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THE BIOLOGICAL PREREQUISITES OF PATHOGENICITY OF DERMATOMYCETES

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In 1933, A. v. Mallinckrodt-Haupt publishod her basic work under the title Der Stoffwechsel der pathogenen Hautpilze und sein Zusammenhang mit der Pathogenese der Mykosen [The Metabolism of the Pathogenic Skin Fungi and Its Connection with the Pathogenesis of Mycoses]. With admirable insight, the author critically reviewed and evaluated the large number of publications available on this topic at that time and she then supplemented these observations on the basis of her own very careful experimental studies although the methodology may appear somewhat outdated today. In the meantime, 30 years have passed and during that time a wealth of new knowledge has been obtained throughout the world on the biology of the dermatomycetes as well as on the pathogenesis of dermatomycoses. Last but not least we must mention here the great advances in the natural sciences which in dermatomycology likewise contributed to the enhancement of our knowledge both with respect to the biology of the fungi and with respect to the morphology and physiology of the terrain in which the dermatomycetes settle as parasites; finally we must also mention the therapy of mycoses which was greatly advanced as a result of this. We therefore think that it would be quite proper for us to say a few words here about the biological foundations of the pathogenicity of dermatomycetes, in the light of what we know today. These comments on the host-parasite relationship (HPR) spring primarily from the viewpoint of the plant physiologist. We therefore want to confine ourselves to the description of the measurable physiological properties of the dermatomycetes and a comparison with apathogenic, partly soil-inhabiting and keratinophile molds. We therefore want to emphasize here the attacking fungus as the agents responsible for mycoses and we want to leave the explanation of the HPR, as regards the host organism, the attacked human individual or animal, to those who are authorities in that particular field, in other words, we want to leave this to the dermatologists. We are of course quite aware that, in the final analysis, all mycological efforts, regardless of what angle they are undertaken, can lead to success only if they are aimed at one single objective and that of course is the combination of all of the individual discoveries that have been made so that we may develop a therapy of dermatomycoses which will be well founded on the basis of a causal analysis and which will thus be quite specific. In order to

describe the physiological foundations of the pathogenicity of dermatomycetes in greater detail, we might first of all look at the special physiological properties of these fungi and we might then report some comparative investigations with molds. However, we do not have enough time here to make any statements as to methodology although it is precisely this methodology which is very often decisive and quite important in judging the results! Since our statements here can give the reader only a rough survey, we want to confine ourselves to a listing of the facts which are largely based on our own investigation results. Undoubtedly, keratinophilia deserves being mentioned first among all of the specific properties of the dermatomycetes, although the capability for keratin reduction (in other words, the possession of keratinase and peptidases) is by no means the domain of the skin fungi; after all, there are some other fungi which can utilize keratin as a primary C-N source although exclusively in the form of saprophytes. As regards the biochemistry of keratin decomposition, we will report on this later on (Ziegler, 1964). As far as the nutrition-physiological value of other N-sources is concerned, we know that the dermatomycetes can utilize a large number of simple organic N-compounds (amino acids [AS], urea, casein-hydrolysates) but also peptones, gelatins, denatured serum, and other proteins (survey in Nickerson, et al, 1947; Ziegler and Bohme, 1964). We have some rather contradictory information on the suitability of inorganic N-sources (NH_4 salts, nitrates). Apart from the fact that the particular KH-source and base-nutrient solutions (NL) are of significance, we can say that the results of our investigations now make it possible to make a certain decision. Accordingly, non-pleomorphous dermatomycetes require AS or other organic N-sources (urea, peptone, gelatin, keratins) for their initial growth, but they can also include NH_4 ions in their N-metabolism (Ziegler, 1964). An NH_4 titer which rises at first (because of the release of NH_4) or which is high from the beginning of the experiment on and which drops afterward can be considered as an indicator for progressive growth processes. There is no strict order of rank within the carbohydrates (KH) which are suitable for the nutrition of the dermatomycetes. In addition to a few mono- and oligosaccharides (mannite, glucose, saccharose, cellobiose, fructose, maltose, raffinose, lactose), these fungi can convert polycondensed KH (starch, glycogen, malt extract, pectins, and probably also cellulose, among others) partly even better than the oligosaccharides (Stockdale, 1953; Bereston, Robinson, and Williams, 1958; Koehne, 1962; Ziegler, 1961; 1964; Goddard, 1934; Hejtmanek, 1959, 1960, 1961, 1961a). But the pathogenic skin fungi certainly do not play a role as nutrition rivals or competitors for the fast-growing fungi which decompose cellulose, pectin, or starch. Their more or less pronounced amylo-, pectino-, or cellulo-lytic capacities should be considered as original characteristics. Furthermore we know that dermatomycetes can use various lipids as a primary C-source (bibliography in Ziegler, 1964). At this point we would like to emphasize particularly the capability for the decomposition of wool fat (Adeps lanae anhydricus) which we proved beyond doubt. From this we can conclude that dermatomycetes attack or at least can break through the protective fat deposits of the skin and its appendixes. The requirement for vitamins varies; most of the skin fungi are vitamin-autotrophic (Area Leao and Cury, 1951). The supply with mineral nutrients (Ziegler, 1964)(Na, K, Mg, Ca, Cu, Fe, Zn, Mn, among others, as well as P, S, Cl) can be kept rather short. The phosphate

requirement of the skin fungi, which is strikingly low in comparison to other microorganisms, should be interpreted as an adaptation to the relatively unfavorable mineral supply conditions in keratinized terrains. Another characteristic which has a rather selective significance here is the "alkalizing tendency" of the dermatomycetes. When the composition and the buffer capacity of NL or of the terrain attacked so permit, these fungi, like numerous human-pathogenic bacteria, raise the pH values into the range of pH 6-8. Elsewhere (Ziegler, 1963, 1964, 1964a) we reported on the enzymes of the skin fungi, in other words, their ecto-enzymes, which are separated for nutrient substrate decomposition or conversion respectively for the mobilization of nutrients; and we emphasized the tremendous significance of these enzymes for both the saprophytic and the parasitic phases. In dermatomycetes, particularly in microsporum canis and trichophyton metagrophytes, the following ecto-enzymes have been established: amylases, pectinase, cellulase, lipases, alkaline phosphatase, peptidase (proteinases) and keratinase. As we know, specific nutrient substrates generally lead to an increase in the synthesis and in the separation of the adequate enzymes. In our enzyme studies, we were also able to observe a series of coupled physiological mechanisms. In cultivating the fungi on keratins, wool fat, or polysaccharides, it was not only the enzymes directly required for this that were separated more abundantly than under simple nutrition conditions (STD-NL) but it was also at the same time a series of enzymes that was formed here to an increased extent for other reactions ("nonspecific induction"). For instance, the lipase and phosphatase titers in keratin-NL were increased although no substrates required for these enzymes were present; on the other hand, the peptidase titer in protein-free NL with complex, respectively, polycondensed C-sources (polysaccharides, wool fat, etc) was increased. Over-all we can say that the small groups of dermatomycetes has a series of characteristic properties: (1) they require very little in terms of nutrition-physiology; (2) they reveal a pronounced keratinophilia; (3) they grow relatively slowly; (4) they are capable of surviving on organic materials in the ground; (5) they reveal a tendency toward and tolerance of alkalization; (6) they have polyenzymatic capabilities; (7) they reveal coupled enzymatic mechanisms (nonspecific induction). To what extent are we dealing here with specific properties of dermatomycetes? We tried to answer this question by means of comparative investigations with molds. Such investigations have unfortunately been extremely rare in the past (for instance, Volkonovsky: Ernahrungsphysiologische Studien 「Nutrition Physiology Studies」, 1934; White, Darby, Stechert, and Sanderson: Zelluloseabbau 「Cellulose Decomposition」, 1948; Rzucidlo, Stachow, Nowakowska and Kubica: Zellwandchemie und Immunologie 「Cell Wall Chemistry and Immunology」, 1958, 1963; De Vries: Keratinabbau 「Keratin Decomposition」, 1962; Jung and Gerhart: Redoxferments 「Redox Ferments」, 1963; Chesters and Bull: Laminarinabbau 「Laminarin Decomposition」, 1963). We (Ziegler, 1964 unpublished) compared the nutrition-physiology behavior of microsporum canis and trichophyton mentagrophytes with the behavior of some molds (Absidia glauca, Aspergillus niger, Stemphylium tetraedricoglobosum, Fusarium oxysporum, Cladosporium spec, Penicillium janthinellum, and Penicillium lilacinum). These experiments are still in progress and they are being expanded. Such molds are generally extremely vital and they

very fast; they are encountered relatively frequently in soil samples (Cladosporium, Stemphylium, Penicillium janthinellum, Penicillium lilacinum); some of them form antibiotics (Penicillium janthinellum, Janthinellin); most of them are very active decomposers of cellulose, hemicelluloses, and pectin. Our series of experiments produced the following results: in the cultivation of the fungi in STD-NL (Ziegler, 1964), the growth rate, the maximum mycelium weights, the relative and the absolute mineral requirement, especially the phosphate requirement, as well as the carbohydrate (KH) requirement were greater for the molds (particularly for Penicillium janthinellum and Penicillium lilacinum) than for the dermatomycetes. In the cultivation of fungi in hair keratin NL, the growth rate, the percentage-wise keratin decomposition, and the relative peptidase activity were greater in the dermatomycetes than in the fungi (for details, see Ziegler, 1964). The easily attacked horn keratin was rapidly decomposed by keratinophile molds (Penicillium janthinellum, Penicillium lilacinum), although this happened just as fast as in the case of microsporum canis and trichophyton mentagrophytes. Molds furthermore differ from skin fungi by virtue of the fact that, in the case of the former, poorly suitable or unsuitable N-sources, such as nitrates or ammonium salts, can be used and that the former, because of their many-sided enzymatic capabilities, are more adaptable in terms of nutrition physiology than the dermatomycetes [sic]. The latter have probably lost a number of their original properties in the course of the development of their capability for keratin decomposition. Our past comparative investigations tell us that we have primarily quantitative differences between the characteristics of the dermatomycetes and those of the keratinophile molds. Just exactly whether an accumulation of these differences during the course of the development of the fungi leads to a change in the quality so that pathogenic dermatomycetes developed from keratinophile molds -- that is something that can be expressed only in the form of an assumption here. But if this assumption were to apply in fact, then such molds could be considered as potential parasites from which new pathogenic forms could develop. To what extent can the knowledge obtained in vitro with respect to the general and special physiology of the dermatomycetes be applied to the in vivo conditions? Mineral nutrients, including trace elements, are present in the epidermis and in the perspiration only in small quantities (Mitchell and Hamilton, 1949; Dutcher and Rothman, 1951; Anke and Schneider, 1962). But they are sufficient for the requirements of the dermatomycetes (English and Barnard, 1955) which, moreover, can mobilize organically bound phosphate (Szakall and Weber, 1959; Blakey, Earland and Stell, 1963) by means of their ecto-phosphatases. Organic compounds in the form of proteins, lipids, and secondarily, also polysaccharides constitute the main portion of the potential nutrients in the epidermis. For the hydrolysis of these compounds, the skin fungi have specific enzymes. The pH values of the skin are constant but they are mostly in ranges which can be tolerated by the dermatomycetes (the underarm bending side pH 4.0-6.0). Moreover, these fungi are in a position to raise the environmental pH values to the levels required for their ecto-enzymes because of their alkalizing tendency. This is particularly important for enzymatic keratin decomposition, which takes place rather briskly only under conditions ranging from neutral to slightly alkaline. In addition to the previously mentioned nutrition-physiological qualities of the epidermis, we have a number of other properties of the host organism that are very important for the

spread and the course of fungus infections (Mallinckrodt-Haupt, loc cit, Blank, Sagani, Boyd, and Roth, 1959; Barlow, Chattaway, Brunt and Townsley, 1961). For instance, the penetration of the fungi is either promoted or delayed by the speed of the normal keratinization processes and the subsequent scale removal "loss". On the basis of some individual observations we know that dysproteinemia promotes chronic trichophytia (Desai, Modi, and Bhat, 1962) and that presumably qualitative differences in the composition of the keratin can cause a partial onychomycosis (Langhof, Ettig, and Lemke, 1963). On the other hand, Burke (1962) was able to establish only very small quantitative and no qualitative differences in the AS spectrum of the skin of healthy persons and fungus patients. So far, we have hardly any accurate and generally applicable details on the defensive reactions of the host organism. In the skin fat and particularly in the hair fat of adults (for instance, Rothman, Smilganic, and Shapiro, 1945; Vilanova and Casanovas, 1949) and in the serum (for instance, Memmesheimer, McNall, and Sternberg, 1962; Bielunska, 1963) we do find fungistatic components but so far no one has been able to establish a basically higher serum fungistasis titer in patients than in healthy people. Likewise it was impossible to determine any reliable differences between the quantity and the components of the skin fat in healthy people and in fungus patients. With respect to the etiology of mycoses, however, it was possible to identify a certain predisposition for those infections in constitutionally determined relatively high ($pH > 5.5$) pH values, combined with a reduced buffer capacity in the epidermis (Jolly, Hailey, and Netick, 1961; Zucker, 1964). There are no fundamental differences between the nutrition-physiological requirements of the dermatomycetes in vitro and those in vivo. We hope that we have presented a survey of the current status of special metabolism physiology of dermatomycetes in this article. We hope that the comprehensive knowledge of the characteristic properties of this fascinating fungus group will lead not only to a better understanding of the biological foundations of their pathogenicity but also to new points of departure for the fight against these fungi.

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