Influence of X-ray irradiation on the ultrastructure of rat submandibular gland striated-duct cells

Edward B. Messelt and Erik Dahl
Department of Anatomy, Dental Faculty, University of Oslo, and Norsk Hydros Institute for Cancer Research, Department of Biophysics, Oslo, Norway

Messelt, E. B. & Dahl, E. Influence of X-ray irradiation on the ultrastructure of rat submandibular gland striated-duct cells. *Acta Odontol. Scand.* 1983, 41, 277–282. Oslo. ISSN 0001–6357.

Previous investigations have indicated that striated-duct cells react to stimulation with an apocrine secretion, morphologically demonstrated by bleb-like projections of the apical cytoplasm. Since bleb formation as an ultrastructural feature also has been debated and sometimes interpreted as a fixation artifact, it was considered essential to extend the studies by exposing the submandibular gland to X rays to establish whether such treatment would have any influence on the formation of blebs. The material used in the present study consisted of rat submandibular glands exposed to X rays in the range of 200–1800 rad. The glands were examined by both SEM and TEM. The duct cells exposed to 200 rad appeared normal, with no sign of alteration in their ability to produce blebs, whereas duct cells exposed to 750 rad showed no sign of bleb formation. Some of the duct cells exposed to 1800 rad showed considerable morphological changes, consistent with oncotic transformation. The results support the conclusion that bleb formation is a normal morphological feature and not an artifact. This study also indicates that the functional activity of the cells is reduced after exposure to X rays. □ *Salivary glands; ultrastructure; X-ray*

Edward B. Messelt, Dept. of Anatomy, Dental Faculty, University of Oslo, P.O.B. 1052 Blindern, Oslo 3, Norway

The apocrine glands are defined as producing their secretion by decapitation of a cytoplasmic protrusion extending from the free surface of the gland cell. Several studies have shown that cytoplasmic protrusions of different sizes and shapes are present in striated ducts of salivary glands (1, 4, 6, 10, 11, 14, 18–21). A survey of the literature reveals that these bleb-like protrusions have been variously interpreted as either signs of a fixation artifact (9), a local weakening of the apical plasma membrane which results in a ballooning structure caused by an increased hydrostatic pressure (22), or, simply, as different stages of apocrine secretion (6, 7). Previous investigations have shown that rats stimulated by starvation for 24 h and feeding shortly before death develop blebs on the luminal surface of their submandibular gland striated-duct cells, whereas striated-duct cells of unstimulated animals mostly fail to show apical swelling (6, 11). These observations were suggested to imply that blebs are not artifacts but the result of a naturally occurring physiological process. Investigations have also shown that these blebs most likely are separated from their cells of origin and the content emptied into the duct lumen (7). Apical blebs of rat submandibular gland striated ducts have therefore been suggested to represent a form of apocrine secretion.

The salivary glands are comparatively sensitive to ionized radiation (17). The effect of X rays is clinically recognized as a distressing mouth dryness observed in patients receiving X-ray treatment of the oral tissues (3). Phillips (12) found that 24 h after X-ray exposure the volume of rat parotid gland salivary flow decreased by 50%. Morphologically, atropy, necrosis, and degenerative nuclear changes have been described 24 h after irradiation (12, 13). In striated-duct cells Chomette et al. (2) found striking mitochondrial changes with crystal lysis and ballooning degeneration 3 days after irradiation. The infoldings of the basal plasma membrane and the cellular granules were also reported to disappear. The present

study is a continuation of investigations of the nature of bleb formation of submandibular gland striated ducts and is designed to establish the effect of exposure to X rays on bleb development at the apical aspect of these cells.

Materials and methods

Nine adult female Wistar White rats were used in these experiments. They were all from the same strain and the same generation. All the animals received a single dose of either 200, 750, or 1800 rad, delivered by a standard Stabilipan® X-ray machine using 210 kV at 20 mA, with added filtration of 1 mm Cu at an exposure rate of 183 rad/min. Radiation exposures were calibrated before each exposure. Before the irradiation procedures the animals were lightly anesthetized by intraperitoneal injection of pentobarbital sodium (Nembutal®). Only the heads and necks were exposed, the bodies being protected by lead sheeting. To produce an autonomous nervous stimulation, the animals were, after the exposure, starved for 24 h and then fed 15 min before being killed. The submandibular glands were fixed under pentobarbital anesthesia by vascular perfusion through the carotid artery with 5% glucose, followed by 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3. After perfusion for 15 min the submandibular glands were exised and cut into thin slices under the dissecting microscope. The tissues were then fixed for an additional period of 24 h by immersion in the fixative. Subsequently the specimens were rinsed for 10 min in 0.15 M phosphate buffer at pH 7.3 and post-fixed in 1% osmium tetroxide at 4°C for 2 h (8). After fixation the blocks were rapidly dehydrated in graded series of acetone solution and embedded in Vestopal W (16). Ultrathin sections were cut on an LKB Ultrotome III. The sections were treated with uranyl acetate for 30 min, followed by lead citrate (15) for 5 min. From the same plastic blocks 1-μm-thick sections were cut for light microscopy. These sections were stained with the Ponceau de Xylidine/Giemsa method (5). The ultrathin sections were examined in a Philips 400 T electron microscope. The tissues prepared for scanning electron microscopy were critical-point dried with carbon dioxide. The dried specimens were attached to metal stubs with silver paste and coated with gold in a vacuum evaporator. Coated samples were examined in a Jeol 50 A scanning microscope with an accelerating voltage of 10–15 kV. Micrographs were recorded on Polaroid Type 52 film.

Results

The examination showed that striated-duct cells of rat submandibular glands exposed to 200 rad developed apical blebs in large numbers (Fig. 1). There seemed to be no visible difference either in number or in size between these blebs and those of unirradiated striated ducts (6, 7). The blebs varied in shape but appeared equal in electron density. They did not contain cytoplasmic organelles. Bundles of filaments, previously described in unirradiated ducts as the separating zone, ran horizontally across the cell and divided the blebs from the rest of the cytoplasm (Fig. 1). Bordering the separating zones on their basal aspect were numerous membrane-bound granules. The basal plasma membrane of the striated-duct cells showed a large number of deeply penetrating infoldings, which returned to a clearly visible basal lamina (Fig. 2). Numerous radially arranged mitochondria were found in the basal cytoplasm between the membranous indentations.

The luminal membrane of duct cells exposed to 750 rad were microvillous, but they did not show any sign of apical blebbing (Figs. 3 and 4). Only a few granules were seen, most of which were smaller than those of duct cells exposed to 200 rad (Fig. 3). The separating zone no longer seemed to be prominent. The mitochondria associated with the basal infoldings appeared to be reduced in number. A condensed electron-dense material was present basally to the duct cells, where the basal lamina normally is found (Fig. 5). In duct cells exposed to 1800 rad the number of mitochondria was

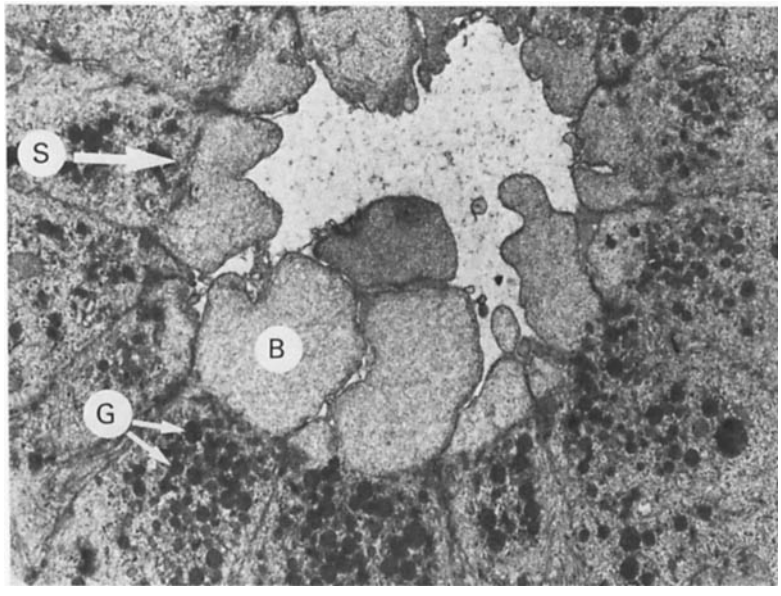

Fig. 1. Luminal part of striated-duct cells showing well-developed blebs after being exposed to 200 rad. A separating zone divides the blebs from the rest of the cytoplasm. B = bleb; G = granule; S = separating zone. (×7200).

increased, and they occupied most of the cytoplasm (Fig. 6). The duct lumen was decreased in size and was usually filled with electron-lucent ballooning structures. The nuclei showed extensive pleomorphism with regard to both size and electron density.

Discussion

The present study focused attention on more obvious signs of cytoplasmic changes and especially changes that are supposed to be of importance to secretion and was not con-

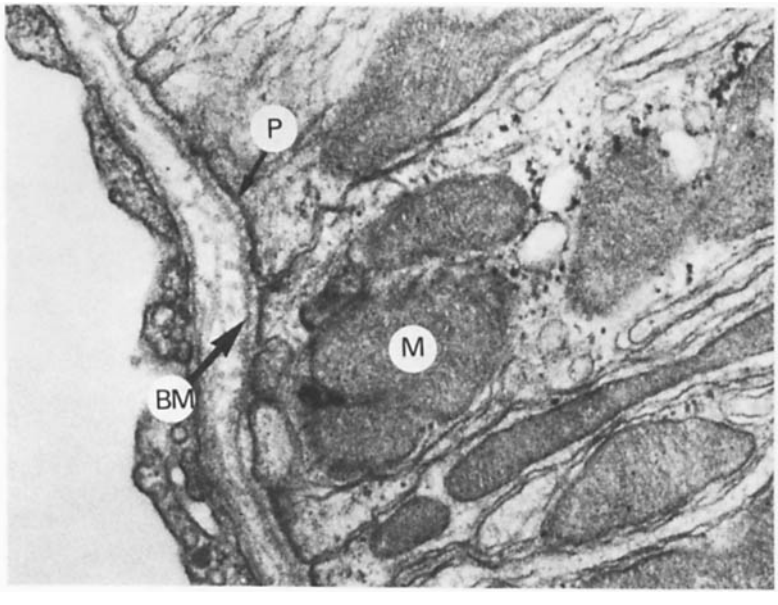

Fig. 2. Basal part of the striated-duct cell after exposure to 200 rad. The plasma membrane has deeply penetrating infoldings that return to the basement membrane. BM = basement membrane; P = plasma membrane; M = mitochondria. (×36,000.)

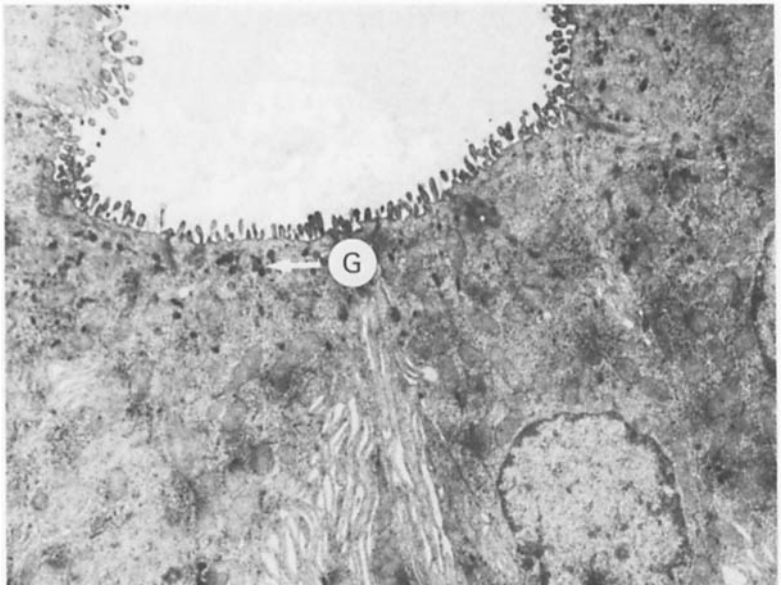

Fig. 3. Striated-duct cells exposed to 750 rad. The luminal cell membrane is here seen to be microvillous. The apical granules are few and small. G = granules. (×8000.)

cerned with very subtle alterations in cellular organelles. The striated ducts exposed to 200 rad appeared unaffected, and the cells did not differ notably in ultrastructure from unirradiated cells previously described (6, 7). Since no sign of damage was apparent, it is felt that the striated-duct cells are resistant to experimental irradiation at 200 rad. Structural changes such as reduction of the number of basal mitochondria and the lack of normal basal lamina, after a glandular exposure to 750 rad, might be interpreted as a reduction in the function of the duct cells. The loss of apical blebs and cytoplasmic

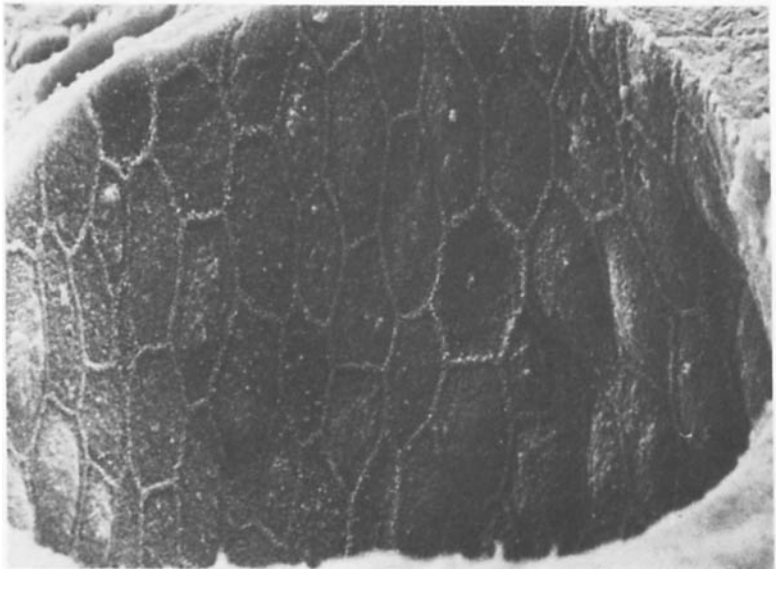

Fig. 4. Scanning electron micrograph of the luminal surface of striated duct exposed to 750 rad. The cell margins are well demarked, but there is no bleb present. (×5000.)

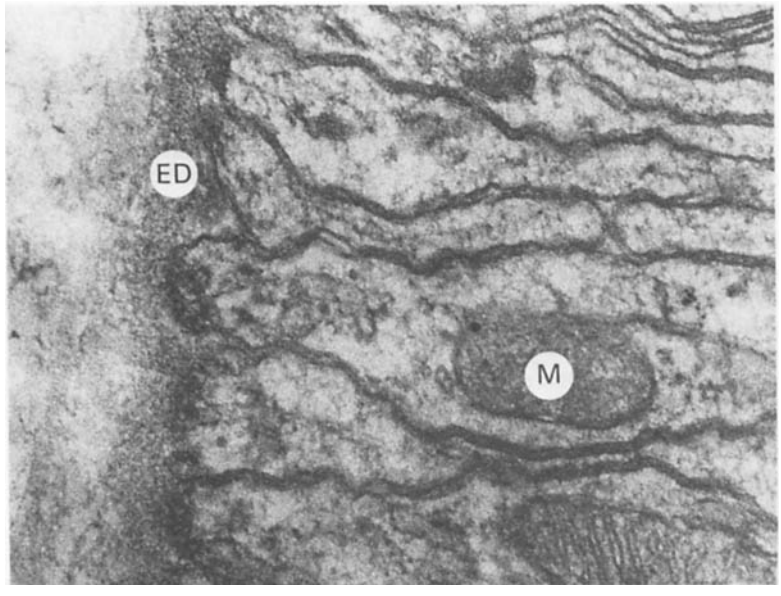

Fig. 5. Basal part of striated-duct cell exposed to 750 rad. An electron-dense material is present where the basement membrane normally is found. The mitochondria are reduced in number compared to what is found in 200-rad-exposed cells. ED = electron-dense material; M = mitochondria. (×58,000.)

granules seems to confirm this assumption.

Santangelo & Toto (17) have previously found that duct cells show the most marked alterations of the glandular cells, and they assumed that these cells were the most susceptible to damage by irradiation. It is felt that the distressing effect of irradiation therapy on the oropharynx known as dry mouth to some extent might be caused by this cell damage and the apparent failure of these cells to release secretion in an apocrine manner. The structural changes of striated-duct cells exposed to 1800 rad manifested themselves as dramatic. These alterations were

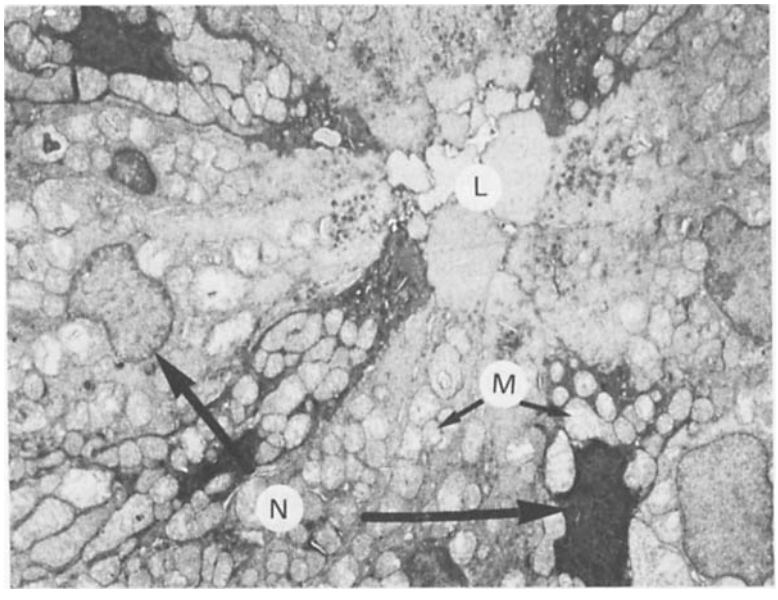

Fig. 6. This micrograph shows the luminal part of a 1800-rad-exposed striated duct. The cells are heavily loaded with mitochondria, and the nuclei show pleomorphism. The duct lumen is decreased in size. L = lumen; M = mitochondria; N = nucleus. (×4600.)

characterized primarily as degenerative, to some extent giving the impression of a transformation of the duct cells to oncocytes. Since bleb formation occurs in unirradiated (6, 7) and low-dose X-ray-exposed cells but is absent when the cells are exposed to higher doses, it is assumed that this formation is not caused by unbiological conditions during fixation and embedding but rather represents a normal physiological response that is destroyed when the tissue is exposed to high doses of X rays.

References

1. Caramia, F. Ultrastructure of mouse submaxillary gland. J. Ultrastruct. Res. 1966, 16, 505–523
2. Chomette, G., Auriol, M. & Vaillant, J. M., Bertrand, J. C. & Chenal, C. Effects of irradiation on the submandibular gland of the rat. Virchows Arch. pathol. Anat. 1981, 391, 291–299
3. Frank, R. M., Herdly, J. & Philipe, E. Acquired dental defects and salivary gland lesions after irradiation for carcinoma. J. Am. Dent. Assoc. 1965, 70, 868
4. Kurtz, S. M. The salivary glands. In: Electron microscopic anatomy. Kurtz, S. M., ed. Academic Press, New York 1964, 97–122
5. Messelt, E. B. A new differential staining method of semithin sections of polyester-embedded salivary glands. Acta Odontol. Scand. 1981, 39, 67–70
6. Messelt, E. B. Ultrastructural studies on the bleb formation in seal and rat submandibular gland striated ducts. Acta Odontol. Scand. 1982, 40, 25–33
7. Messelt, E. B. Ultrastructural studies on apical blebs of striated ducts in the rat submandibular gland. Acta Odontol. Scand. 1982, 40, 103–112
8. Millonig, G. The advantages of a phosphate buffer OsO_4 solution in fixation. J. appl. Physiol. 1961, 32, 1637
9. Munger, B. L. The cytology of apocrine sweat glands. I. Cat and monkey. Z. Zellforsch. 1965, 67, 373–389
10. Parks, H. F. On the fine structure of the parotid gland of mouse and rat. Am. J. Anat. 1961, 108, 303–329
11. Parks, H. F. Morphological study of the extrusion of secretory materials by the parotid glands of mouse and rat. J. Ultrastruct. Res. 1962, 6, 449–465
12. Phillips, R. M. X-ray-induced changes in function and structure of the rat parotid gland. J. oral Surgery 1970, 28, 432–437
13. Pratt, N. E. & Sodicoff, M. Ultrastructural injury following X-irradiation of rat parotid gland acinar cells. Archs. oral. Biol. 1972, 17, 1177–1186
14. Reinecke, M. Lichtmikroskopische-histochemische und electron-mikroskopische Untersuchungen am abführenden Gangsystem der Parotis des Meerschweinschens. Arch. Klin. Exp. Ohr. Nas. Kelkopfheilk. 1967, 189, 9–20
15. Reynolds, E. S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 1963, 117, 208–212
16. Ryter, A. & Kellenberger, E. L'inclusion au polyester pour l'ultramicrotomie. J. Ultrastruct. 1958, 2, 200–214
17. Santangelo, M. V. & Toto, P. D. Radiation effects on mouse submandibular gland. J. dent. Res. 1965, 40, 1291–1297
18. Scott, B. L. & Pease, D. C. Electron microscopy of the salivary and lacrimal glands of the rat. Am. J. Anat. 1959, 104, 115–161
19. Tamarin, A. & Sreebny, L. M. The rat submaxillary gland. A correlative study by light and electron microscopy. J. Morphol. 1965, 117, 295–352
20. Tandler, B., Denning, C. R., Mandel, I. D. & Kutscher, A. H. Ultrastructure of human labial salivary glands. III. Myoepithelium and ducts. J. Morphol. 1970, 130, 227–246
21. Wilborn, W. H. & Shackleford, J. M. The cytology of submandibular glands of the opossum. J. Morph. 1969, 128, 1–34
22. Young, J. A. & van Lennep, E. W. The morphology of salivary glands. Academic Press, London 1978, 85–86

Received for publication 5 May 1982