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The Ratio of Soluble to Insoluble Collagen in Normal Chick Dermis*† (32811)

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Weiss and Matoltsy (1) reported a total absence of wound healing in chick embryos prior to the tenth day of incubation. The epithelial cells of the wound margin, instead of covering the wound surface as they do in older embryos, penetrate the underlying mesenchymal stroma. We thought that the explanation for this behavior of the epithelium should be sought in the embryonic mesenchyme.

The importance of the mesenchyme, e.g., the dermis, for the differentiation of the epidermis and its derivatives during embryogenesis has been well established (2–5). Differentiation and proliferation of epidermal cells in newts was found to be dependent on mesenchymal factors (6). McLoughlin (7) was able to alter specifically the differentiation of embryonic epidermis by combination with mesenchyme from various organs. Epidermal cells cultured without mesenchyme do not differentiate but degenerate, whereas frozen–thawed mesenchyme is able to support epidermal differentiation (8). This property of frozen–thawed mesenchyme, however, is lost after its exposure to trypsin (8). This finding supports the assumption that large molecules are responsible for the inductive action of the mesenchyme. Wessells (9) succeeded in inducing and maintaining cornification of embryonic chick epidermis in the absence of mesenchyme by explanting it on top of a Millipore membrane and supplying it with a suitable concentration of embryo juice. Other substrata failed to support epidermal differentiation. These findings are regarded as evidence for the importance of the physical structure of the substrate for epidermal cornification.

Collagen is one of the most important structural elements of the dermis. Dodson obtained differentiation of epidermis grown on collagen gells. Enquist and Adamson (10) stated that "a lack of healing means absence of collagen synthesis." In view of these data, a correlation between collagen polymerization in granulation tissue and epidermal wound coverage appears plausible. Our investigations, reported in this paper, were concerned with

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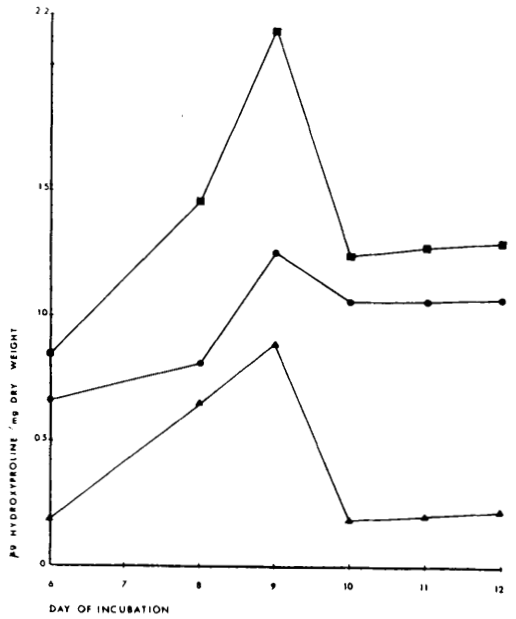

FIG. 1. Hydroxyproline content in collagen fractions in the skin of chick embryos. ▲ Neutral, salt-soluble; ● insoluble; ■ total.

the question of the extent to which changes in collagen polymerization are correlated with changes in wound healing in chick embryos.

Materials and Methods. The dorsal skin was taken from chick embryos from days 6 to 12 of incubation (10–20 animals for each day) and defatted in ether for 24 hours with occasional shaking and changing. Soluble collagen was extracted using the methods of Hosoda (11) in neutral salt buffer or citric acid buffer, respectively. Precipitation of the various collagen fractions was achieved by addition of sodium chloride to a final concentration of 20%. The precipitated collagen was spun down at 18,000*g* for 1 hour. It was then hydrolyzed in 6 *N* hydrochloric acid at 115°C for 12 hours. The hydroxyproline content was determined according to Neuman and Logan (12). The insoluble collagen remaining after salt and acid extraction was also hydrolyzed and the hydroxyproline determined by Newman and Logan's method. The proportion of total collagen which was incorporated into dermal fibers was estimated by determining the respective ratios of insoluble and soluble collagen to the total collagen. This was done at intervals between days 8 and 12 of development. The results presented here were gathered from 4 series of experiments.

Results. The total content of skin collagen doubled from the sixth to the tenth day of embryonic life. On day 10 the collagen content dropped to a value of 60% from day 9 (Fig. 1). There was virtually no change in the amount of collagen from days 10 to 12 (Fig. 1). Between days 9 and 10 of incubation, however, there was a marked increase in insoluble collagen and a simultaneous decrease in soluble collagen (Fig. 2). On day 9, insoluble collagen constituted 60%; and on day 10, 85% of the total collagen. This ratio of 85% insoluble to 15% neutral salt soluble collagen in the skin persisted until day 12 of development when our studies terminated. Acid soluble collagen was only found in a few cases in very minute amounts.

Discussion. If it is assumed that the structure of the dermis is responsible for the control of the epidermis, the total collagen values are less meaningful than the amount of collagen which is polymerized into fibers. In

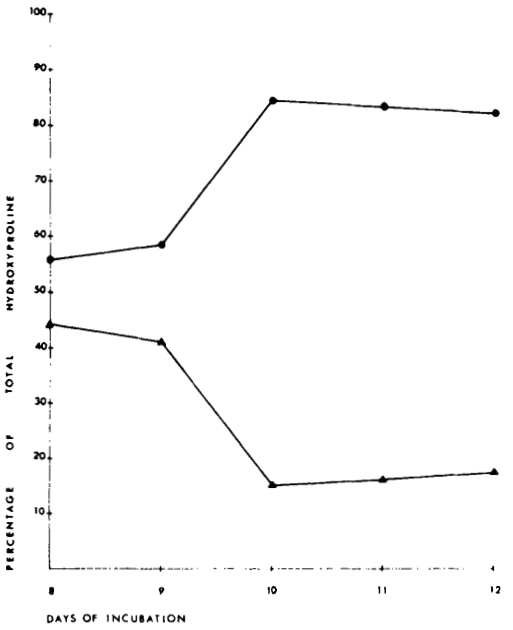

FIG. 2. Ratio of each fraction to total collagen content in chicken embryo skin between days 8 and 12 of incubation. ▲ Neutral, salt-soluble; ● insoluble.

our studies, the collagen content of 10-day embryonic skin dropped to 60% of that on day 9. This is because hydroxyproline values were determined for *whole* skin, not for the dermis alone. On day 9, the epidermal component grew much more rapidly, due to feather formation, than the dermis. The *relative* amount of the collagen is therefore reduced without a decline in the absolute values. The shift in the ratio of insoluble to neutral salt soluble collagen, in the developing dermis, parallels a similar change observed in whole chick embryos (13). A marked increase in insoluble collagen was also found in wound healing of mammals between 8 and 12 days after wounding, especially around day 10 (11). According to Gillman *et al.* (14) and Lindsay and Birch (15), this coincides with the time when the wound epidermis stops its infiltrative downgrowth and begins to cover the wound surface.

The epidermal wound infiltration observed by Weiss and Matoltsy in chick embryos less than 10 days old could be related to the poor collagen polymerization at these early stages. The close time relationship between an increase in collagen polymerization and the first appearance of wound healing suggests that the collagen structure of the dermis strongly influences the behavior of epidermal cells.

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Serologic Grouping of *Cryptococcus neoformans* (32812)

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Evans (1,2) divided the species *Cryptococcus neoformans,* a pathogenic fungus, into three antigenic types, A. B, and C by agglutination, precipitation, and capsular reactions. Agglutinins in the sera of rabbits immunized with killed cryptococcal cells have appeared to be directed against both type specific (2) and species specific (3) antigens. The antigens concerned with type specificity have been associated with the capsular polysaccharide (4). The purpose of this study was to examine further the antigenic variation among isolates of *C. neoformans* and to determine whether serotyping would have value as an epidemiological tool.

Materials and Methods. Cultures tested. All cultures were designated *C. neoformans* by previously published criteria (5). In this scheme, *C. neoformans* was separated from

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