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Lesions of the Enamel Organ of Developing Dog Teeth Following Experimental Inoculation of Gnotobiotic Puppies with Canine Distemper Virus

R. R. DUBIELZIG, R. J. HIGGINS and S. KRAKOWKA

University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology, Philadelphia, Pa. and The Ohio State University, College of Veterinary Medicine, Department of Pathobiology, Columbus, Ohio

Abstract. Ten 7-day-old gnotobiotic Beagle puppies were inoculated intraperitoneally with virulent canine distemper virus (R252-CDV). The dogs were killed and perfused with paraformaldehyde/glutaraldehyde from eight to 36 days after inoculation. The developing teeth of the mandibles were examined by light microscopy, and the teeth from three dogs were examined by electron microscopy. Necrosis of individual cells in the stratum intermedium of the developing tooth was the first change, detectable at day 9 post-inoculation. At day 16 post-inoculation, there was disorganization of the ameloblasts. In the stratum intermedium, multinucleate giant cells and large eosinophilic cytoplasmic viral inclusions were prominent. Ultrastructurally, these inclusions consisted of clusters of tubular aggregates typical of canine distemper virus nucleocapsids. At 28 to 36 days post-inoculation, the changes were seen in the reduced enamel epithelium. Multinucleate cells were seen, but no inclusions. Some necrotic cells were seen. In these teeth, ameloblastic cells of the root were morphologically normal. Our results suggest that distemper virus affects developing teeth by direct infection of the enamel organ.

Dogs naturally infected with distemper virus while the adult teeth are developing often have defects in the enamel when the adult teeth emerge (fig. 1) [1, 9]. Called enamel hypoplasia, these defects range from focal depressions in the enamel to segmental lack of enamel formation. Similar changes can be caused by any disruptive effect on the enamel organ. Other authors have stated that dogs infected with canine distemper virus develop enamel hypoplasia because of nonspecific effects of the disease such as circulatory changes, edema, exudate formation, metabolic disturbances, and fever, rather than specific virus infection of the ameloblast [1, 9]. Recently, the jaws of three puppies that died of encephalitis caused by canine distemper virus were examined histologically [4]. Disruption, cystic degeneration, multinucleate giant cells, and eosinophilic cytoplasmic inclusions were seen in the enamel organ [4]. The results of that study suggested that the enamel organ *per se* is

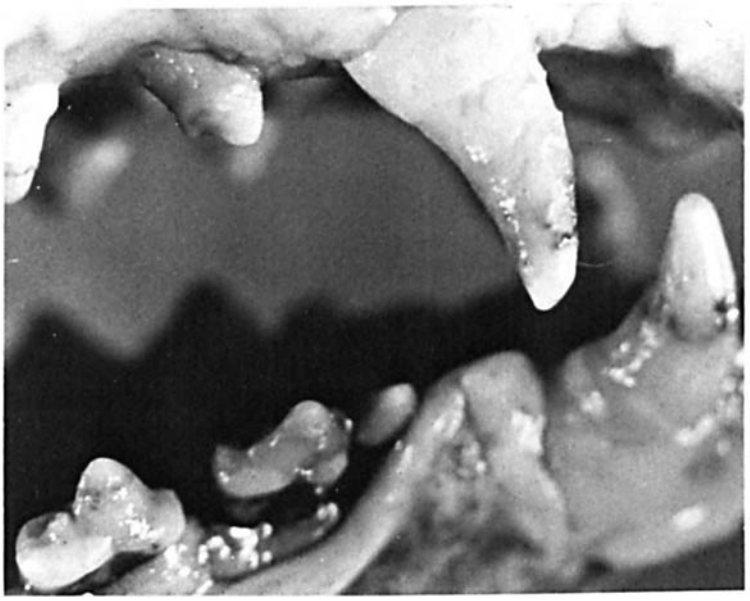

Fig. 1: Enamel hypoplasia in dog surviving canine distemper virus infection during development of adult teeth.

directly affected by the virus. We examined morphologically the lesions in the enamel organ following experimental infection with virulent canine distemper virus.

Materials and Methods

Ten gnotobiotic Beagle dogs [5, 7] were infected with 0.2 ml of spleen suspension containing $10^{4.5}$ infective particles/ml virulent canine distemper virus (R252-CDV) by intraperitoneal inoculation. Eight were 7 days old and two were 5 days old at the time of inoculation. One uninoculated dog was used as a control. The experimental dogs were killed from eight to 36 days post-inoculation. The control dog was killed at 16 days after inoculation of principles. All dogs were perfused by vascular perfusion with 4% paraformaldehyde followed by perfusion with 5% glutaraldehyde. The mandibles were stored in 10% buffered formalin, radiographed to aid in sectioning, then decalcified for 12 hours in 15% formic acid. Sections from all mandibles were embedded in paraffin for routine histologic evaluation. Other sections from each mandible were embedded in methacrylate and sectioned routinely. Enamel organs were dissected from dogs killed at 9, 16, and 36 days post-inoculation. Minced sections were processed routinely for electron microscopic evaluation.

Results

The earliest lesion in the developing teeth (fig. 2), seen by light microscopy in the dog killed nine days post-inoculation, was necrosis of individual cells in the stellate reticulum and stratum intermedium (fig. 3). The ameloblasts were unaffected.

Dogs killed at 10, 16, and 18 days post-inoculation had more severe necrosis of individual cells in the stratum intermedium. Multinucleate syncytial cells were seen in the stratum intermedium but only occasionally among the ameloblasts (fig. 4).

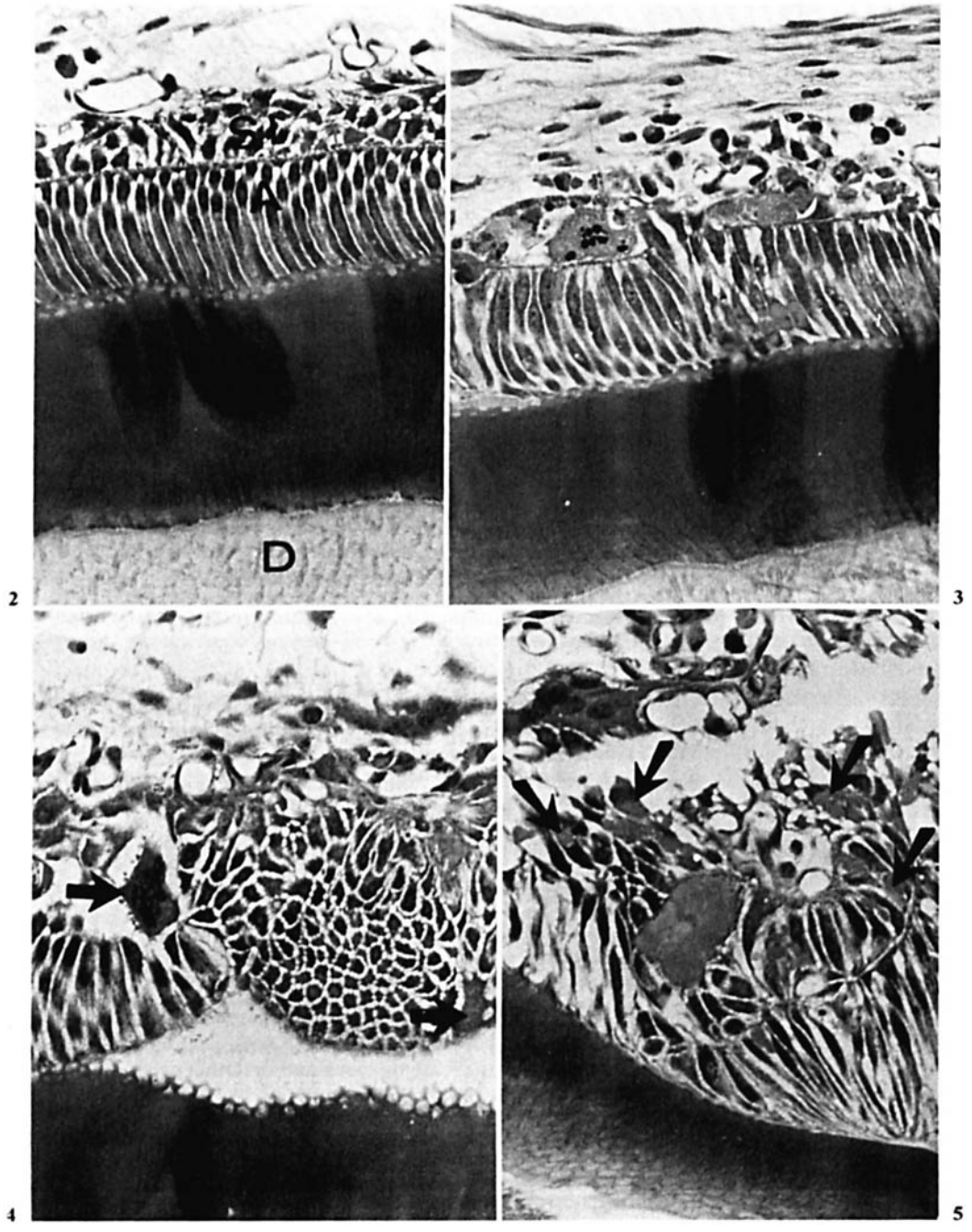

Fig. 2: Normal enamel organ of developing tooth. D = Dentin, E = Enamel matrix, A = ameloblasts, SI = stratum intermedium. HE.

Fig. 3: Enamel organ, day 9: necrosis of cells in stratum intermedium, relative sparing of ameloblasts. HE.

Fig. 4: Syncytial cells (arrows) and ameloblastic disruption in enamel organ, day 16. HE.

Fig. 5: Eosinophilic intracytoplasmic inclusion bodies (arrows) in enamel organ, day 16. HE.

Large eosinophilic intracytoplasmic inclusions were in all epithelial layers (fig. 5). The ameloblasts became progressively shorter and more disorganized.

Dogs killed at 21, 28, and 36 days post-inoculation showed premature loss of ameloblasts which became incorporated with the mucosal epithelium to form the reduced enamel epithelium (fig. 6). Syncytial cells persisted and the enamel organ became more squamous. Fusion with the gingival epithelium took place. In the same sections, the ameloblasts surrounding the roots appeared to have developed and functioned normally (fig. 7).

Sections of the enamel organ from dogs killed 9, 16, and 36 days post-inoculation were examined by electron microscopy. Ultrastructural examination of the nine-day and 36-day teeth confirmed the light microscopic findings, but no virus particles were seen. The 16-day teeth showed syncytial cells and some necrosis (fig. 8). Large clusters of tubular aggregates of viral nucleocapsids were seen in the cytoplasm of all the epithelial cells, but most markedly in those of the stratum intermedium (fig. 8). These clusters corresponded in position to the eosinophilic inclusions seen by light microscopy.

Discussion

Other authors have stated that post-distemper enamel hypoplasia is caused by the nonspecific effects of systemic disease, particularly vascular changes, edema, exudate formation, problems in mineral metabolism, and fever [1, 9]. A morphologic study of the developing teeth from three dogs with naturally occurring encephalitis caused by canine distemper showed syncytial cells, inclusions, and necrosis [4], suggesting that the effect on the developing tooth is due to direct virus infection of the enamel organ. We show here that typical lesions occurring in nine experimentally infected puppies were similar to those seen in field cases.

Ameloblasts developing subsequent to the infection looked normal. Disruption of the enamel organ was attributed to the direct effects of canine distemper virus infection of this tissue.

The tubular particles seen in the cytoplasm of diseased cells in the enamel organ were similar to those seen in paramyxovirus-infected cells [2] and cells with canine distemper virus inclusions [3, 8].

Since canine distemper virus infection in animals 6 to 7 days old invariably results in fatal neurotropic viral encephalopathy [3, 6] 20 to 40 days after infection, the consequences of R252 canine distemper virus infection of ameloblasts (i.e. enamel hypoplasia) could not be ascertained in this series. It is likely, however, that a proportion of animals infected at 4 to 8 weeks old, when 30% to 40% incidence of chronic encephalitis is expected [3, 6], would develop similar lesions and resultant pitting and scarring of tooth enamel. Thus, it is evident from these findings that this strain of canine distemper virus causes a segmental hypoplasia of the enamel by direct infection of the cells of the enamel organ. Dental structures developing in the convalescent period after infection seem to develop and function normally.

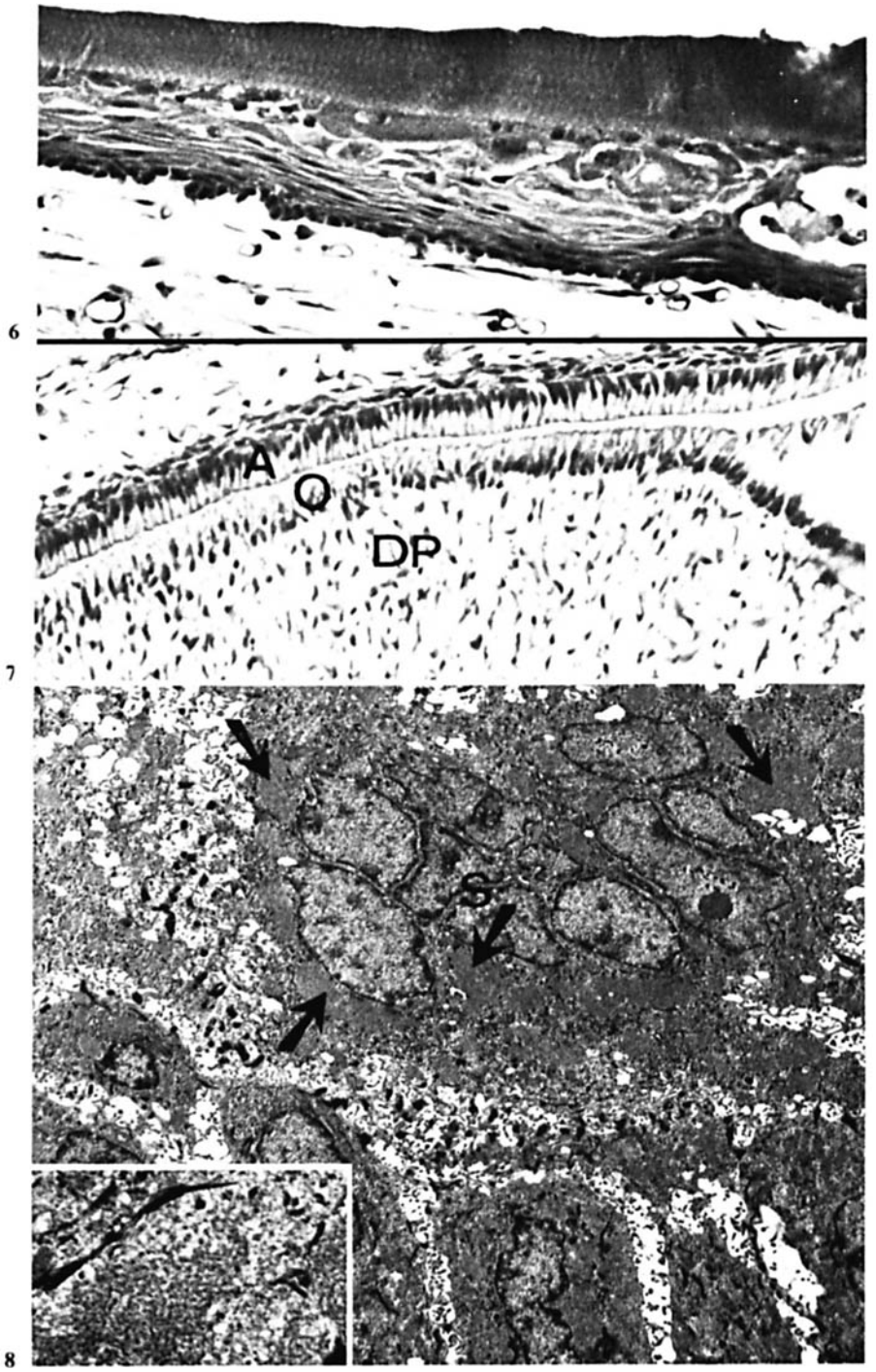

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Fig. 6: Twenty-one days after inoculation with distemper virus, syncytial cells persist as ameloblasts, become incorporated with surface mucosa. HE.

Fig. 7: Formation of functional ameloblastic tissue on dental roots, day 28. Ameloblasts (A), odontocytes (O), dental pulp (DP). HE.

Fig. 8: Enamel organ 16 days after inoculation with distemper virus: syncytial cells (S), cytoplasmic inclusions (arrows). *Inset:* higher magnification of viral particles.

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Request reprints from Richard R. Dubielzig, University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology, Philadelphia, PA 19104 (USA).