COLLAGEN SYNTHESIS BY THE EPITHELIAL ENAMEL ORGAN OF THE EMBRYONIC RABBIT TOOTH*

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SUMMARY

Epithelial enamel organs and dental papilla mesenchyme were separated from 26-day embryonic rabbit molar tooth rudiments. The isolated epithelia and mesenchyme were incubated overnight in the presence of H^3-proline and the collagens synthesized by both tissues were characterized by CM-cellulose chromatography. The epithelium produced 3 times as much collagen per tissue as the mesenchyme. This epithelial collagen contained an excess of $\alpha 1$ chains ($\alpha 1/\alpha 2 \sim 3.0$) suggesting the presence of two species of collagen, probably types I and IV. The mesenchyme appeared to produce only type I collagen. The extracellular matrix between the enamel organ and dental mesenchyme plays an important role in tooth morphogenesis and the present data indicate that a major portion of the collagenous component of this material is derived from the epithelium.

Epithelial-mesenchymal interactions in embryonic tissues have been the subject of extensive study in the tooth and the interdependence of the enamel organ epithelium and dental mesenchyme has been well described (1, 2). Particular attention has focused on the role of the extracellular matrix between these two dental tissues since it seems capable of promoting morphogenesis in both isolated epithelia and mesenchyme (3).

The possibility that collagen may be involved in embryonic tissue interactions has received considerable attention (4, 5, 6) and the biosynthetic capacity of several embry-

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onic epithelia to produce collagen is well documented (5, 7, 8, 9). The present report demonstrates that the morphogenetically important extracellular collagenous matrix, interposed between tissue interactants during embryonic mammalian tooth development, is produced by both the epithelial enamel organ and the adjacent dental mesenchyme.

MATERIALS AND METHODS

Rabbit embryos were removed from pregnant New Zealand white rabbits on the 26th day of gestation. Maxillary and mandibular molar tooth rudiments were excised and placed in calcium-magnesium free phosphate buffered saline, pH 7.2, and the epithelial enamel organ, including both inner and outer enamel epithelia, separated from the adjacent dental mesenchyme (3). The isolated tissues were incubated for 4 hours at 37°C in Eagle's Minimum Essestial Medium (Hank's balanced salts) supplemented with 50 μg/ml β-aminopropionitrile, 292 μg/ml L-glutamine, 100 μg/ml ascorbic acid and 50 μCi/ml H^3-proline (NEN-323). The combined medium and tissue was extracted in 0.1M acetic acid at 4°C, dialyzed versus the same to remove salts and un-incorporated H^3-proline, and then lyophilized. The lyophilized extracts were redissolved in 0.5M acetic acid and combined with an acetic acid solution of previously purified, pepsin-solubilized, New Zealand white rabbit embryonic skin collagen (10). The radioactively labeled collagens from the cultures were then purified along with the carrier collagen as previously described (7, 8, 10). The purified collagens were heat denatured (50°C for 20 minutes) and chromatographed on columns of carboxymethyl (CM)-cellulose as previously described (7). The isolated α chains were desalted, lyophilized and hydrolyzed in 6N HCl under N_2 at 110°C for 24 hours. The radioactive amino acids were isolated on a Jeolco 5AH amino acid analyzer equipped with a stream splitting device which permitted a portion of the eluent to be counted for radioactivity. All determinations of radioactivity in both the α chains and amino acids were done in Aquasol in a Beckman liquid scintillation counter.

In a separate set of experiments, intact tooth rudiments were incubated with H^3-

Table I

	ENAMEL ORGAN EPITHELIUM	DENTAL PAPILLA MESENCHYME
COLLAGEN SYNTHESIZED		
Hydroxyproline cpm/ tissue-medium extract	25, 194	6, 872
$\alpha 1/\alpha 2$ CHAIN DISTRIBUTION		
cpm from CM-cellulose chromatography	3	2
% RADIOACTIVITY IN 4-HYDROXYPROLINE		
$\alpha 1$ chains	44%	42%
$\alpha 2$ chains	38%	35%

proline as described above without prior separation into epithelial and mesenchymal components. The extracellular matrix between the tissues was then isolated essentially cell-free by a sonication procedure as previously described (11) and the collagen in the matrix isolated and analyzed as described above.

RESULTS AND DISCUSSION

The enamel organ epithelium and the dental mesenchyme both synthesized collagen in vitro, but on a tissue basis the isolated epithelia were 3 to 4 times more productive in the net amount of acid soluble collagen produced than the equivalent number of isolated fragments of mesenchyme (Table I). This result suggests that the collagen synthesis observed in the epithelia does not result from a small mesenchymal contaminant. Histological controls showed no mesenchymal contamination of the isolated epithelia.

The collagen produced by the epithelium chromatographed on CM-cellulose with an excess of $\alpha 1$ chains over $\alpha 2$ ($\alpha 1/\alpha 2 \backsim 3$) (Fig. 1; Table I). The $\alpha 1$ chain peak was not

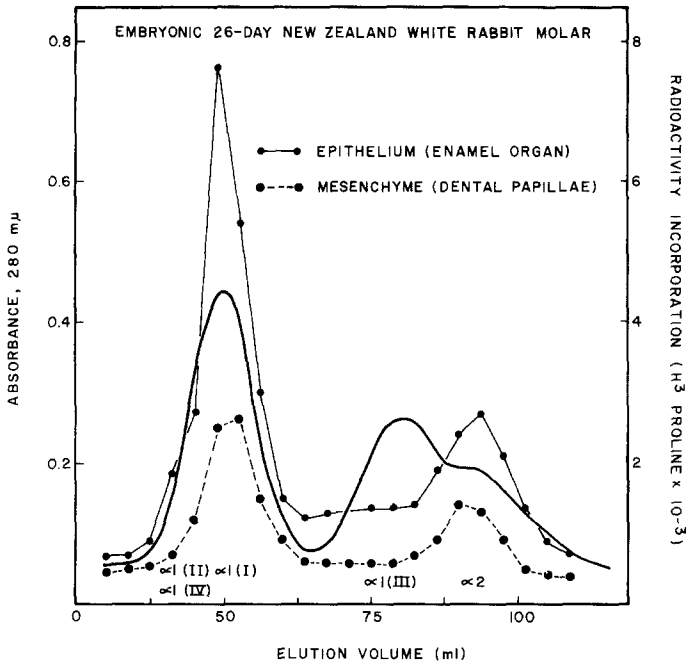

Figure 1: CM-cellulose chromatogram of collagens produced by equivalent numbers of isolated enamel organ epithelia and dental mesenchyme. Three times as much collagen is made by the epithelia and the collagen produced has an excess of $\alpha 1$ chains. The approximate elution positions of five different collagen α chains are indicated.

apparently heterogeneous, although it most likely contains both $\alpha 1$ type I and $\alpha 1$ type IV (vide infra), and it co-chromatographed with the carrier $\alpha 1$ chain. The $\alpha 2$ chain eluted from the column in its usual position. There was no significant radioactive material co-chromatographing with the large peak seen in the embryonic rabbit skin carrier preceding the $\alpha 2$ chain which is the elution position of both β_{12} and the recently described $\alpha 1$ type III (10, 12, 13).

The isolated mesenchyme produced a collagen with a chromatographic profile typical of type I collagen, or $[\alpha 1(I)]_2 \alpha 2$, containing twice as many $\alpha 1$ chains as $\alpha 2$ (Fig. 1; Table I). No radioactivity was present in the $\alpha 1$(III) region.

The principal collagen in the extracts of extracellular (progenitor dentine) matrix isolated by sonication of 26-day molar tooth organs, which had been previously incu-

bated as intact organ rudiments for 4 hours with H^3-proline, was indistinguishable from that synthesized by the isolated dental papilla mesenchyme (Fig. 1).

Analysis of the isolated α chains by hydrolysis and separation of the hydroxyproline isomers and proline on an amino acid analyzer indicated that 42.5% of the proline residues in all $\alpha 1$ chains were hydroxylated in the 4-position, and less than 1% in the 3-position (Table I). The values for the epithelial $\alpha 1$ were slightly higher than those for the mesenchyme on four determinations, but the differences were not significant. The $\alpha 2$ chains contained a lower percentage of proline hydroxylation than the $\alpha 1$ chains with 36.8% and with the same distribution between the 3 and 4 isomers (Table I).

The elution profiles on CM-cellulose of the four known types of mammalian collagens have now been fairly well established. Using the profile of type I collagen as a reference, $\alpha 1$ types II and IV elute slightly in advance of, or with, $\alpha 1$ type I (10, 14, 18) whereas $\alpha 1$ type III elutes slightly before $\alpha 2$ (10, 12, 13). The chromatographic profile of unknown radioactively labeled collagens can, therefore, give some indication of the molecular species of collagen extracted from a labeled tissue. The present results suggest that the principal collagen in the extracellular organic matrix of embryonic mammalian molar tooth organs is type I and that it is synthesized in large part by the inner enamel epithelium. In addition the isolated epithelium also appears to be producing an $(\alpha 1)_3$ type molecule since an excess of $\alpha 1$ chains were present. This most likely represents the type IV molecule present in the basal lamina adherent to the undersurface of the inner enamel epithelia. Similar results in which both types I and IV are probably produced by an epithelium has previously been described in the embryonic cornea (9).

These data indicate that the enamel organ epithelium, prior to the initiation of amelogenesis, is producing two types of collagen and presumably depositing type I at the interface between its basal surface and the preodontoblast mesenchyme. This extracellular matrix, considered to represent early dentine, is a collagenous connec-

tive tissue stroma of both mesenchymal and epithelial origin. The production of collagen, therefore, does not seem to represent a cell function unique to either cell type in contrast to the specific synthesis of dentine phosphoprotein by the odontoblasts (15) or enamel protein(s) by the ameloblasts of the inner enamel epithelium (16). The developmental importance of this early extracellular matrix in tooth development has been well described (17) and the present data indicate that the collagenous portion of this material is clearly derived from both of these interacting cell types.

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