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Corneal Calcification

Abstract. *Superficial calcification was produced in the normal rabbit cornea by mild irradiation with a carbon dioxide laser. The calcification was entirely extracellular and closely resembled that observed in human band keratopathy, which was characterized as hydroxyapatite by x-ray diffraction. The electron-microscopic appearance of calcific spherules and conglomerates in early corneal calcification is presented. The calcific spherules arise at the basal plasma membrane surface of the epithelial cells in close relation to their basement membrane.*

In studies on the effects of continuous carbon dioxide laser irradiation (wavelength 10.6 μ) (*1*), a number of rabbit eyes developed clinical and histopathologic changes identical with band keratopathy as observed in the human cornea. In the human cornea, this calcification appears to be wholly extracellular and is generally most prominent in the region, known as Bowman's membrane, lying beneath the epithelium. In the rabbit cornea, which does not have a structure completely analogous to Bowman's layer in the human cornea, early calcification also appears by light microscopy to lie beneath the epithelium and is wholly extracellular.

The rabbits were exposed to CO_2 laser irradiation of approximately 0.35 watt/cm^2 for 10 minutes over most of the corneal surface. The corneas developed superficial opacification. In two eyes the typical clinical appearance of band keratopathy was seen when examined 12 days later by both gross inspection and biomicroscopy. In another animal exposed at 0.48 watt/cm^2 for 7 minutes, calcification within a more dense corneal opacity was noted only upon histologic and electron-micro scopic examination from samples taken 14 days after irradiation. Histopathologic studies revealed the calcareous deposits [stains with alizarin red (*2*) (Fig. 1)] just beneath the epithelium. By electron microscopy, myriad spherules (Fig. 2a) appearing as concentric rings of exceedingly fine crystals (Fig. 2b) were observed within the basement membrane region of the epithelium, in the superficial stroma, and near the superficial keratocytes. Also visible were conglomerates of spherules that were presumably formed by fusion of the spherules. The superficial keratocytes engulfed a number of these spherules, which were then seen lying in membrane-bound cytoplasmic vesicles. No spherules could be found within the cytoplasm of the overlying epithelial cells, although a number could be seen to lie almost against the basal plasma membrane of the cells (Fig. 2c). In more severe cases, the space between the two outer rings of the spherule became more dense. It is possible that the basement membrane of the epithelium plays a major role as the initial nidus for the calcification. This might explain the double outer layering of the spherules or conglomerates, much like that seen in calcification of basement membrane of the peritubular capillary of the kidney in experimental calcinosis (*3*). These electron-microscopic observations were similar to those made in cases of human band keratopathy (*4, 5*). X-ray diffraction studies of samples of human cornea exhibiting band keratopathy revealed a crystal pattern characteristic of hydroxyapatite (*5*).

The cornea is normally a transparent and avascular structure, and band keratopathy can often be clearly observed in the central region, where there is no evidence of vascularization. Because the calcified spherules are found over almost the entire extent of the cornea just beneath the epithelium, and in a single plane beneath the epithelium, it was clear that the deposition of calcium in this region was related to some activity

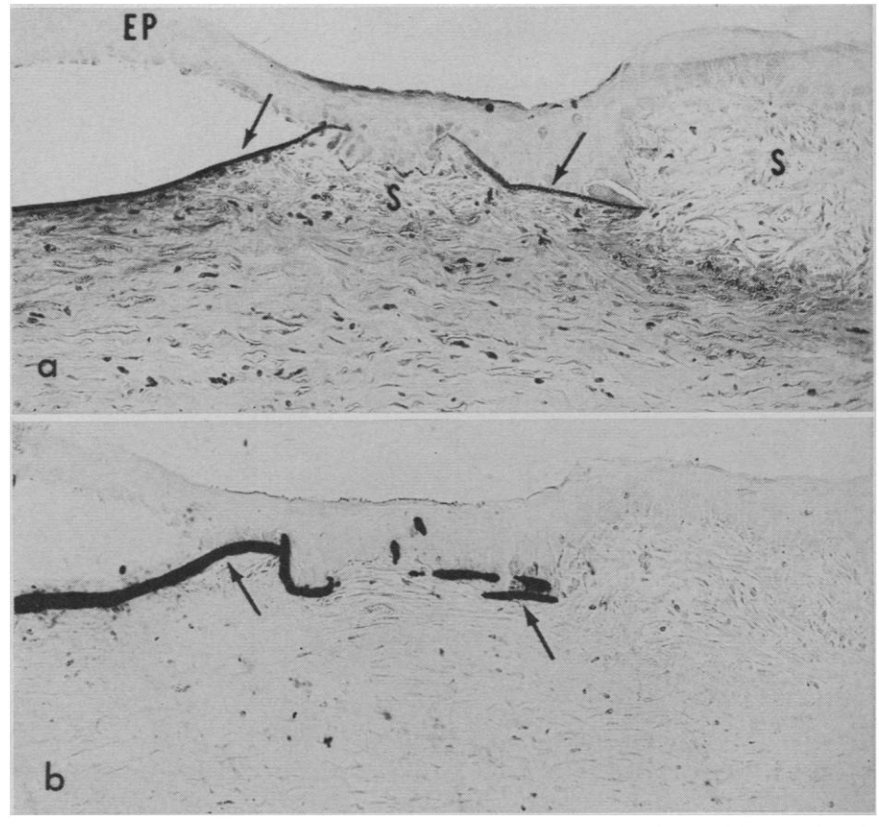

Fig. 1. (a) Thin section (1.5 μ) of Epon-embedded cornea stained with paraphenylenediamine (*7*) to show an interrupted, dark-staining layer in the anterior stroma (free arrows). Subepithelial stromal scarring is present in two locations (*S*), and the epithelium (*EP*) is lifted free of this dense layer on the left (× 115, AFIP negative 68-5142). (b) A section taken from the same block used for Fig. 1a and stained with alizarin red (*2*) to show the heavy concentration of calcium (free arrows) (× 115, AFIP negative 68-5143).

of the overlying epithelium. The production of these spherules can occur as close as the basal plasma membrane surface of the cells, presumably by accumulating cations at the cell surface, possibly within a surface layer of mucopolysaccharide. These conclusions would be in accord with experimental demonstrations by Borle (*6*). The calcifying spherules in our rabbits then aggregated into conglomerates that, in the human cases (a later stage of band keratopathy), became a massive amount of extracellular calcification which by x-ray diffraction showed the crystal pattern of hydroxyapatite. This form of extracellular calcification produced by the cells at their surface is in contrast to another form of calcification that is almost wholly intracellular, produced by excessive parathyroid hormone (*6*). This latter observation also agrees with experimental demonstrations of Borle (*6*).

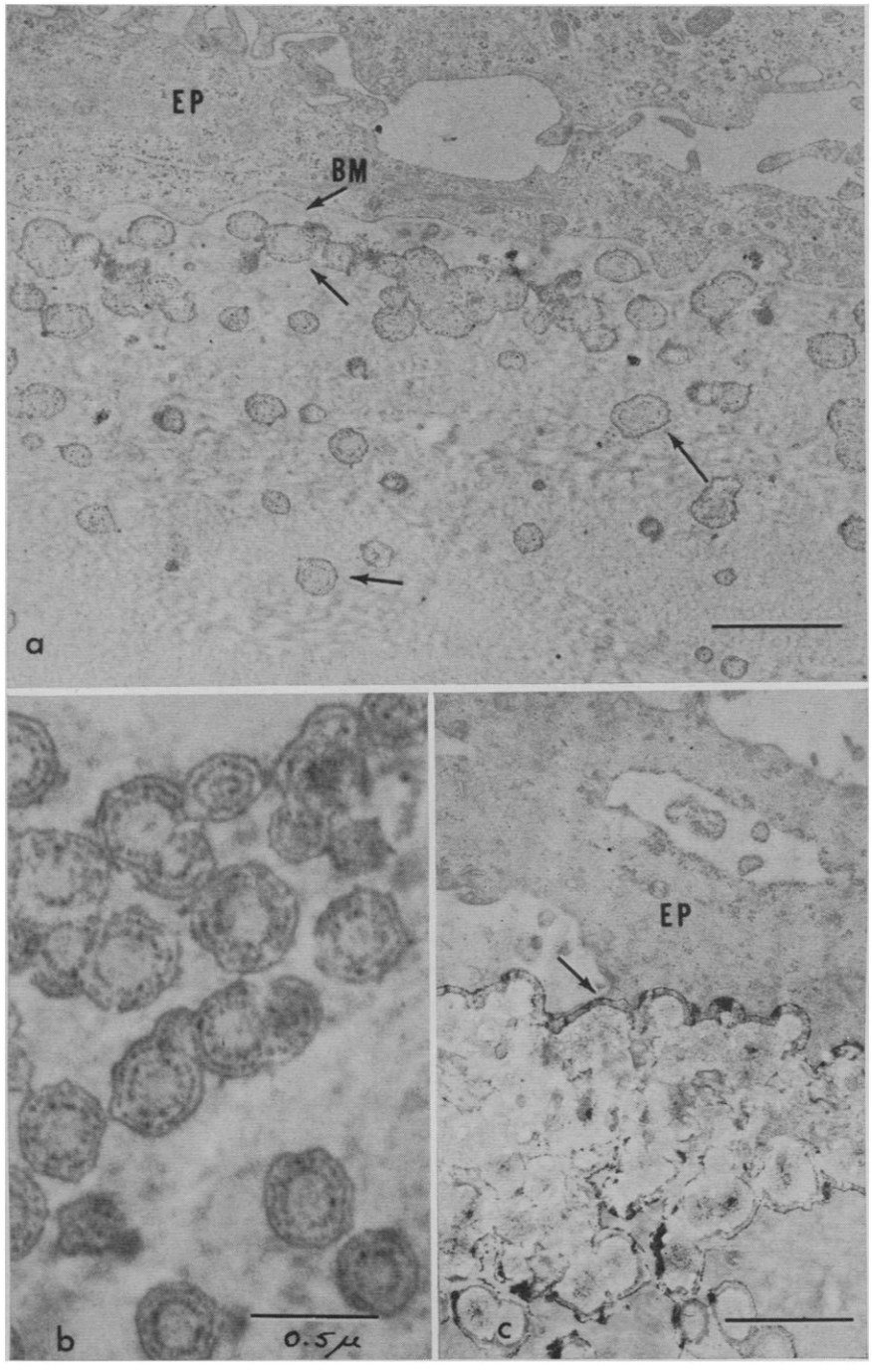

Fig. 2. (a) Corneal basal epithelial cells (*EP*), small portions of the epithelial basement membrane (*BM*), and the many calcific spherules in the basal region (free arrows) as well as in the superficial corneal stroma (× 16,500). (b) Higher magnification of calcific spherules observed in the cornea sampled in Fig. 2a. The concentric and laminated arrangement of these structures is evident (× 33,000). (c) A sample of superficial cornea from a rabbit receiving slightly greater injury. In the subepithelial region, there is a massive conglomerate of spherules. The basal epithelial cell (*EP*) has partially pulled away, and the extremely close relation of the outer double layer of the spherules to the cell surface and region of the basement membrane can be seen (free arrows) (× 16,500, AFIP negative 68-5286-2).

It appears, therefore, that (i) band keratopathy can be produced in nonvascularized rabbit corneas by a simple combination of trauma and exposure; (ii) the epithelial cells of the cornea are closely related to the accumulation and formation of the extracellular calcific spherules near the cell surface; (iii) the extracellular calcifying spherules can aggregate into large conglomerate masses; (iv) the underlying keratocytes can be activated and can phagocytose some of the calcifying spherules as well as some of the smaller conglomerates; and (v) this extracellular form of calcification differs in morphologic features from the intracellular calcification that has been seen in hyperparathyroidism, but both show the characteristic x-ray diffraction pattern of hydroxyapatite.

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