

# THE PROTEOLYTIC ACTIVITY OF NON PATHOGENIC AND PATHOGENIC FUNGI

By

B. S. VERMA,\* Baroda.

PART II

**Pathogenic fungi**

(Dermatophytes)

Verujsky (1887) was the first to initiate studies of the physiology of ringworm fungi in his work with *Trichophyton tonsurans*. In 1894 Roberts inoculated an "artificially-reared" *Trichophyton* species on to human and animal hairs which had been removed. He observed that the fungus penetrated and disintegrated much as occurs in the natural process of ringworm infection. For the first time he was impressed that the process of disintegration was likely to be essentially one of digestion. Thereafter, there has been a continuing interest in the proteolytic activity of the metabolic products of pathogenic fungi.

Macfadyen (1894) working with some strains of ringworm fungi (species not named) drew conclusions from findings using different "soils" for growing them. He found that the solution of gelatin in which the fungi were grown possessed an active proteolytic property, and that such activity could be destroyed by heating for two minutes at 100°C. Acid reaction retarded this enzyme activity, while alkali enhanced it. He was not, however, able to satisfy himself that the proteolytic substance had any action on fibrin or hair, in spite of the fact that his *Trichophyton* strains would grow on "soils" composed almost entirely of keratin.

Roberts (1899) attempted to show that *Trichophyton* cultures in dried form would decompose gelatin by enzyme action. He concluded that "amylotic ferment" was not produced by the trichophyta and "*Trichophyton* microsporon" directs its keratolytic powers first at the cuticle and later to deeper keratin of hair, while "*Trichophyton* megalosporon" acts mainly by digesting the inner substance of hair leaving a relatively unaffected hollow shell of cuticle. He also emphasised that the enzymes he described, and which he called 'keratolytic', were different from the 'proteolytic' ferments previously described by Macfadyen. Roberts went on to criticise Sabouraud's division of trichophyta into "ectothrix" and "endothrix" types (Sabouraud, 1910) and suggested a reclassification according to their enzyme production.

Tate (1929) concluded from his studies that proteolytic enzymes are present in the dermatophytes which possessed properties very similar to trypsin. Using congo red fibrin he did not find any evidence that trypsin was amongst them. He also found that these fungi could readily split tributyrin into fatty acids, presumably due to the presence of a lipase. His results suggested that urease and amygdalin were present in greater amount in pleomorphic forms than in normal cultures. He did not think that pleomorphism per se changed the enzymatic activities of these fungi,

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\* Head of Skin and V. D. Dept., Medical College, Baroda.

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Verujsky's (1887) comparative studies of the morphology and physiology of *T. tonsurans* and *Achorion schoeleinii* was the earliest work on the physiology of dermatophytes. He found that a neutral or slightly acid medium was the most favourable for growth of the fungi and that the optimum temperature was about 33°C; he found that both fungi would liquify gelatin. *Achorion schoeleinii* was found to assimilate sugar in any form, while *T. tonsurans* utilized glucose but not saccharose. On malt medium he found that the ratio of "weight of *Trichophyton* to sugar consumed" is 1:2 but when 1% glycerin was added to the 'cospora' burm of microsporon of the medium this ratio was increased to 2:3.

Bodin (1899) found that the cospora form of "microsporon of the horse" (*M. equinum*) had an optimum temperature for growth of about 35°C. Glucose, dextrin and maltose were assimilated in this order of preference, but sucrose was not utilized. Later, he and Lenormand (1901) showed that this fungus produces two enzymes in the culture fluid, one which clots the milk and the other which dissolves the clot.

Tate (1929) studied a number of dermatophytes for their enzyme contents, but his results were inconclusive. More recently, Bentley (1953) and Chattaway et al (1954) demonstrated amino acid oxidase and asparaginase activity in ringworm fungi. In 1956, Cruickshank and his colleagues were able to demonstrate the skin-splitting property of filtrates of *T. mentagrophytes*.

Barlow and Chattaway (1955) studied the effect on susceptibility to fungal attack of changing the molecular structure of hair keratin. They showed that measures which encourage the breakdown of disulphide linkages and hydrogen bonds facilitate fungal invasion *in vitro*. The reverse is the case when cross-linkages are increased between free amino and carboxy groups. Raubitschek (1961) in challenging the thesis that the increase in amino acids observed in substrates by the action of ringworm fungi is due to keratolysis, has suggested that they originate from fungal autolysis. This problem was further studied using electronmicroscopy (Mercer and Verma 1963), Polarised microscopy (Verma 1965) and fluorescent microscopy techniques (Verma 1966). It was found that *T. mentagrophytes* perforate the human hair *in vitro* by a process of enzymatic digestion.

#### REFERENCES

- Barlow, A. J. E., and Chattaway, F. W. (1955). *J. invest. Derm* 24, 65.  
 Bentley, M. L., (1953). *J. gen. Microbiol.*, 8, 365.  
 Bodin, E. (1899). *Arch. Parasitol.*, 2, 362-376.  
 Bodin, E., and Lenormand, C. (1901). *Ann. Inst. Pasteur.* 15, 279-288.  
 Chattaway F. W., Thompson, C. L. and Barlow, A. J. E. (1954). *Biochem. biophys. Acta.* 14, 583.  
 Cruickshank, C. N. D., and Trotter, M. D. (1956). *Nature, Lond.* 177, 1085.  
 Macfayden, A. (1894). *J. Path. Bact.*, 3, 177-183.  
 Mercer, E. H. and Verma, B. S. (1963) *A. M. A. Arch. Derm*, 87, 357.  
 Raubitschek, F. (1961) *Sabouraudia*, 1, 87-90.  
 Roberts, L. (1894). *J. Path. Bact.* 3, 300-309.  
 Roberts, L. (1899). *Britt. Med. J.*, 1, 13-14.  
 Sabouraud, A. (1910). *Less Teignes*, 1st ed., Paris L. Masson.  
 Tate, P. (1929). *Parasitology*, 21, 31-53.  
 Verujsky, D. (1887) *Ann. Inst. Pasteur*, 1, 369-391.  
 Verma B. S. (1965) *Acta. Derm-Venerol.* 45, 196.  
 Verma, B. S. (1966) *Brit. J. Dermat.* 78,222.