Localization and Distribution of Tritiated Histidine in Growing Mouse Incisor

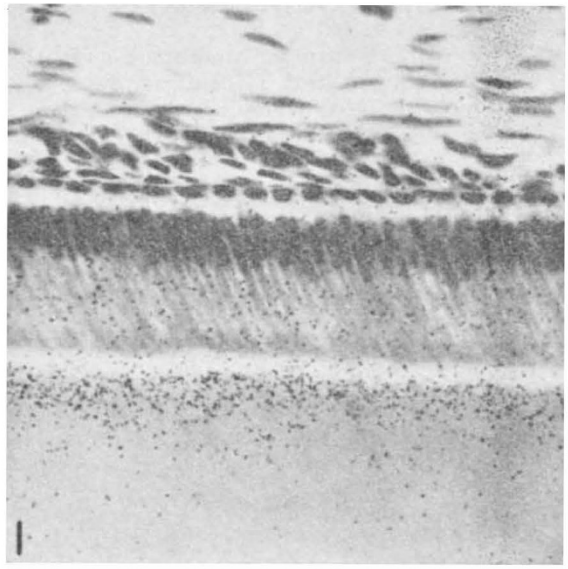
Histidine has been reported as being the predominant basic amino-acid of developing human enamel[1]. At present specific histochemical methods for *in situ* demonstration of histidine are not available[2]. With the recent availability of tritiated histidine, autoradiography appears to offer an adequate method for tracing its utilization in the growing incisors of mice.

Twenty-four female Brookhaven National Laboratory Swiss albino mice, 16 days of age, were used in this work. The mice received a subcutaneous injection of 1 μc. of tritiated histidine per gram of body-weight 1, 4, 8, 24 hr., 7 and 14 days prior to killing with ether. Immediately after killing the lower jaws were removed and fixed for 1 week in cold buffered formalin and washed for an additional 24 hr. in running tap water. Tissue samples were decalcified in a solution of 10 per cent 'Versene'. Paraffin blocks were prepared and sections cut at 5μ. Autoradiographs were prepared using Kodak 'NTB_3' liquid emulsion. The preparations were kept in a cold, dry atmosphere and exposed for 30 days. After proper exposure the autoradiographs were developed, stained with hæmatoxylin and eosin, and grain counts were made. These were based on a unit area of 750 μ^2.

Autoradiographic results showed that labelling of various incisor cells was intense after 1 hr. (20 grains). Histidine labelling was predominantly seen at the dentinal end of the cytoplasm of ameloblasts and the adjacent enamel matrix forming a band of reduced silver grains (Fig. 1). With increasing time the grain count over the cytoplasm of ameloblasts became diminished while additional grains appeared in the enamel matrix. As the grain count increased in the matrix the band of reduced silver grains became wider. At seven days the band appeared about 3–4 times wider (Fig. 2). At 4 hr. a marked decrease in labelling was observed in ameloblasts (9·6 grains). The labelling intensity of the enamel matrix adjacent to ameloblasts was maintained at 4 hr., but at 8 hr. the grain density was diminished. Enamel matrix grain distribution was altered with time so that at 7 days the grain density was higher at the widening band front (Fig. 2), and at 14 days the entire width of the enamel matrix was covered with grains. The total population of grains, however, was smaller than at earlier periods. Only a few grains were observed in ameloblasts at this time (1·1 grains).

Odontoblasts (11·1 grains) and dentinal matrix (7·5 grains) were labelled most strongly at 1 hr.; however, the total grain density was much lower than that of the ameloblast (20 grains) and enamel matrix (62·4 grains). Changes in grain distribution with time were essentially similar to that described for the ameloblasts and enamel matrix, except for the reverse direction of the wave front of dentine proper. The pulp, which is composed of several cell types, revealed a broad distribution of grains. At the base of the incisor the ameloblasts and odontoblasts were weakly labelled.

The presence of a high concentration of histidine in the developing enamel revealed by biochemical analysis was confirmed autoradiographically[1]. Histidine turn-over is more rapid in ameloblasts than in odontoblasts during matrix formation. Turn-over rate of these cells is quite rapid since labelled matrix was seen within 1 hr. Gould *et al.*[3] stated that certain tissues, such as liver, bone and periodontal membrane, exhibit a relatively rapid turn-over of collagen. The present studies reveal that the population of silver grains present over enamel and dentinal matrix was far less at 14 days than the number observed at earlier periods. This fact is taken to represent a fairly rapid turn-over of histidine in the incisor matrix of the mouse.



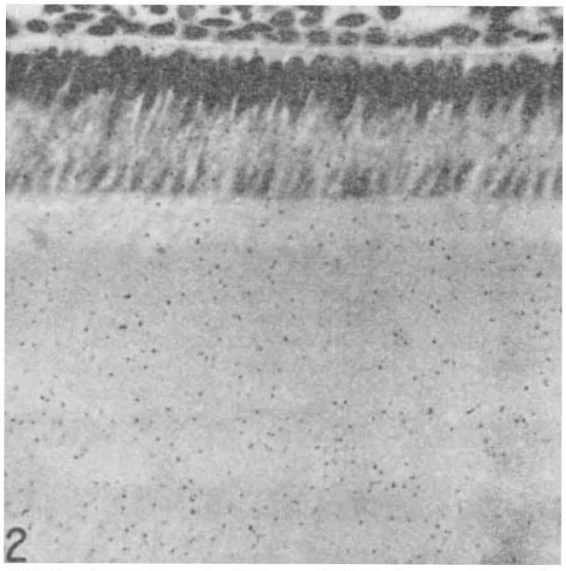


Fig. 1. Autoradiograph of the ameloblastic layer and enamel matrix of mouse incisor labelled with tritiated histidine 1 hr. following the administration of the isotope. (Oil immersion, × *c.* 480)

Fig. 2. Autoradiograph of the ameloblastic layer and enamel matrix of mouse incisor labelled with tritiated histidine 7 days following the administration of the isotope. (Oil immersion, × *c.* 480)

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W. S. S. Hwang
E. A. Tonna
E. P. Cronkite

Medical Research Center,
Brookhaven National Laboratory,
Upton, New York.

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