Elastase: Production by Ringworm Fungi

Abstract. *Isolants of nine species of* Trichophyton, *one of* Epidermophyton, *and four of* Microsporum *were assayed for elastase activity. The species or isolants with elastase activity were obtained from patients with inflammatory ringworm infection. In* Nannizzia fulva (M. fulvum), *plus-mating-type strains were elastase-positive and minus-mating-type strains elastase-negative. A genetic study of mating type and elastase activity indicated a monogenic basis for both mating type and elastase activity.*

Many pathogenic fungi produce an extracellular enzyme, elastase, which degrades elastin, a scleroprotein (*1*). Isolants of dermatophytes from inflammatory or noninflammatory ringworm infections were surveyed for elastase activity by demonstrating their ability to clear particulate elastin (1 percent) suspended in nutrient agar.

Only organisms from the initial cultures isolated from clinical ringworm infections were used in the survey. Four of 18 isolants of *Trichophyton mentagrophytes* originated from patients with a marked or severe inflammatory infection; two of these patients exhibited pitted scarring and areas of permanent alopecia. These four strains had elastase activity, but the remaining 14 had no detectable activity. Twelve isolants of *T. tonsurans*, eight of *T. verrucosum*, four of *T. schoenleinii*, as well as five isolants of *T. soudanense*, four of *T. gourvilii*, and three of *T. yaoundei*, recently obtained from patients in Africa, had elastase activity. Twenty-three isolants of *T. rubrum*, two of *T. concentricum*, two of *T. megninii*, ten of *Epidermophyton floccosum*, seven of *Microsporum audouinii*, eight of *M. canis*, and five of *M. gypseum* (*Nannizzia gypsea* and *N. incurvata*) did not have detectable elastase activity. In *M. fulvum* (*N. fulva*), however, two isolants had elastase activity, and one did not. These observations were confirmed by assaying filtrates from cultures grown in tryptose-yeast-glucose broth, with the use of the orcein-elastin procedure (*2*).

The discovery of the perfect stage of *M. fulvum* indicated that this species, as *N. fulva*, is heterothallic; that is, strains of both plus and minus mating types are required for the formation of cleistothecia (*3*). A collection of 21 isolants, including the three that were recently obtained from ringworm infections and used in the survey, were tested for elastase. Each isolant was paired with a standard mating type and grown on soil-hair agar medium (*3*). Twelve isolants were minus mating type and elastase-negative, and nine were plus mating type and elastase-positive.

Strain 2181 (plus mating type and elastase-positive) and strain X644 (minus mating type and elastase-negative) were crossed. One mature cleistothecium was crushed and 100 ascospores were isolated at random (*4*). The mating type and elastase activity of each of the 100 monoascosporic cultures were determined. Fifty-eight cultures were minus mating type and elastase-negative, and 42 were plus mating type and elastase-positive. These observations indicate a good fit to a 1 : 1 ratio for both mating type and enzyme activity. No evidence is available at this time to suggest that elastase activity and the plus mating type are determined by a single locus. These phenotypes could be determined by very closely linked genes.

Of the three strains of *M. fulvum* recently isolated from patients and included in the survey, the two plus-mating-type and elastase-positive isolants came from inflammatory infections and the one minus-mating-type and elastase-negative isolant from a noninflammatory infection. Although these observations indicate a relation between elastase activity and inflammatory infection, additional evidence would be required to demonstrate a causal relationship. Rippon and Peck (*5*) have recently shown an association between collagenase activity and the ability to produce disease in the human mycetoma agent, *Streptomyces madurae*.

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Embryonic Morphogenesis: Role of Fibrous Lattice in the Development of Feathers and Feather Patterns

Abstract. *In the morphogenesis of embryonic feather germs the formation of dermal cell groupings is associated with the development of a highly regular pattern of birefringence in the dermis. This birefringence is due to a lattice-like system of collagenous tracts along which dermal cells become progressively aligned and grouped in regularly spaced sites. The experimental results suggest that this fibrous lattice is of major significance in the morphogenesis of feather germs and in their characteristic pattern of distribution.*

Inductive interactions between dermis and epidermis during the development of feather germs in the chick embryo have been studied extensively (*1*). However, little is known about the morphogenetic mechanisms which determine the localization of feather germs and their characteristic patterns of distribution. During a recent study of feather-germ formation, certain hitherto unknown organizational features relevant to this problem were observed and examined. We found that in the feather-forming area of the dorsal skin a portion of the dermal cell population becomes organized into distinctly oriented tracts of elongated cells; this process coincides with the deposition of tracts of fibrous material which closely correspond in location to the tracts of the dermal cells. The analysis of these events suggests that morphogenesis in the feather-forming areas is closely associated with the formation of the fibrous tracts and that the latter may provide an integrating framework for the distribution pattern of developing feathers in the dorsal skin.

Sheets of mid-dorsal skin from chick embryos at stages 29 through 32 (approximately 6 to 8 days of incubation) were studied immediately after removal from the embryo and following a period of cultivation in organ

culture. Continuous observations on skin sheets developing in organ culture revealed that the first noticeable change in the dermis in connection with feather development is the appearance in the midline of cells elongated in an anterior-posterior direction. This precedes the formation of the condensations of dermal cells (dermal papillae); however, small clusters of cells appear early in this dermal tract and mark the sites of the first row of dermal papillae.

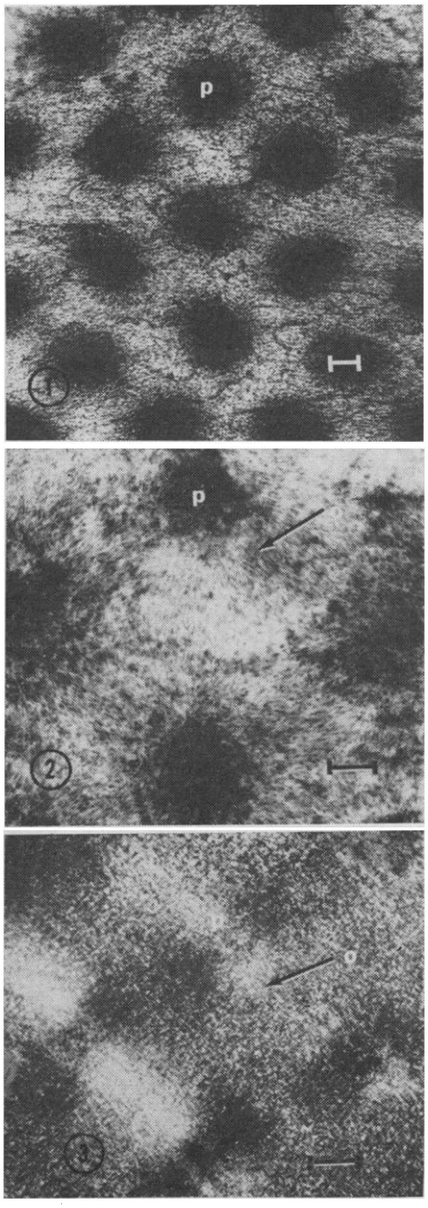

Fig. 1. Whole mount of the central portion of the dorsal skin from an 8-day chick embryo; pattern of developing feather papillae (*p*). Fig. 2. Portion of the feather field showing four dermal papillae (*p*) and oriented interpapillar cells (*o*); 8-day chick embryo. Fig. 3. Whole mount of the dermal fibrous mat after digestion with trypsin, stained with Chromosome Red and photographed through polarizing optics, showing the birefringent lattice and oriented fibers (*o*) between papillar sites (*p*). Bar represents 0.01 mm.

By stage 32, the entire dorsal feather field had become progressively organized into a lattice-like system of tracts of oriented dermal cells linking the dermal papillae or their presumptive sites. Figure 1 shows the distribution of feather germs as seen in whole mounts of dorsal skin; Fig. 2 shows tracts of elongated cells between the papillae. The elongation of the dermal cells resembles that shown by cells growing in vitro on a substrate of oriented fibers (*2*, *3*); the overall appearance suggests that the arrangement of dermal cells in this macropattern is related to the microstructure of the substratum.

To study the localization of the fibrous material, we separated the dorsal skin from early embryos into epidermis and dermis by treatment with trypsin; these were stained with Chromosome Red (*4*) and examined with a polarizing microscope as whole mounts. The epidermis in all stages examined contained a meshwork of generally distributed, birefringent material with no preferential localization. The dermis also had such general birefringence; however, in addition a distinct pattern of birefringence appeared in the dermis as its development progressed; it was first noticeable as a streak in the midline, then it extended laterally in the form of a lattice. The tracts of oriented dermal cells described above followed this lattice closely.

To examine further the nature of the birefringent lattice, we mounted sheets of dermis on slides, fixed them in 70 percent alcohol and subjected them to prolonged treatment with trypsin. The residual material consisted of a fibrous mat which showed a pattern of birefringence entirely similar to that observed in whole skin or in isolated dermis and which coincided with the tracts of the oriented, interpapillar cells (Fig. 3). At the sites of dermal condensations, the tracts of birefringent fibers intersected. Preparations stained with Van Gieson's stain for collagen revealed an identical pattern of fibers.

The collagenous nature of this fibrous lattice was further suggested by its sensitivity to collagenase (CLSP-A, Worthington; 0.1 mg per milliliter of Tyrode's solution; treatment for 1¼ hours at 45° to 50°C). Since the CLSP-A preparation contains only the monomer form of collagenase and a very small proportion of caseinase (*5*), it appears that collagen constitutes a major component of the fibrous lattice.

The relationship of this fibrous lattice to skin morphogenesis is suggested by observations on embryos treated with hydrocortisone in which feather development is either totally or partially inhibited (*3*). In cases of total inhibition, neither oriented dermal cell tracts nor an organized fibrous framework were found. In cases of partial inhibition, fibrous strands and oriented dermal cells were found only in regions containing dermal papillae. Furthermore, when skin, at a sensitive period of development, is cultured in vitro in the continuous presence of collagenase, the development of feather germs is inhibited; short exposure to collagenase delays their development for approximately 24 hours. These findings are of special interest in the light of recent evidence which implicates collagen in the morphogenesis of embryonic salivary glands (*6*).

The organization of the fibrous material in the dermis into detectable tracts and lattices of aligned fibrils precedes the appearance of the dermal papillae and thus foretells incipient dermal morphogenesis; this suggests, as a concept for further exploration, that the lattice functions in the alignment of dermal cells in early embryonic skin and thus in organizing the macropattern of the developing feather tracts. These findings also raise the possibility that cell movements and cell aggregations play a significant role in the morphogenesis of dermal papillae and in feather development.

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