Histochemical Demonstration of Calcium in Rat Tracheal Cartilage (33784)

E. KAELLIS
(Introduced by S. Fedoroff)
Department of Oral Biology, College of Dentistry, University of Saskatchewan, Saskatoon, Canada

During the course of a histochemical study of the localization of calcium in the thyroid glands of rats using the GBHA [glyoxal bis(2-hydroxyanil)] method of Kashiwa and Sigman (1), tracheal hyaline cartilage rings adjacent to the gland absorbed heavy deposits of stain in a central core running through each ring, indicating that calcium had been deposited within them. Since only young, healthy rats had been used in the study, the identification of calcium in the trachea seemed at variance with the generally accepted belief that calcium deposition in hyaline cartilage is a phenomenon of aging. For example, Moss (2) states, ". . . it seems that calcification is related to the aging of cartilage and consequent nutritional difficulty." Leeson and Leeson (3) also state, "The most important retrogressive change within cartilage is *calcification*." and Copenhaver (4) remarks, "Calcification is likewise of common occurrence in old cartilage, and is usually associated with degenerative changes of the cartilage cells." A review of several texts (5–7) revealed similar descriptions of calcification in hyaline cartilage as being associated with, or caused by, senile degeneration.

Further study of calcium deposition in tracheal cartilage of young rats was undertaken in an effort to confirm and extend the original observations.

Procedure. Thirty-five normal CFN rats ranging in age from 1 day to 27 weeks, fed standard laboratory chow and given water, both *ad libitum*, were used in this study. About half the animals used fell within the age range of 1–4 weeks when maximum calcium deposition seemed to occur. The rats were killed with ether and the entire trachea from the larynx to the bronchi was dissected free from each animal and stained for calcium using the alizarin red S method of Dahl (8), modified by the use of an FCF fast green counterstain and by mounting in Clearmount. This method was chosen because it has as much sensitivity and comparable discreteness as the GBHA method but gives greater color contrast.

As a control, sections were treated with either an alkaline Versene solution or a solution of equivalent alkalinity with no Versene and then subjected to the staining process.

Results. The results with the alizarin red S method were completely comparable to those obtained with rats of comparable age by the GBHA method mentioned in the introduction.

The first histochemical indication of calcium deposition was evident in the cephalad tracheal rings, i.e., adjacent to the larynx, in rats about 2 weeks old. In successively older animals staining was evident in rings further caudad on the trachea, and, by the sixth week, all rings showed evidence of calcium.

In each cartilaginous ring calcium deposition began in the ventral midline area, and with age spread dorsally, so that by 6 weeks all portions of each ring stained for calcium. Only the central core of the rings stained, and this core was surrounded on all sides by a layer of noncalcium-containing cartilage (see Fig. 1).

Chondrocytes appeared viable and nonhypertrophic in both the central calcium containing core and in the surrounding noncalcium-containing matrix. When the staining was so intense as to obscure cellular detail, examination of the Versene-treated sections from the same trachea showed cells normal in appearance in all areas.

During the earlier stages of the process of calcium deposition there was considerable variation in time of appearance, progression, and extent of staining from one rat to another. By 8 weeks, however, heavy staining appeared in all tracheal rings in all animals.

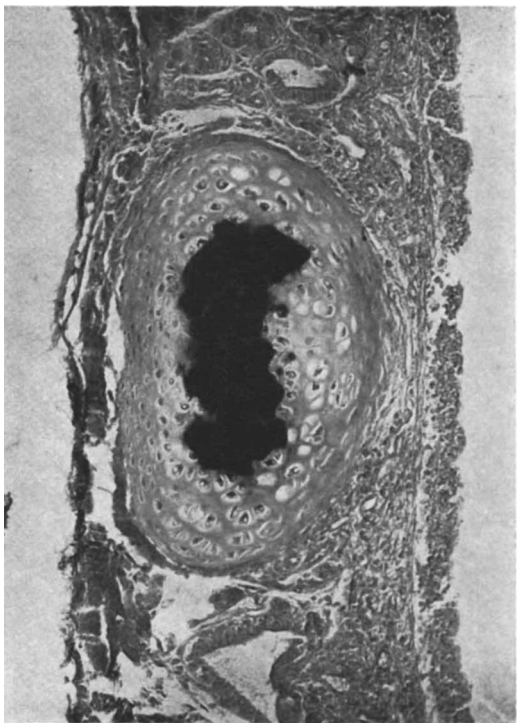

FIG. 1. Longitudinal section through trachea of 6-week-old rat, stained with alizarin red S; scale 1:165; dark area in cartilage denotes calcium.

Prior to deposition of calcium in the matrix, tiny stained granules were found within the cytoplasm of chondrocytes, and as this process advanced the perilacunar area took up the stain, eventually there being a confluence of stained areas. This is essentially the same process described by Matthews *et al.* (9), and others (10–12), in epiphyseal plate and cartilage templates, except that there was no hypertrophy.

Control sections pretreated with Versene were negative for staining, and alkaline pretreated sections without exposure to Versene stained normally.

Discussion. The significance of this early uptake of calcium in hyaline cartilage of trachea is unknown, but clearly it cannot be attributed to degenerative changes accompanying old age. It is conceivable that the infusion of calcium salts into the cartilage matrix bestows a certain presumably desirable rigidity to the tracheal rings. If calcium deposition in cartilage matrix leads to hypertrophy and death of chondrocytes as is thought to happen in epiphyseal cartilages and in the early models of long bone, then the pattern of deposition in the trachea must be different from the others. In the present instance, although it seems that calcium was deposited, cells appear to remain alive.

Summary. A histochemical study of calcium deposition in rat tracheal cartilage showed that deposition begins at about 2 weeks of age, and that by 6 weeks all the rings were involved. The deposition begins in the midventral area of the cephalad rings and extends caudally to all the rings, simultaneously extending posteriorly in each ring. It begins intracellularly and spreads to the matrix. The chondrocytes within these areas appear normal.

The author wishes to acknowledge the advice and interest of Dr. K. J. Paynter, and a grant from the National Research Council of Canada.

1. Kashiwa, H. K. and Sigman, M. D., Jr., J. Dental Res. **45**, 1796 (1966).

2. Moss, M. L., *in* "Histology" (R. O. Greep, ed.), p. 151. McGraw-Hill, New York (1966).

3. Leeson, C. R. and Leeson, T. S., *in* "Histology" p. 110. Saunders, Philadelphia, Pennsylvania (1966).

4. Copenhaver, W. M., *in* "Bailey's Textbook of Histology," Williams and Wilkins, Baltimore, Maryland (1964).

5. Ham, A. W. and Leeson, T. S., *in* "Histology" p. 271. Lippincott, Philadelphia, Pennsylvania (1961).

6. Bloom, W. and Fawcett, D. W., *in* "Histology" p. 149. Saunders, Philadelphia, Pennsylvania (1962).

7. Ham, A. W., *in* "Histology," p. 378. Lippincott, Philadelphia, Pennsylvania (1965).

8. Dahl, L. K., Proc. Soc. Exptl. Biol. Med. **80**, 474 (1952).

9. Matthews, J. L., Martin, J. H., Lynn, J. A., Collins, E. J., Calcified Tissue Res. 1, 330 (1968).

10. Johnston, P. M., J. Biophys. Biochem. Cytol. 4, 163 (1958).

11. Bohatirchuk, F., Am. J. Anat. **117**, 287 (1965).

12. Kashiwa, H. K., Stain Technol. **41**, 49 (1966).

Received Dec. 9, 1968. P.S.E.B.M., 1969, Vol. 130.