody titers rose after several reinfections. No apparent relationship between the circulating antibody titer and susceptibility to reinfection in the rabbits was found.

Kielstein (123, 124) regularly detected antibodies to *T. verrucosum* in sera of infected cattle by passive hemagglutination tests by using coated, tanned sheep erythrocytes. Sera of infected cattle had titers above 1/32, whereas normal animals had titers below 1/32. Antibodies were detected less regularly by complement-fixation or precipitation in agar.

The gamma globulins of 4 of 5 sera of infected cattle significantly inhibited the oxygen uptake of *T. mentagrophytes* "in vitro" (122). Again, no apparent relationship between the antibody titer and the severity of infection was found. The author speculated that strong immune reactions in trichophytosis of cattle are probably due to cell-bound antibodies which are responsible for recovery and rarity of reinfection.

Evidence for cell-bound antibodies at the site

of previous infections was obtained in guinea pigs infected with *T. mentagrophytes* var. *granulosum* (43). By indirect fluorescent antibody tests, using fluorescent rabbit antibody to a keratinase isolated from this species, antibody reactions to the keratinase could be detected. The reactions were at the level of the external sheath of the hair follicle in sections of the previously infected skin. These reactions might be due to circulating antibodies with high affinity for tissue.

Humans. Kolmer and Strickler were the first to demonstrate circulating antibodies in human dermatophytosis in 1915 (136). They detected complement-fixing antibodies in 78% of sera from children with tinea capitis due to *M. audouinii*. They also claimed that 2 of 3 sera of patients with favus due to *T. schoenleinii* had complement-fixing antibodies. Sutter (222) stated that there are no complement-fixing or precipitating antibodies detectable in sera from most cases of human ringworm nor in any cases of animal ringworm. He found antibodies only in sera from isolated cases of deep-seated tinea of humans and only at the height of infection. Later, Carol (37) detected complement-fixing antibodies in sera from severe cases of deep trichophytosis by using "trichophytin" as the test antigen. Sera from patients with superficial infections were occasionally positive, and sera from favus cases were always negative.

Nathan (172) found complement-fixing and precipitating antibodies in sera from cases of deep trichophytosis, but considered them non-specific. Blumenthal and von Haupt (32) also detected complement-fixing antibodies in sera from cases of deep-seated tinea, but rarely in those from superficial infections. They observed that the circulating antibody titers were related to the severity of the disease. Similar results were obtained by Földvári (65).

Greenbaum (87) found that sera from patients with superficial ringworm did not give clearly positive complement fixation, even though he used antigens prepared from the infecting species.

Sulzberger and Kerr (218) and Sulzberger (216) demonstrated circulating antibodies in the sera of patients with positive immediate "trichophytin" skin tests by using the Prausnitz-Küstner test. These results were later confirmed by Jadassohn and Suter (110) who also demonstrated circulating reagins by this method.

Jessner and Hoffmann (113) observed that serum of patients with deep-seated trichophytosis contained substances which decreased the virulence of the dermatophyte when inoculated

into guinea pigs. The serum also inhibited the growth of the fungus on culture media. The inhibitor was stable when heated at 56 C. Later, Ayres and Anderson (5) demonstrated "fungicidal" antibodies in sera of patients with dermatophytids. The growth of dermatophytes taken from a primary focus was inhibited on Sabouraud medium containing 8.0% of such patient serum. Sera from patients without dermatophytids were not inhibitory.

Miller et al. (166) used "undenatured trichophytin" extracted from washed mycelium as test antigens. They detected mainly precipitins in 11 of 15 sera, but rarely complement-fixing antibodies in sera of patients.

Kallenberg (117) detected complement-fixing antibodies in sera from 52 of 84 patients, but no such antibodies in sera from 60 noninfected adults.

Later, Kuroda (140) used a crude polysaccharide antigen from *T. mentagrophytes* var. *asteroides* and found complement-fixing and precipitating antibodies in sera of patients infected by *Trichophyton interdigitale*, *T. rubrum*, or *E. floccosum*. All patients with dermatophytid eruptions had circulating antibodies. Similar results were obtained by Tomomatsu (228) with sera from 20 of 26 patients with *T. mentagrophytes*, *T. rubrum*, or *Microsporum ferrugineum* infections. These sera cross-reacted with antigens from 11 different species.

Pepys et al. (184) demonstrated precipitins in 50.0% of sera of patients with *T. rubrum* infections, which not only reacted with mycelial extracts and culture filtrates of *T. rubrum* and *T. mentagrophytes*, but cross-reacted with antigens of *Cladosporium herbarum*, *Penicillium notatum*, and *Aspergillus fumigatus*. Later, Longbottom and Pepys (150) showed that their glycopeptide fractions prepared from *E. floccosum* and *T. mentagrophytes* even reacted with C-reactive protein of human serum.

Studies of experimental *T. rubrum* infection in man (96) showed that precipitins are detectable up to 5 weeks after inoculation. They decreased at the time when immediate cutaneous hypersensitivity developed.

Reyes and Friedman (188) detected antibodies in sera of patients by passive hemagglutination tests with sheep erythrocytes coated with mycelial extracts. These antibodies also reacted with antigens of species of *Penicillium* and *Hormodendrum* and could be completely absorbed with mycelia of these saprophytes. The antibodies detected in sera from patients by the indirect fluorescent antibody test also proved to be nonspecific for dermatophytosis (137, 168, 236). Cross-reactions were obtained

with other fungi and with sera from noninfected controls.

Voldánová et al. (235) analyzed sera of patients with *T. rubrum* infections whose clinical manifestations resembled those described by H. Blank and Smith (26) with inflammatory processes in the deep layers of the skin. Precipitins were found in 9 of 12 sera by using a commercial "trichophytin" as test antigen. No complement-fixing or agglutinating antibodies were detected. More recent investigations including those of Schetsiruli (205) and Balogh et al. (6) have indicated the presence of circulating antibodies to the causative organism in sera of patients with allergic manifestations.

Antibodies to dermatophytes were demonstrated in sera of patients with tinea capitis and tinea corporis by charcoal agglutination, immunodiffusion, and complement-fixation tests by Grappel et al. (82, 83). The test antigens used were saline extracts of autoclaved mycelia and polysaccharides which did not react with C-reactive protein or antiserum to C substance. The lower titers of the control sera which reacted by charcoal agglutination tests indicated that the reactivities of sera from patients were not due merely to cross-reacting antibodies present in serum, but were specifically induced during dermatophytic infection. Complement-fixing and precipitating antibodies were absent in all of the adult control sera. Precipitins were found in sera from patients with tinea capitis due to *M. audouinii*, *T. mentagrophytes* var. *granulosum*, *T. schoenleinii*, and *Trichophyton tonsurans*, and from cases of tinea corporis due to *T. mentagrophytes* var. *granulosum* and var. *interdigitale*, and *T. rubrum*. Patients' sera with precipitating antibodies reacted with saline extracts of *M. audouinii*, *T. mentagrophytes* var. *granulosum* and var. *interdigitale*, *T. rubrum*, *T. schoenleinii*, and *T. tonsurans*. These observations were not surprising because many investigators have shown that dermatophyte species have many antigenic components in common. In tinea capitis (83), the humoral antibodies were not restricted to patients with deep-seated infections as has often been reported, and, in tinea corporis, the zoophilic variety of *T. mentagrophytes* induced antibody formation more frequently than the anthropophilic one. Chronic *T. rubrum* infections were often accompanied by the formation of precipitating or complement-fixing antibodies. Antibodies were also found in most patients who developed a trichophytid. In some cases, the antibodies persisted for only a short period of time. Therefore, the time at which the blood was drawn was

crucial for the detection of circulating antibodies.

The results of tests for hypersensitivity and circulating antibodies give indirect evidence for the immunological induction of inflammatory tissue reactions such as kerion Celsi and of allergic regional and disseminated eruptions. Zaslow and Derbes (251) obtained direct evidence for the presence of antibodies in kerion tissue from nine patients by using a direct fluorescent antibody test with rabbit antihuman gamma globulin. Unfortunately, no information was obtained as to the specificity of the cell-bound gamma globulin.

The occurrence of autoimmune-type reactions in dermatophytosis has also been reported. Brusilovskaia (35) reported an autoimmune process in patients with onychomycosis due to *T. rubrum* and with other mycoses due to *T. gypseum* and *Trichophyton faviforme*, which also occurred in experimentally infected animals. Autoimmune antibodies were detected by complement fixation tests with skin antigens. Recently, Peck et al. (179) reported on the results of fluorescent antibody tests on sera of a patient with a *T. rubrum* infection. They showed that complement-fixing antibodies to a commercial "trichophytin" had an affinity for epithelial tissue. Complete absorption of complement-fixing antibodies with "trichophytin" removed the antibodies which adhered to the epithelial cells.

Seeliger (206, 207) pointed out that serological techniques have not been of value for diagnostic purposes due to the cross-reactivity of some dermatophyte antigens with those of other microorganisms. However, as purified antigens which are more specific for the dermatophyte group become available, rapid, sensitive serological tests may prove to be useful.

Nonspecific Resistance

Many nonspecific factors may account for natural resistance to infection in humans or animals. This section will be limited mainly to the "serum factor," a fungistatic substance in serum of normal individuals and animals. This factor is believed to limit the growth of the dermatophytes to the keratinized layers, i.e., prevent their invasion of living tissues (91).

Several investigators have demonstrated the antidermatophytic activity of normal serum. Sera of newborns, adults, and animals show this inhibitory activity (13, 73, 152, 180, 195).

Lorincz et al. (152) and Blank et al. (25) characterized the "serum factor" as an unstable, dialyzable, heat-labile component of fresh serum and tissue fluid.

Lorincz et al. (152) and Goodman et al. (73) planted dialysis bags containing live *T. mentagrophytes* in the peritoneal cavity of mice or dogs. The growth was inhibited in the cavity, but growth resumed once the bags were removed and the contents were planted on media.

Rippon and Scherr (191) planted dialysis bags containing suspensions of *T. rubrum* and *M. audouinii* in the peritoneal cavity of rabbits. They observed proliferation of the dermatophytes, accompanied by a change to a yeast-like growth, especially with *T. rubrum*. The change in morphology was accompanied by the ability of the dermatophyte to invade deep tissue when injected into rabbits and mice, thus resembling the yeast phase of some dimorphic fungi. They speculated that the protection afforded by the plastic bag allowed the gradual induction of a cultural phase whose environmental optima were more consistent with those of the host. When cultured on Sabouraud agar at 25 C, the dermatophytes reverted to their original mycelial form.

The dermatophytes do not usually grow in internal organs of the living body. However, they can easily be cultured on these organs once they have been removed (104). Blank et al. (25) cultured human skin, both newborn and adult, as an organized tissue and infected the skin with dermatophytes to simulate natural human infection. After incubation with or without serum, the infected tissues were sectioned and the localization of the dermatophytes in the tissues was studied. The authors found that the hyphae readily invaded all layers of the skin when no serum was present, but that the growth of the fungus was restricted to the keratinized layers of the skin when serum had been added to the culture medium. This activity was lost after dialysis or heating of the serum at 56 C. The "serum factors" were not identified, but neither gamma globulin nor other protein fractions, such as albumin and Cohn fractions II, III, or IV, showed any activity.

Wilson et al. (243) described subcutaneous granulomatous infections of the leg due to *T. rubrum*. Bizarre forms of the dermatophyte were observed in the lesions. Later, Blank and Smith (26) reported a case with widespread granulomatous lesions due to *T. rubrum*. They also found abnormal forms of the dermatophyte in the lesions. Their patient had an abnormally low titer of "serum factor."

Normal sera from human newborns and adults, as well as from guinea pigs and rabbits, inhibit the proteolytic enzymes isolated from *T. mentagrophytes* var. *granulosum*. The inhibitor from human serum was isolated and identified

as alpha-₂ macroglobulin. Its significance in the process of dermatophytosis is not yet known (77, 246; S. F. Grappel, unpublished data).

Other factors may contribute to resistance to dermatophytic infection. Rothman et al. (197) explained the spontaneous cure of tinea capitis at puberty as due to an increase in fungistatic fatty acids present in the sebum of adults. However, this hypothesis has not been generally accepted (133). Rothman and Lorincz (196) also pointed out a possible relationship between the absence of sebaceous glands and selective localization of lesions in tinea pedis.

Kligman (131) observed the necessity for active keratinization for infection of animals with skin cycles. Animal hairs were not susceptible while in the resting phase.

Chronic widespread dermatophyte infections often occur in hosts whose general resistance has been reduced. A low rate of desquamation of skin (196), the presence of underlying diseases including Cushing's syndrome (36, 174, 185), malignant lymphoma (148), or diabetes (153) and treatment with immunosuppressive agents (74) may all lead to such widespread, intractable infections.

Anergy, as manifested by a negative "trichophytin" test (56, 243), serum-dysproteinemia, including hypoalbuminemia and hypergamma-globulinemia (24, 26, 56, 148), and atopy (116) have also been associated with chronic infections.

IMMUNOCHEMISTRY OF DERMATOPHYTES

Chemical Composition

Blank (22) used X-ray diffraction to study the cell walls of 15 species of dermatophytes; he found chitin, but not cellulose. Shah and Knight (209) analyzed purified cell walls of four species by paper chromatography. They found these to consist mainly of a glucose-containing polysaccharide, an *N*-acetyl glucosamine polymer (chitin), mannan, protein, and small amounts of galactosamine and lipid.

Merkel (162-164) analyzed whole mycelia from *T. gypseum*, *A. schoenleinii*, and *A. quinckeanum* which were frozen and thawed 20 times or more and then extracted. He found that lipids comprised 60 to 74%; polysaccharides, 17 to 30%; and proteins, 8 to 10% of his extracts. Each species contained glucose, mannose, and glucosamine and had similar amounts of nitrogen and protein in the form of nucleoprotein.

Analyses of polysaccharide fractions by many other workers also revealed that the sugars most

frequently present were glucose, mannose, galactose, and glucosamine (100, 101, 125, 161, 188).

Götz and Pascher (76) studied the amino acid compositions of three dermatophyte species, *T. mentagrophytes*, *T. schönleinii*, and *T. rubrum*, and found that they did not differ qualitatively or quantitatively to any significant extent, except for a larger amount of phenylalanine in *T. mentagrophytes*. This was also the only species which produced α -aminobutyric acid.

The chemical composition may be altered by growth conditions and age of cultures. Swanson and Stock (223) studied the metabolic activity of *M. quinckeanum* and found that dermatophytes have a slower rate of metabolism than saprophytic molds. The maximal total nitrogen, protein, and ribonucleic acid contents were found at the end of the log phase, whereas trichloroacetic acid-soluble nitrogen increased with increasing age and could be part of structural polysaccharides. The rate of carbohydrate synthesis was found to be relatively uniform during the entire growth cycle. By starvation experiments, they concluded that lipid rather than carbohydrate was the primary storage material in this dermatophyte.

Stuka and Burrell (214) studied the influence of nitrogen content of the growth medium and age of the culture on the antigenic composition of *T. rubrum*. They concluded that young cultures grown in low concentrations of multipiptone are most antigenic. Fluorescent antibody studies indicated that the hyphal tips were most reactive and that extracellular antigenic material was exuded from the tips.

Flaherty and Burrell (63) showed later that the carbohydrate source influenced the polysaccharide composition of the mycelium, especially with regard to molecular weight of the major antigenic components. The optimal antigenic activity was obtained in medium with low carbohydrate concentrations of 15.0 g/liter. This is less than the amount in routine Sabouraud dextrose medium.

Antigenic Composition

Interest in the separation and characterization of antigenic components from dermatophytes began with the work of Bloch et al. (30). These investigators were the first to identify the active component of "trichophytin" as a nitrogen-containing polysaccharide.

Since that time there have been numerous publications dealing with the isolation of purified "trichophytins" and the serology of antigenic preparations. However, in most instances the antigen preparations that were isolated

were heterogeneous or were not tested for homogeneity.

Differentiation of species. Jadassohn et al. (107-109) initiated the concept of group-specific and species-specific antigens of dermatophytes and stimulated interest in isolation of antigens for serological differentiation of species as well as for identification of factors involved in hypersensitivity reactions and the cutaneous manifestations of dermatophytosis.

Jadassohn et al. (108) used an extract, prepared according to the method of Bloch et al. (30), termed "Trockentrichophytin" or "desiccated trichophytin." They showed that infection of guinea pigs with *A. quinckeanum* induced not only a delayed cutaneous reactivity, but also an immediate-type hypersensitivity to this antigen preparation which was apparent 40 days or more after infection. They also found that the uterus of guinea pigs could be sensitized by subcutaneous injection of the "desiccated trichophytin." Uterine strips from guinea pigs sensitized by this method were used for analysis of "trichophytins" prepared from four species of dermatophytes (107, 109). It was found that the "trichophytins" thus prepared from *A. quinckeanum*, *T. mentagrophytes*, *Epidermophyton Kaufmann-Wolf*, and *T. schoenleinii* were mixtures of antigens and that each species had one or more antigens in common as well as antigens that were characteristic of the species. However, they had no success in purifying the species-specific antigens. Nevertheless, their demonstration of group-specific and species-specific antigens led to many attempts at differentiation of dermatophyte species by immunological methods.

Sharp (211) found extensive cross-reactions between mycelial extracts of dermatophyte species by precipitin tests with rabbit antisera. Differences in relative titers could not be used as a basis for species differentiation.

Keeney and Erickson (118) isolated protein and carbohydrate antigens from culture filtrates of *T. mentagrophytes* and *T. rubrum* grown in synthetic medium. They claimed that the antigens were species-specific by serological methods (precipitin tests), but showed cross-reactions in tests for dermal sensitivity.

Fischer (62) compared "desiccated trichophytins" from additional species by the Schultz-Dale technique. He found both species-specific and common antigens in *T. rubrum* and *E. floccosum*.

Tomomatsu (226) compared crude polysaccharide antigens from 11 different species of dermatophytes by precipitin reactions with rabbit antiserum with each of these species. He

concluded that the *Trichophyton* species could be separated from the *Epidermophyton* and *Microsporum* species by differences in serological reactivities.

Miura (167) and Miura and Kasai (169) showed that fluorescent antibody techniques using rabbit antisera to different dermatophyte species could be used to detect dermatophytes and differentiate them from other groups of fungi. However, their technique could not be used to distinguish species.

Dyson and Landy (58) grouped species on the basis of gel diffusion tests with antisera to sonically treated mycelium. Their results showing the antigenic differentiation of *T. mentagrophytes* and *T. rubrum* paralleled the results of species differentiation by "in vitro" hair invasion tests (1).

Kielstein (120) analyzed crude polysaccharides from 12 dermatophyte species by Ouchterlony gel diffusion tests. He found three antigens that were common to all species and several others that showed partial identity. The results could not be used as a basis for serological classification.

Sagara (201) also used gel diffusion to compare mycelial extracts and found that *T. mentagrophytes* var. *interdigitale* and var. *asteroides* could be differentiated from *T. rubrum*, but not from each other by this method.

Biguet et al. (17) used immunoelectrophoresis of saline extracts to show that *M. ferrugineum* was more closely related to species of the genus *Microsporum* than to *Trichophyton* species. These results agreed with its classification in the genus *Microsporum* based upon the formation of spindle-shaped, thick-walled macroconidia, which were described later for this species by Vanbreuseghem et al. (233).

Andrieu et al. (3) also used immunoelectrophoresis for analysis of 17 species. More than 10 antigens were detected in extracts of each species by using homologous antisera. Many of these antigens cross-reacted with antisera to other species. The large number of antigens detected by this method indicated that the antigenic components in dermatophytes were more complex than had been supposed previously.

Hypersensitivity factors ("trichophytin"). Most of the early efforts aimed at purification of antigenic components of dermatophytes were directed toward obtaining a pure "trichophytin." Although most investigators isolated crude nitrogen-containing polysaccharide fractions which elicited "trichophytin" reactions in sensitized animals (11, 70, 128, 129, 162, 224, 227, 228), some also suggested that protein

fractions elicited cutaneous "trichophytin" reactions (67, 138). Protein "trichophytins" containing 12 amino acids were isolated from five species of dermatophytes by Meyer et al. (165).

Shechter et al. (212) separated protein constituents of six species of dermatophytes by disc electrophoresis. However, these fractions were not isolated or characterized. Fractions specific for *E. floccosum* were detected.

Interest in components of dermatophytes involved in pathogenicity led to detection of proteolytic enzymes by several investigators (27, 40, 189, 190, 192). Yu et al. (247, 249) isolated antigenic proteolytic enzymes from *T. mentagrophytes*. These enzymes also had "trichophytin" activity in infected guinea pigs (77). Lipid fractions have also been reported to have "trichophytin" activity (203).

Ito (100, 101) showed the complexity of "trichophytin" by isolating 22 fractions with "trichophytin" activity from mycelium and culture filtrate of *T. mentagrophytes* var. *asteroides* by 0.5% phenol-water extraction and chromatography on diethylaminoethyl (DEAE)-Sephadex A-50. In addition to carbohydrate-peptide complexes, these active fractions included ribonucleic acid, peptides, and polysaccharides.

Purified Antigenic Components

Beginning about 1962, two groups started definitive studies on the structures of antigens from dermatophytes and have attempted to establish relations between structure and immunological activity. For this reason, the present section will be concerned primarily with the work of those two groups. Barker, Cruickshank, and associates (7-9, 14, 92) concentrated all of their earlier efforts on glycopeptides, but have recently studied some polysaccharides. Bishop, Blank, and associates originally confined their studies to polysaccharides, but later investigated other antigenic components including proteolytic enzymes (18-21, 23, 78-81, 84, 247-250). More recently, a third group, Nozawa et al. (176-178) has concentrated its efforts on purified polysaccharide-peptide complexes.

Isolation of antigens. Glycopeptides, polysaccharides, and keratinases were isolated as described below.

Glycopeptides. Immunologically active glycopeptides have been extracted from submerged cultures of dermatophytes with ethylene glycol by Codner et al. (42). This is a procedure developed originally for extraction of the "O" antigen of *Shigella dysenteriae* (171). The material in the extract was purified by Barker et al. (9) by dialysis and subsequent fractional pre-

precipitation by cetyltrimethyl ammonium bromide from a borate buffer solution of increasing pH according to the method of Barker et al. (10). Under these conditions, the fraction precipitated at pH 7 to 9.5 contained most of the biological activity (cutaneous hypersensitivity tests) and was homogeneous by cellulose acetate strip electrophoresis, by ultracentrifugation, and by gel filtration. However, a subsequent investigation by Barker et al. (7) showed that this material could be fractionated further into two glycopeptides by gradient elution from DEAE-Sephadex A-50. In the same investigation, three glycopeptides were isolated from surface cultures of that organism (*T. mentagrophytes*) by the same procedures. The ethylene glycol extraction procedure has also been used for cultures of *T. rubrum*, *M. canis*, *T. schoenleinii*, *Keratinomyces ajelloi*, and *E. floccosum* to yield crude antigens; fractionation by the same procedures gave glycopeptides from *T. rubrum* and *M. canis* (14).

Recently, How et al. (92) have isolated glucans from four species of dermatophytes, *T. mentagrophytes*, *T. rubrum*, *M. canis*, and *T. schoenleinii*. The glucans were purified from ethylene glycol extracts by mild fractionation, at neutral pH, on Bio-Gel P-300 and DEAE-Sephadex A-50.

Nozawa et al. (177, 178) have concentrated their efforts on isolation of immunologically active polysaccharide-peptide complexes from *T. mentagrophytes*. These investigators used the phenol method of Westphal et al. (237) to extract defatted mycelial mats of *T. mentagrophytes* grown in Sabouraud broth. The crude extracts were separated and purified by gel filtration and DEAE-cellulose column chromatography.

Polysaccharides. The procedure used by Bishop, Blank, and associates for isolation of polysaccharides was purposely more drastic than that used to isolate the glycopeptides, because the objective was to obtain the total, soluble polysaccharides, free from protein and lipid, for serological studies. The mycelia from culture growth were defatted by extraction with solvent and deproteinized by digestion with trypsin, and the polysaccharides were extracted from the insoluble residue by hot, dilute alkali (18). The individual polysaccharides in the mixtures thus obtained were separated by precipitation of one as its insoluble copper complex and subsequent separation of the other two by chromatography on DEAE-cellulose (19). These procedures yielded three polysaccharides from each of the following organisms: *T. granulosum*, *T. interdigitale*, *M. quinckeanum*, *T. rubrum*,

T. schoenleinii, *Microsporium praecox*, *T. ferrugineum*, *Trichophyton sabouraudii*, and *T. tonsurans*. Each of the polysaccharides isolated was homogeneous by free-boundary electrophoresis in borate buffer.

In addition to the neutral polysaccharides, a pyruvated glucan was isolated from autoclaved mycelium of *M. quinckeanum* (61). The mycelium was extracted with ethylene glycol, yielding a mixture of peptidoglycans. The pyruvated glucan was separated from the mixture by ethanol precipitation and was purified by chromatography on DEAE-Sephadex A-25.

Keratinases. One extracellular and two cell-bound proteases with keratinolytic activity on guinea pig hair were isolated from cultures of *T. mentagrophytes* var. *granulosum* grown in a liquid keratin medium with horse hair as sole source of nitrogen (247-249). The extracellular enzyme, keratinase I, was isolated from the culture filtrate by chromatography on DEAE- and carboxy methyl-cellulose columns and purified by gel filtration on Sephadex G-100. The mycelial enzymes, keratinase II and keratinase III, were extracted with phosphate-buffered 1.0 M NaCl, pH 7.8. They were separated by column chromatography on DEAE-cellulose and carboxy methyl-cellulose and were purified by gel filtration on Sephadex G-100.

Chemistry of antigens. The chemistry of glycopeptides, polysaccharides, and keratinases was as described below.

Glycopeptides. Definitive structural work on the glycopeptides of Barker et al. was done on a preparation (8) which was shown subsequently to be a mixture (7). However, the results are valid in showing the main structural features of this group of allergens. The yields and constitutions of the glycopeptides varied with different cultures, the chief variation being in the D-galactose content which accounted for 9% to 20% of the glycopeptides. The particular sample examined contained D-galactose (9%), D-mannose (73%), and protein (9%). Evidence for a limited number of peptide chains was obtained by the finding of three N-terminal amino acids, glycine, alanine, and threonine. Furthermore, extensive (80 to 90%) hydrolysis of the amino acids by proteolytic enzymes indicated a predominance of peptide rather than carbohydrate-amino acid linkages. The glycopeptides contained the following amino acids: aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, and lysine. In the subsequent investigation (7), the glycopeptides from submerged cultures and from surface cultures of *T. mentagrophytes* were resolved into two and

three galactomannan peptides, respectively. The ratios of amino acids were similar in each of these products, with the exception of high contents of proline in the three galactomannan peptides from the surface cultures. It was suggested that this difference between the surface and submerged cultures could have been caused by a difference in proteolytic activity of the organism under these conditions. It was noted that the glycopeptides of high proline content were more resistant to pronase and ficin than the glycopeptides obtained from submerged cultures. Because proteolytic activity is a necessary property of any dermatophytic organism, it is possible that the peptide moieties of the glycopeptides represent a resistant core.

The carbohydrate portion of the glycopeptide was analyzed by partial hydrolysis, periodate oxidation, and methylation studies (8). Mild hydrolysis of the glycopeptides caused release of all of the galactose, and its presence as the D-enantiomer was confirmed by the specific reaction with D-galactose oxidase. On periodate oxidation, all of the D-galactose was destroyed and there was an immediate production of 0.13 moles of formaldehyde. All of this evidence indicated that the D-galactose was present in the furanoside form, probably as terminal units. Periodate oxidation also reduced the mannose content of the glycopeptides from 89% to 36.5% and, after reduction and hydrolysis of the oxidized product, glycerol and erythritol were detected. During methylation analysis, the D-galactose units were lost, probably by hydrolysis during work-up, and the following O-methyl ethers of D-mannose were found: 2,3,4,6-tetra-O-methyl; 3,4,6-tri-O-methyl; 2,3,6-tri-O-methyl; and unidentified mixtures of di-O and mono-O-methyl derivatives. The ratios of the tetra-tri-di-mono were 23:27:25:14. From these results, it was inferred that the main linkages in the mannan portion of the molecules were 1 → 2 and 1 → 4.

Nozawa et al. (177) obtained a major glycopeptide from their initial crude mycelial extract by gel filtration on Sephadex G-100. They confined their studies to this first fraction and discarded subsequent minor fractions. The major fraction which amounted to 68% of the crude extract, and contained 1.8% nitrogen, was subsequently chromatographed on a DEAE-cellulose column equilibrated with 0.01 M borate buffer and yielded seven glycopeptides.

The glycopeptides were analyzed for amino acid content. Most of the glycopeptides contained a large amount of serine, threonine, proline, glycine, alanine, and aspartic acid and

smaller amounts of glutamic acid, valine, isoleucine, lysine, and leucine.

Successive digestion of two of the separated glycopeptides with trypsin and papain reduced the nitrogen content from 0.32 to 0.04% and from 0.91 to 0.13%, respectively.

Each of these polysaccharide-peptide complexes contained mannose, galactose, glucose, and glucosamine. However, no fine structural analyses were done on the sugar moieties.

Polysaccharides. The structures of the three polysaccharides isolated from each of five organisms have been described in a series of six papers by Bishop, Blank, and associates (2, 18, 19, 20, 21, 23). The dermatophyte species examined were *T. granulorum*, *T. interdigitale*, *M. quinckeanum*, *T. rubrum*, and *T. schoenleinii*. A subsequent publication by Grappel et al. (81) described the structures of similar polysaccharides from an additional four species of dermatophytes: *M. praecox*, *T. ferrugineum*, *T. sabouraudii*, and *T. tonsurans*.

The isolation and fractionation procedures described earlier yielded the same pattern of polysaccharides from each of the nine organisms listed above, namely two galactomannans (I and II) and a glucan. Their properties and the results of methylation studies provided definitive evidence of the structures of these polysaccharides. Thus, the occurrence of D-galactose in the furanose ring form, inferred in the study by Barker et al. (8), was confirmed by the isolation of 2,3,5,6-tetra-O-methyl-D-galactose in amounts that accounted for all of the D-galactose that was present originally. The results also showed conclusively that these organisms contained two different galactomannans. They were similar in having nonreducing end groups of D-galactofuranose and D-mannopyranose units and in containing 1 → 6-linked D-mannopyranose units in the chains. However, galactomannans II contained a predominance of 1 → 2-linked D-mannopyranose units in the linear portions, and no such units were present in the galactomannans I. This represents a basic structural difference between the two groups of polysaccharides that could not have arisen by inadvertent chemical modification (e.g., removal of the acid-labile D-galactofuranoside units) during extraction or fractionation.

The galactomannans I (20) are branched polysaccharides with the branch points through the C-2 and C-6 hydroxyls of D-mannopyranose predominantly, except for those from *T. sabouraudii* and *T. tonsurans* where the major branch points are through the C-6 and C-3 hydroxyls. The linear portions of those polysac-

charides are formed almost exclusively by 1 → 6-linked D-mannopyranose units with only a small number of 1 → 3 linkages; the chains are terminated by D-mannopyranose and D-galactofuranose groups.

In the galactomannans II (19), branch points occur through C-6, C-3 and C-6, C-2 disubstituted D-mannopyranose units to a more equal extent. In the galactomannan II from *T. ferrugineum*, the branching is exclusively through the C-6, C-2 positions and in that from *T. tonsurans* exclusively through C-6, C-3. The linear units are predominantly 1 → 2-linked D-mannopyranose, and there are a significant number of 1 → 6 linkages in all except those from *M. praecox*, *T. ferrugineum*, *T. sabouraudii*, and *T. tonsurans*. As in the galactomannans I, the galactomannans II have nonreducing terminal groups of D-galactofuranose and D-mannopyranose.

The glucans (21) were also branched polysaccharides, but with a more consistent pattern of substitution. All branches occurred through the C-6, C-3 positions, and linear chains were formed by 1 → 6 and 1 → 3 linkages. The variation in the ratio of these linkages is probably not significant because 1 → 3-linked polysaccharides are labile to alkali (239), and alkali was used to extract these polysaccharides.

The results indicated that the polysaccharides within each of the three groups were remarkably similar with only minor variations in their structural features. Thus, in the galactomannans I, only three of the nine polysaccharides show a significant difference from the others, and one of the differences is common to two of these three. In the galactomannans II, there are three significant differences, one of them (the linear units) common to four of the polysaccharides, one (the end groups) shared by two polysaccharides, and two distinct differences in branch points. The maximal variations from a common structure occur in the polysaccharides from *T. tonsurans*, followed in order of decreasing differences by *T. sabouraudii*, *T. ferrugineum*, *T. rubrum*, and *M. praecox*. There were no significant differences between the glucans except for the positive specific rotation of the one from *T. granulorum*. The specific rotations may indicate varying proportions of α - and β -anomeric linkages, but this has not been confirmed.

The pyruvated glucan (61) isolated from *M. quinckeanum* contained 0.6% pyruvic acid. Methylation studies showed that it was present as a 4,6 ketal (4,6-O-1-carboxyethylidene) attached to nonreducing terminal D-glucose

units of the polysaccharide. The glucan had a specific rotation of -15° and contained no nitrogen. Most of the glycosidic linkages were in the β -configuration. From methylation analyses, it was determined that the glucan contained 1 → 3 and 1 → 6 linkages in a ratio of 2:1.

The glucans isolated by How et al. (92) differed from those reported earlier by Bishop et al. (21) in that all contained $\alpha(1 \rightarrow 4)$ -linked D-glucose residues as determined by their infrared spectra. Only the nitrogen content of the glucan from *T. rubrum* was reported. It contained less than 0.1% nitrogen.

Keratinases. The extracellular keratinase isolated from *T. mentagrophytes* var. *granulosum* by Yu et al. was a protein containing 18 amino acids (250). These were alanine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. The molecular weight of this enzyme was 48,000.

The two cell-bound enzymes isolated from *T. mentagrophytes* var. *granulosum* were both glycoproteins having molecular weights of 440,000 (keratinase II) and 20,300 (keratinase III) (249). Each contained the same 18 amino acids as the extracellular keratinase I, but these also contained galactose, mannose, and glucose (Yu et al., unpublished data).

Immunological reactivity. Immunological reactivity in relation to structure and taxonomic importance is as described below.

Relation to structure. Hypersensitivity reactions have been classified as immediate and delayed, the former based upon the presence of circulating antibodies, the latter as a manifestation of cellular immunity (72, 221).

Barker, Cruickshank, and their colleagues were able to demonstrate both immediate- and delayed-type hypersensitivity reactions in guinea pigs sensitized to *T. mentagrophytes*. The immediate and delayed hypersensitivity reactions were related to the carbohydrate and peptide moieties, respectively, of their glycopeptides (7, 9, 14). It was found that degradation of the carbohydrate portion of the glycopeptides from *T. mentagrophytes* by periodate oxidation greatly diminished the immediate hypersensitivity reaction; degradation of the peptide portion by proteolytic enzymes diminished the delayed reaction (9). These results were confirmed with the purified glycopeptides (7). Similar studies were made with glycopeptides from *T. mentagrophytes*, *T. rubrum*, *T. schoenleinii*, *M. canis*, *K. ajelloi*, and *E. floccosum* by Basarab et al. (14). On the basis of

cutaneous hypersensitivity reactions in animals sensitized with homologous and heterologous species, it was concluded that the glycopeptides were not species-specific, but indeed were very similar in different genera of keratinophilic fungi. It was also shown that polysaccharides (such as yeast mannans) that had structural features in common with the carbohydrate portion of the glycopeptides gave strong immediate hypersensitivity reactions in dermatophyte-sensitized animals. Although these results clearly demonstrated the presence of cross-reacting antigens in the dermatophytes examined, the results could not be quantified, and low-level reactions could well have been missed. It has been suggested that "the established techniques of determining delayed hypersensitivity, either by skin reaction or cytotoxicity, have a high threshold and, therefore, low levels of cellular hypersensitivity escape detection. Claims that immunity is separable from delayed hypersensitivity may be true if the presence or absence of a skin reaction serves as the measure for desensitization" (221). For these reasons it would seem that the immediate hypersensitivity reaction or, even better, the quantitative interaction of antigens with antibodies, would offer better opportunities for assessing the antigenic specificity of the compounds.

Nozawa et al. (177, 178) demonstrated both immediate- and delayed-type cutaneous reactivities to their polysaccharide-peptide complexes from *T. mentagrophytes* in patients with trichophytosis. By using their phenol-water extract partially purified on Sephadex G-100, they showed that proteolytic digestion decreased the delayed hypersensitivity by 54%, but hardly affected the immediate hypersensitivity reaction in sensitized humans. Precipitin reactions with rabbit antiserum to the phenol-water extract were hardly affected by the proteolytic digestion, indicating that the circulating antibodies produced were specific mainly for the carbohydrate moieties.

Noguchi et al. (176) studied the rabbit antibodies produced to phenol-water extracts of *T. mentagrophytes*. They found that IgG gave precipitin and complement fixation, but not passive hemagglutination reactions. IgM gave passive hemagglutination and less precipitation, but no complement-fixation reactions.

These investigators did not isolate polysaccharide-peptide fractions from any other species for comparison.

The N-free neutral polysaccharides isolated by Bishop et al. did not elicit cutaneous hypersensitivity reactions in guinea pigs sensitized by cutaneous infection (200). However, these poly-

saccharides were studied immunochemically and compared according to serological reactivities.

The first paper in that series of investigations by Grappel et al. (78) showed that each of the three groups of polysaccharides (galactomannans I and II and glucan) from five organisms reacted with antiserum produced in rabbits to autoclaved mycelial suspensions of *M. quinckeanum*. The serological reactions were monitored by qualitative precipitation in gels, by quantitative complement fixation, and by immunoelectrophoresis. The immunodiffusion results indicated that, within each group, the polysaccharides from each of the heterologous species contained antigenic groups with reactivities that were very similar to those of the homologous organism *M. quinckeanum*. Quantitative complement fixation showed that the five galactomannans I were very similar, but that differences occurred in both the galactomannans II and the glucans. These differences could not be related to the minor structural variations revealed by methylation studies.

The role of the D-galactofuranoside end groups in the immunological specificities of galactomannans from five dermatophytes was also investigated (79). Because of the acid lability of furanosidic linkages, it was possible to remove the D-galactose from the galactomannans I and II leaving mannans I and II that were still serologically active and that could not, therefore, have been degraded extensively. The serological activities, qualitative and quantitative, of the mannans I were the same as those given by the parent galactomannans I, showing that the D-galactofuranoside units were not important determinants in that group of polysaccharides. The reverse situation was found in the mannans II, which displayed considerable differences from their parent galactomannans II by immunodiffusion and complement fixation analyses. Probably the amount (0 to 13%) of the galactofuranoside units in the galactomannans I was not large enough to induce specific antibodies, but the higher contents of that unit (22 to 33%) in the galactomannans II were recognized. The same study revealed a strong cross-reaction between mannans I and mannans II by using antisera absorbed with galactomannans I or II. This cross-reaction could be explained if the removal of the D-galactofuranoside units from galactomannans II unmasked some 1 → 6-linked D-mannopyranose units. Mannans from three *Candida* species demonstrated cross-reactivity with the antiserum to *M. quinckeanum*. This was attributed to the presence of 1 → 2-linked α-D-mannopyranose units

in the *Candida* mannans (245), thus providing a structural feature in common with the galactomannans II.

The pyruvated glucan isolated by Fielder et al. (61) reacted with rabbit antiserum to autoclaved mycelium of *M. quinckeanum*. It also elicited immediate-type cutaneous reactions in guinea pigs sensitized to *M. quinckeanum* by dorsal cutaneous infection.

This glucan did not cross-react with type XXVII pneumococcal antiserum containing antibodies directed toward the pyruvic ketal, probably because of the low pyruvate content of the glucan. Other species of dermatophytes have not yet been examined for the presence of similar glucans.

How et al. (92) showed that the glucan which they isolated from *T. rubrum* was capable of sensitizing guinea pigs when injected in complete Freund adjuvant. It elicited significant immediate-type cutaneous reactions in guinea pigs sensitized with ethylene glycol extract or the purified glucan of *T. rubrum*. The *M. canis* glucan, but not the *T. mentagrophytes* glucan, elicited immediate cutaneous reactions in guinea pigs sensitized to *T. rubrum* glucan. The ethylene glycol extract, from which the *T. rubrum* glucan was isolated, elicited both immediate- and delayed-type cutaneous reactions in such guinea pigs.

Gel diffusion analyses, by using antisera prepared in rabbits to the active keratinases I and II, showed that these enzymes had some determinant groups in common (249, 250). Rabbit antibodies to these enzymes precipitated, fixed complement, and inhibited their proteolytic activity (S. F. Grappel, unpublished data). Grappel et al. (85) showed that such antibodies could also cause a retardation in the growth and an alteration in the structure of *T. mentagrophytes* in culture. The inhibitory effect of rabbit antibodies on growth and structure was also demonstrated with antibodies to autoclaved mycelium. However, in the presence of guinea pig complement, antibodies could actually cause a change in the morphology of dermatophytes (85, 86).

Antibodies to the keratinase II were occasionally detected in sera of infected guinea pigs by complement fixation tests, but they did not persist (S. F. Grappel, unpublished data). By using fluorescent antibody tests, Collins et al. (43) demonstrated local antibody reactions to keratinase II at the level of the external sheath of the hair follicle in biopsy sections of previously infected sites of guinea pigs.

Austwick (4) speculated that such dermatophyte-specific antibodies diffusing into the hair

bulbs could be responsible for the degenerative changes observed in the intrapillary hyphae in healing ringworm lesions.

Grappel and Blank (77) heat-inactivated the keratinases I and II before using them as skin test antigens. Both of the heat-inactivated keratinases, the extracellular and the cell-bound, elicited delayed cutaneous hypersensitivity reactions in guinea pigs sensitized by dorsal cutaneous infection with *T. mentagrophytes* var. *granulosum*. The reactions obtained were greater than those to a commercial "trichophytin." Eleuterio et al. (59) studied the nature of the cutaneous reactions to the keratinases. They used macrophage migration inhibition tests with peritoneal exudate cells according to the method of David et al. (49). The results of their investigation confirmed the specific cellular nature of the reactions to the keratinases.

Guinea pigs were also sensitized to the active keratinases by immunization with the enzymes in Freund complete adjuvant (77). These guinea pigs had both circulating antibodies and cutaneous hypersensitivity. The antibodies that were produced inhibited the proteolytic activity of the enzymes in addition to fixing complement and precipitating with the keratinases.

Taxonomic importance. Thus far, the chemical structures and the cutaneous reactivities of dermatophyte glycopeptides in sensitized guinea pigs have not shown any evidence of species specificity. However, most of the preparations isolated from more than one species were mixtures and were not compared immunologically.

Further studies on the cross-reactivities of the three groups of neutral polysaccharides by Grappel et al. (80, 81) revealed that there were differences in the antisera that were prepared in rabbits to each of the organisms. The cross-reactivities of the 27 polysaccharides with antisera to each of the 9 dermatophyte species from which they were isolated were determined by complement fixation (84). The results showed that the galactomannans I were the most reactive of the three groups of polysaccharides and differed most in their reactivities with antisera to *T. interdigitale*, *T. sabouraudii*, and *T. schoenleinii*. The galactomannans II were less reactive than the galactomannans I and differed in their cross-reactivities with antisera to each species. The structural differences are probably sufficient to classify *T. tonsurans*, *T. sabouraudii*, *T. ferrugineum*, *T. rubrum*, and *M. praecox* as distinct species. However, the significance of the serological variations, determined by complement fixation, is more difficult to assess. The antisera were produced in rabbits. Antiserum

for each species was taken from a single animal for the tests. Minor differences could therefore represent individual differences in the animals' responses. There is also the possibility of variation in the antigen composition in relation to the time at which the growth was harvested. Changes in the composition of extracellular fungal polysaccharides with time during growth have also been reported (159, 160).

The keratinolytic enzymes have only been isolated from one species thus far. However, these antigenic proteolytic enzymes may offer the most promising tools for an improved classification of the dermatophytes as well as for elucidating the mechanism of resistance and its relationship to hypersensitivity in dermatophytosis.

IMMUNOPROPHYLAXIS AND THERAPY

The evidence presented thus far has indicated that acquired resistance to dermatophytic infection is accompanied by cutaneous sensitization (delayed-type hypersensitivity).

Animals

Early investigations showed that inoculation of living or killed dermatophyte cells by routes other than the cutaneous could produce hypersensitivity and resistance to subsequent cutaneous infection. However, injection of guinea pigs with whole cells by intracardial, intramuscular, subcutaneous, or intraperitoneal routes induced only a poor or partial protection. Subsequent cutaneous infections took an abortive course, but protection was of relatively short duration. No protection was obtained by injection of culture filtrates (31, 38, 114, 135, 143, 199).

Hypersensitivity and partial resistance could also be obtained in guinea pigs by rubbing their skin with killed mycelium (222) or by repeated intradermal injections of a "toxic substance" obtained from the supernatant fluid of mixtures of extracts of infected skin and mycelium incubated at 37 C for 24 h (155, 156, 158).

Later, Wharton et al. (238) injected rabbits subcutaneously with a killed *T. rubrum* suspension in Freund complete adjuvant. The immunized rabbits were completely resistant to infection for up to 17 months or more. This resistance was greater than that obtained in rabbits after cutaneous infections. Precipitating antibodies were detected in the resistant animals. Injection of culture filtrate or extract of the fungus did not give protection.

Reiss and Leonard (187) showed that intradermal injections of a "trichophytin," even if

incorporated in Freund complete adjuvant, produced hypersensitivity in guinea pigs, but no resistance to infection. Without the adjuvant, neither hypersensitivity nor resistance was observed.

Topical application of killed, disintegrated *T. mentagrophytes* mycelium in an ointment base was found to induce both hypersensitivity and partial resistance in guinea pigs. The immunity was not of long duration (16, 119).

Hydrolysates of killed *T. verrucosum* mycelium have been used successfully for prophylaxis for cattle. Florian (64) inoculated 2-week-old calves subcutaneously with a vaccine prepared by extraction of mycelium of *T. verrucosum* ground in CO₂ snow. The vaccinated calves had a 50% lower incidence of disease than controls. The lesions in vaccinated calves which became infected were less extensive and less severe than those in the controls.

Kielstein and Richter (126, 127) prepared several vaccines with *T. verrucosum* or *T. mentagrophytes* cultures by modifications of the method of Florian (64). Extraction of the ground mycelium of *T. verrucosum* with 0.12 N HCl provided the most effective vaccine. Intracutaneous or subcutaneous immunization gave partial or relative protection in heifers when they were exposed to infected cattle. The degree of immunity could not be correlated with circulating antibody titers.

Sarkisov et al. (204) demonstrated complete resistance of calves to experimental infection 2 weeks after immunization with *T. verrucosum* vaccine. The immunity lasted 3 to 5 years. When 107 immunized calves were mixed with ringworm-infected animals, only 16 (14.9%) developed small lesions which healed spontaneously.

Humans

Plato and Neisser (173) made the first attempt to produce a therapeutic vaccine for humans. However, their "trichophytin" from filtrates of ground *Trichophyton* cultures, as well as the later Truffi (231) "trichophytin" from culture filtrate, had no significant therapeutic value.

Strickler (213) prepared a vaccine from killed and powdered mycelium of *T. tonsurans*. He cured 14 of 20 cases of tinea capitis by numerous injections. Later, Jausion and Sohler (112) prepared the claso-vaccine by hydrolyzing mycelium of several species with nitric acid. Repeated subcutaneous or intramuscular injections cured 36 of 45 cases. Da Fonseca and de Área Leão (48) also used a claso-vaccine, known

as "dermatomycol." They cured resistant onychomycosis and tinea capitis. Lewis and Hopper (146) also used this vaccine as well as other "trichophytin" vaccines for cases of tinea capitis. The cure rate was disappointing with all preparations and toxic side reactions were observed.

Sulzberger and Wise (220) injected a "trichophytin" intradermally in order to desensitize 18 patients with "id" reactions. Partial desensitization was observed in 15 patients, and 10 of these patients benefited from the treatment. Traub and Tolmach (230) treated 135 patients with "id" reactions by repeated intradermal injections of "trichophytins." They found that desensitization had little effect both on the "id" eruptions and on the course of the disease. Moreover, desensitization was only temporary. Miller et al. (166) also had little therapeutic success with injections of their "undenatured trichophytin." Later, Bazyka (15) found that small intracutaneous doses of fungal vaccines made with *T. rubrum* or *T. mentagrophytes* could reduce dermatoallergic reactions, whereas large doses increased resistance of patients.

Harada (89) reported that vaccine therapy with polysaccharide-nucleic acid antigens was successful in 50% of patients with dermatophytosis. Longhin and Olaru (151) also reported successful treatment of 680 patients with profound trichophytosis by repeated subcutaneous injections of trichloroacetic acid extracts of *Trichophyton mentagrophytes* mycelia. The extracts contained polysaccharides, phospholipids, and polypeptides and were capable of desensitization of some patients with dermatoallergic symptoms. Five hundred and thirty patients (78%) recovered without any additional therapy. In association with griseofulvin, 33 cases which had recurred after treatment with griseofulvin alone were cured.

Although few attempts at immunoprophylaxis have been made in humans, those reported have been successful in producing a local immunity to experimental infection.

Sutter (222) produced a local immunity in humans by rubbing killed cultures of *A. quinckeanum* into the skin. The resistance to infection diminished with increasing distance from the site of treatment and was accompanied by the development of cutaneous hypersensitivity.

Huppert and Keeney (94) immunized volunteers by repeated topical applications of disintegrated *T. mentagrophytes* mycelium in salve to one foot. Upon challenge, only 14% of the treated feet became infected, whereas 57% of

the untreated contralateral feet became infected. In the controls, 86% infection occurred in the right foot and 71% in the left. As in Sutter's experimental infections (222), resistance was greatest in the immunized area.

SUMMARY

Dermatophytosis may result in acquired resistance to reinfection, both in animals and humans. Immunity is usually local and partial. Complete immunity is rare, but may occur at the infection site. The reinfection of the previously infected site is of shorter duration and shows less inflammation. Fungal elements are rarely detected. Immunity is always accompanied by cutaneous sensitization. Resistance often follows the more inflammatory forms of infection usually caused by zoophilic species; immunity occurs less frequently after infection by anthropophilic species.

The "trichophytin" reaction is the term for the cutaneous hypersensitivity which may develop during infection.

Both immediate- and delayed-type cutaneous hypersensitivities occur in infected animals and in humans. The delayed-type reaction appears early and is usually associated with the resistance to reinfection. Immediate hypersensitivity appears later and is found in chronic infections, especially those due to *T. rubrum*.

The commercially available "trichophytin" preparations are mixtures of crude extracts from dermatophytes and vary in their composition. They are of little value for diagnostic purposes.

The "trichophytin" test is always positive in patients who develop dermatophytids. These are skin eruptions which appear at a distance from the focus of infection. They are believed to be due to the reaction between skin-sensitizing antibodies and fungal antigens carried by the bloodstream. Dermatophytids occur spontaneously in man and have been experimentally induced in rabbits and guinea pigs.

Antibodies are detected in both animals and humans with dermatophytosis. However, due to the crude nature of most antigens used, the antibodies detected by the more sensitive techniques, such as passive agglutination, are not always specific for the infecting organism.

Precipitins and complement-fixing antibodies appear to be more reliably associated with infection, but occur less frequently. Their relationship to resistance has not been established.

A natural "serum factor" is believed to contribute to resistance to dermatophytic invasion.

This component of normal serum is believed to restrict the growth of dermatophytes to the stratum corneum.

Dermatophytes have many antigenic determinants in common with each other as well as with unrelated fungi. Group-specific, species-specific, and "trichophytin"-reactive antigens have been recognized. However, isolation of these components has proved difficult. Attempts to classify dermatophytes according to serological reactivities have had little success.

The same groups of polysaccharides have been isolated from nine species and display only fine differences in structure and serological reactivities. These polysaccharides do not have "trichophytin" activity. Glycopeptides which do have "trichophytin" activity have also been isolated. They are similar in chemical structure, and those from several species give similar "trichophytin" reactions in sensitized guinea pigs. These glycopeptides were not compared by serological methods, nor was their homogeneity demonstrated. Keratinases have "trichophytin" activity. However, these have only been isolated from one species thus far.

Immunization against dermatophytosis has been most successful with whole mycelium or crude extracts. These were injected intra- or subcutaneously or applied topically. The immunity which resulted was usually greatest in the immunized area and was accompanied by cutaneous sensitization.

Progress in the understanding of the immune process in dermatophytosis will greatly depend upon further studies with purified, specific antigens.

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