An Autoradiographic Demonstration of the Effect of the Corneal Epithelium on Amino Acid Incorporation into the Insoluble Constituents of the Corneal Stroma.*

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In previous experiments, (1, 2) it was observed that the incorporation of glycine-1-C^{14} into protein fractions of the corneal stroma of the chick eye was greatly reduced in the absence of the corneal epithelium. Partial removal of the epithelium from the surface of the stroma led to a proportional reduction in the incorporation of the label into the stromal proteins. This finding suggested that the effect of the epithelium was restricted to a limited, closely adjacent area. It seemed desirable, therefore, to ascertain autoradiographically the extent of the epithelial influence on the uptake of label into the stroma. Such autoradiographs actually do show that the area in the stroma with high label uptake corresponds very closely to the area of the epithelial cover.

Methods

The dissection and the conditions for the uptake of glycine-1-C^{14} were described previously (1, 2). The chick corneas were obtained from 8-day hatched chicks of the High Line strain. Each cornea was suspended in a 10 ml. beaker in 2 ml. of a synthetic tissue culture nutrient solution containing glucose, amino acids, vitamins, and electrolytes. Twelve μg. in a solution of 0.01 ml. of glycine-1-C^{14} (Nuclear, Chicago) corresponding to an activity of 0.6 μC. were added to the samples at the same time, and incubation at 37°C. in a Dubnoff shaker was started immediately after addition of the label. In the present series of experiments, one-half of the epithelium was removed with a sharp scalpel before incubation with the label. After an incubation period of 1 hour, the corneas were briefly washed with Ringer's solution. Unbound glycine was removed from the cornea by repeated washings in 70 per cent and 80 per cent alcohol before fixation. After fixation in Bouin's solution, the corneas were imbedded in paraffin and sectioned at 10 μ. Sections were cut perpendicular to the corneal surface and to the line of removal of the epithelium. The sections were transferred to slides dubbed in chrome alum gelatin (3), covered with emulsion from Kodak 35 mm. autoradiographic permeable base safety stripping film, and kept at 4°C. for 4 weeks. After this exposure, the slides with the covering emulsion were developed in Dupont 53-D developer for 2 minutes and fixed and washed in the usual manner.

RESULTS

From the photograph in Fig. 1, it can be seen that the incorporation of glycine into the insoluble fraction of the corneal stroma extends exactly as far as does the epithelial cover. In the area covered with epithelium the label is found in about the same high density throughout the thickness of the stroma. The transition from the high grain density in the epithelium-covered area to the background density in the epithelium-free area of the stroma is very abrupt.

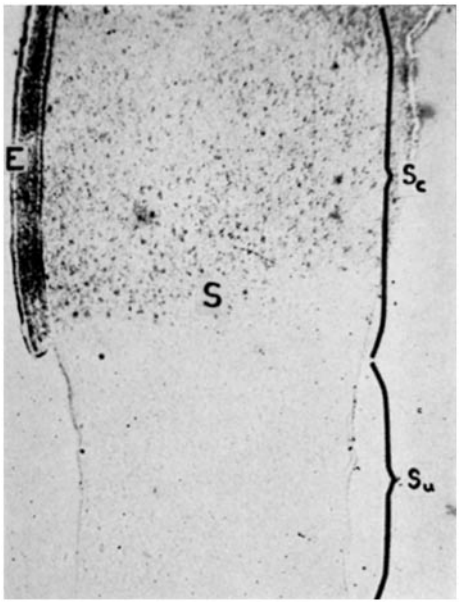

FIG. 1. Cross-section through portion of chick cornea after incubation with glycine-1-C^{14} and radioautographic demonstration of extent of labelling. Epithelial cover partially removed before incubation. × 140.

E, Epithelium. *S*, Stroma. *Sc*, area of stroma covered by epithelium. *Su*, area of stroma not covered by epithelium.

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The autoradiograph of the epithelium as a whole appears to have a much higher grain density than does the stroma. This is due, in part, to the higher cell number per unit area in the corneal epithelium than in the corneal stroma of the young chick. The higher grain density in the epithelium is also due to a markedly higher uptake of glycine-1-C^{14} per cell in the epithelium than in the stroma (unpublished). Within the epithelium a particularly high uptake was observed in the basal cell layer which may be related to its proliferative activity. The demarcation of the epithelium into three separate zones which is apparent on the photograph is an optical artefact which arises from focusing on the gelatin layer of the autoradiograph.

DISCUSSION

The sharply defined delimitation of the high grain density in the area of the stroma covered with epithelium demonstrates the close spatial dependence of the label uptake in the stroma upon some factor contributed by the epithelium. In the direction perpendicular to the surface, the effect of the epithelium extends over a distance of about 500 μ. It is the more remarkable that there is no indication of a spreading of this effect into the area without the epithelial cover. This abruptness of the demarcation of high and low density areas in the stroma gives rise to the question whether this effect is actually due to an agent which is released by the epithelium and diffuses freely through the stroma. This would appear to be the most obvious assumption. However, the picture obtained in the autoradiograph would suggest an effect which is propagated in a non-random fashion and in a direction predominantly perpendicular to the surface of the stroma. Although such a possibility seems at the present time to be without precedent, it would not seem to be altogether impossible and is being considered in the further exploration of this phenomenon which is in progress at this laboratory. Among the insoluble components, the stroma proteins, and in particular a collagen fraction, have been shown previously (1, 2) to incorporate glycine-1-C^{14}. The possibility that other unextracted constituents, *e.g.* nucleic acids, contribute to the sharply defined glycine uptake has not been excluded in the present study. Concentration differences of free glycine-1-C^{14} as a possible cause of unequal labelling in the epithelium-covered and uncovered portions of the stroma have been ruled out by direct measurements of amino acid content.

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