Virchows Arch [Pathol Anat] (1981) 391:291–299

Virchows Archiv A

Effects of Irradiation on the Submandibular Gland of the Rat

An Enzyme Histochemical and Ultrastructural Study

G. Chomette[1], M. Auriol[1], J.M. Vaillant[2], J.Ch. Bertrand[2], and Ch. Chenal[3]

[1] Pathology Department, Hôpital de la Pitié, 83 Boulevard de l'Hôpital, F-75013 Paris, France
[2] Institute of Stomatology, Hôpital de la Salpétrière, 47 Boulevard de l'Hôpital, F-75013 Paris, France
[3] Radiotherapy Department, Hôpital de la Salpétrière, 47 Boulevard de l'Hôpital, F-75013 Paris, France

Summary. Single-dose cervical irradiation by cobalt 60 in rats induced lasting functional disturbances of the submandibular gland which were excessive when compaired with the relative integrity of the gland as seen under the light microscope. Enzyme histochemical and ultrastructural studies revealed severe damage shortly after exposure with appearance of karyolytic bodies and autophagosomes accompanied by increased hydrolase activity. Mitochondrial alterations were concomitant with diminished ductal oxidative enzyme activity. Although most of these alterations resolved rapidly as a result of acinar and ductal cell repair and regeneration originating in the intercalated ducts, secretory abnormalities were still observed two months after exposure as evidenced by the accumulation of granules in acinar cells and the heterogeneity of ductal cell granules. These anomalies, comparable to those observed in sialadenoses, probably result from persistent alterations of intralobular nerve endings.

Key words: Submandibular gland – Rat – Irradiation – Enzyme histochemical study – Ultrastructural study

Introduction

The long-term consequences to the salivary glands of high-dose irradiation of the cervical region are far from uniform. With fractionated doses, sclerosis and atrophy are the rule (Santangelo and Toto 1965; Elzay et al. 1969; Seifert and Geier 1971). This is not the case after single-dose irradiation, which often produces lasting functional disturbances, namely xerostomia, in contrast with the rareness (English et al. 1955) or absence of light microscopic changes (Shafter 1953; Sinn et al. 1972).

Offprint requests to: G. Chomette, Pathology Department, Hôpital de la Pitié, 83 Boulevard de l'Hôpital, F-75013 Paris, France

The authors wish to thank M.F. Baucher, A. Lesot and M. Tacnet for their technical assistance

0340-1227/81/0391/0291/$01.80

In this study, we have used more detailed ultrastructural and enzyme-histochemical investigation methods in an attempt to detect morphological changes which might account for the functional disturbances encountered after single-dose irradiation.

Materials and Methods

Forty-three 15-week-old male Wistar rats weighing an average of 360 g were used. Except for two control rats, all animals received a single dose of either 2,000, 2,500 or 3,000 rads by cobalt 60 irradiation to the mandibular angle and the region immediately beneath it. In some animals, radiomodulation techniques designed to either enhance or antagonize the radiation effects were used (internal carotid ligation, administration of oxygen at atmospheric pressure or hyperbaric oxygen after irradiation). In our hands these techniques failed to produce any significant effects.

The animals were sacrificed at various intervals after irradiation: 7 early (days 2, 4, 9 and 14), 18 on day 45 and 16 on day 70. Specimens were obtained from both right and left submandibular glands to allow comparison and were examined by light microscopy after being fixed in Bouin's fluid and embedded in paraffin. In addition to usual staining procedures (haematoxylin-eosin), a histochemical study of salivary secretion was performed by staining with PAS and alcian blue at pH 2 and 3.

An enzyme-histochemical study was performed on specimens from 12 animals. Tissue fragments were immersed in liquid nitrogen and sections were obtained using a cryostat. Enzyme activities tested included oxidative enzyme activities (diaphorases, succinate dehydrogenase (SD), malate dehydrogenase (MD), isocitric dehydrogenase (ICD), pentose shunt, lactate dehydrogenase), acid hydrolases (acid phosphatase, β-glucuronidase) and nonspecific esterases (naphtol AS), ATPases at pH 8,4 and 9,5 and alkaline phosphatase (Pearse 1972). In addition, the intracellular quantity of RNA was evaluated by Brachet's method.

Specimens for electron microscopy were obtained from the same 12 animals. The specimens were fixed in glutaraldehyde, post-fixed in osmium tetroxide and embedded in Epon. Ultrathin sections were cut on an LKB microtome and examined in a Hitachi H 300 electron microscope.

Results

Light Microscopic Findings

Submandibular glands of control rats exhibited the following features:
- Seromucous acini staining weekly with PAS and alcian blue at pH 3. These acini were surrounded by a dense population of mast cells which stained with alcian blue at pH 2.
- Striated secretory tubules lined with a clear epithelium containing PAS-positive granules (Simson et al. 1973). These curved ducts prolonged small intercalated ducts.
- A large number of intralobular striated ducts.
- Excretory ducts lined with a simple columnar epithelium situated in the interlobular connective tissue.

In irradiated animals, a few focal changes were seen early (between days 3 and 6). These changes included a generally increased density of cell nuclei and tiny focal islets of cell necrosis affecting essentially the striated tubules (Fig. 1). Such changes were sometimes associated with a moderate interstitial inflammatory reaction. Nine days after exposure, numerous large nucleolated nuclei appeared, indicating active regeneration. Beyond day 45, all appreciable changes had disappeared except for one case of chronic sclerosing inflammation with endoductal suppuration.

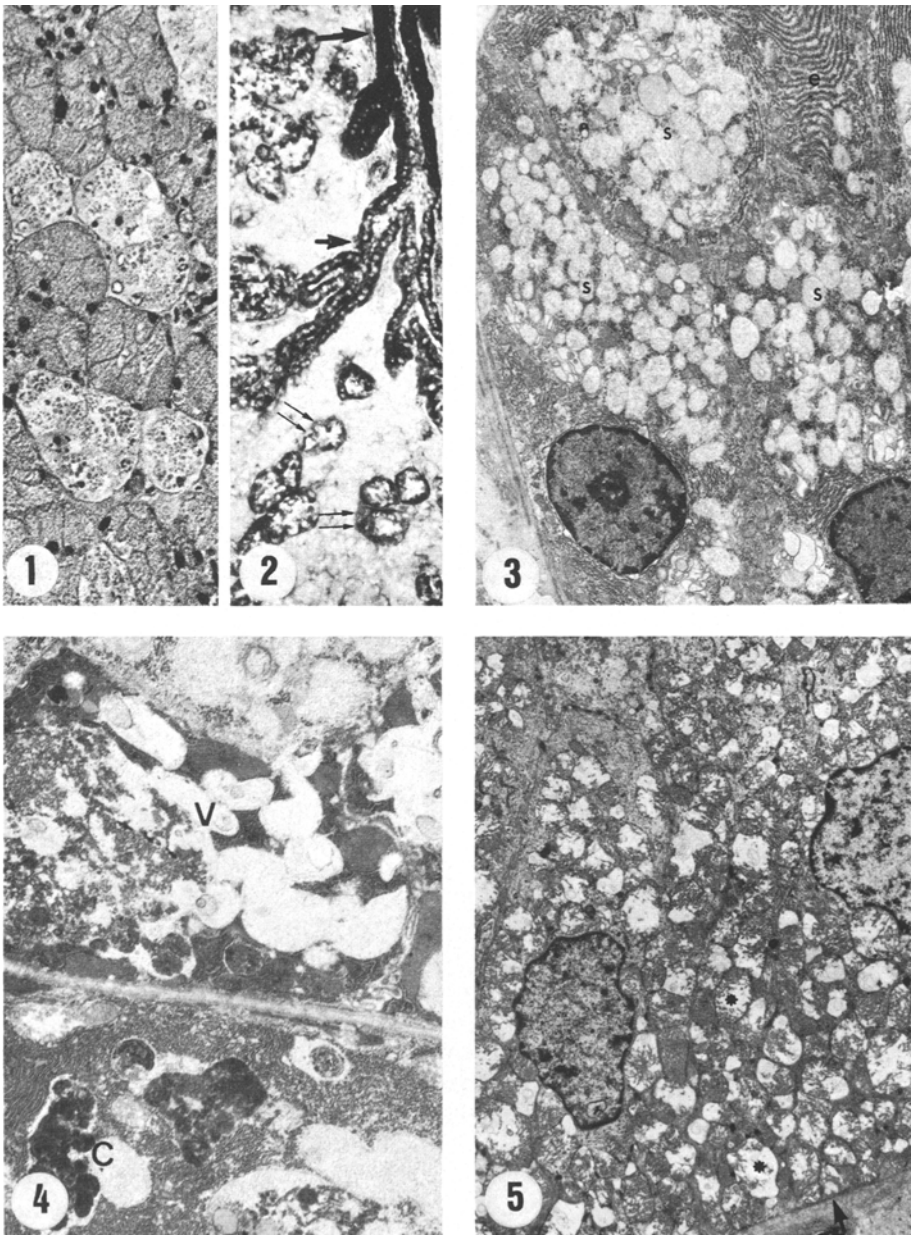

Fig. 1. Rat submandibular gland 9 days after irradiation showing ductal necrosis (→) and apparent integrity of the acini. Haematoxylin-eosin. ×280

Fig. 2. Enzyme histochemical reaction for NAD tetrazolium reductase in the submandibular gland of a non-irradiated control rat. The excretory ducts are strongly positive (➡) with a weaker positive reaction in the striated ducts (➡) and acini (⇉). ×300

Fig. 3. Submandibular acinar cells of a control rat showing apical secretion granules (*S*) and basal ergastoplasm (*e*). ×3,000

Fig. 4. Rat submandibular gland 6 days after irradiation showing a large autophagosome in one of the cells (*V*) and a karyolytic body (*C*) in the ergastoplasm of an adjacent cell. ×5,000

Fig. 5. Ductal cell of rat submandibular gland 9 days after irradiation showing a linear basement membrane (➡) and swollen, vacuolated mitochondria (*). ×10,000

Table 1. Normal and irradiated submandibular gland compared histoenzymological features

	Enzymes	Normal	After irradiation
Excretory ducts	Dehydrogenases Pentose shunt Diaphorase NADP	+++	+++
Excretory ducts and Striated ducts	Dehydrogenases Krebs cycle MD SD	++	++
Excretory ducts, Striated ducts, Acini	Ac. hydrolase Ac. Pase Naphthol-esterase β-glucuronidase	+	
Acini	RNA (Brachet's test)	++	
Myoepithelial cells	ATPase Alk. Pase	++	++

Enzyme-Histochemical Study

In control animals, our findings were similar to those described previously in the rat (Hayashi 1967) and other animal species (Harrison 1974). All duct systems showed strong positivity for oxidative enzymes (MD, LD, SD and diaphorases) (Fig. 2). These enzyme activities, and especially those of pentose shunt enzymes (glucose-6-phosphatase dehydrogenase and 6-phosphogluconate dehydrogenase) were particularly strong in the excretory ducts, where acid hydrolase activity was also intense. In the acini, oxidative hydrolase reactions were much weaker but significant amounts of RNA (Brachet's method) and monoamine oxidase were demonstrated. In the interstitial connective tissue, intense ATPase and alkaline phosphatase activity was detected on vascular walls and in myoepithelial cells.

Little modification of these enzyme activities was noted in irradiated animals. From 3 to 9 days after exposure, ductal oxidative activity remained intense but was irregular with loss of activity in individual cells, particularly in the striated ducts. At this time, hydrolase activity (acid phosphatase and esterase naphtol AS) was strikingly increased in the ducts and even in the acini. After the 9th post-irradiation day, a strongly positive Brachet's test indicated the intensity of acinar regeneration. At a late stage (day 45 and thereafter), enzyme changes were virtually non-existant. At most, there was a slight decrease in ductal oxidative activity and persistence of a moderately enhanced hydrolase activity (Table 1). It is noteworthy that alkaline phosphatase and ATPase activities were comparable in control and irradiated animals for all post irradiation intervals tested.

Ultrastructural Study

In controls, the acini were composed of an assembly of polyhedral cells, flared at their base (Fig. 3). The intercellular spaces, the apical junctional system and the central acinar lumen were all clearly visible (Toner et al. 1971). Secretion in the form of poorly contrasted vacuoles without a limiting membrane accumulated in the cell, surrounding the nucleus. The Golgi complex was not clearly visible. The mitochondria and ergastoplasm arranged in abundant linear figures, tended to occupy the basal portion of the cell. The darker cells of the striated ducts, studded with apical microvilli, exhibited deep infoldings of the basal plasma membrane enclosing numerous mitochondria. Lateral interconnections between adjacent cells were always highly developed. The apical pole of the cell contained a few vacuoles, lysosomes or multivesicular bodies. Tubular cells also contained dense secretory granules 100 to 200 nm in diameter. Bordering on the acini and the ducts were elongated myoepithelial cells, included in the basement membrane, and exhibiting the usual characteristics of muscle cells, i.e., longitudinal system of microfibrils, with denser areas and endocytosis vesicles. These cells formed desmosomal connections with adjacent epithelial cells. The vessels had a discontinuous endothelium. The interstitial tissue, where collagen fibers were sparse, contained fibroblasts and a few granular mast cells.

As early as 3 days after irradiation, changes were seen in the ducts and in the acini. Large autophagosomes (Fig. 4) arising from clear cytoplasmic foci or swelling of organelles appeared in acinar cells on day 3. Within these clear bodies, sometimes bound by a membrane, organelle debris was sequestered. This debris included ergastoplasmic fragments, burst mitochondria, membrane coils and even dense nuclear remnants, Karyolytic bodies (Fig. 4), reflecting nuclear damage, appeared later, on days 6 and 9. They presented as highly osmiophilic granular condensations, sometimes of considerable size (500 to 1,000 nm). The acini and ducts underwent marked alterations. In the striated ducts, striking mitochondrial changes occured (vacuolation of the matrix with cristal lysis and ballooning degeneration). Disappearance of plasma membrane infoldings was also observed (Fig. 5). The acinar and ductal secretory systems were profoundly altered with fragmentation of the ergastoplasm, confluence of acinar granules and disappearance of tubular cell granules. All these transient changes gave way to active regeneration 9 days after exposure. Nuclei of acinar cells were large and nucleolated and the regenerated ergastoplasm was abundant. In addition cell multiplication, giving rise to clear young cells, began to take place in the intercalated ducts from where it spread to the acini and the ducts.

On the 45th post irradiation day, the vacuolar bodies, remnants of the initial exposure, were present in only small numbers. Most of the karyolytic bodies had been expelled from the cells and could be seen in the basement membranes or even in the lumen of the lymphatics. However, a number of overt modifications persisted. Striated ductal cells had not recovered their plasma membrane indentations and the mitochondria remained profoundly altered, swollen and filled with lytic vacuoles (Fig. 6). Moreover, tubular cell secretion remained abnormal, granules being of non-uniform size and density. In the acini, secretory abnormalities were clearly evident despite the abundance of

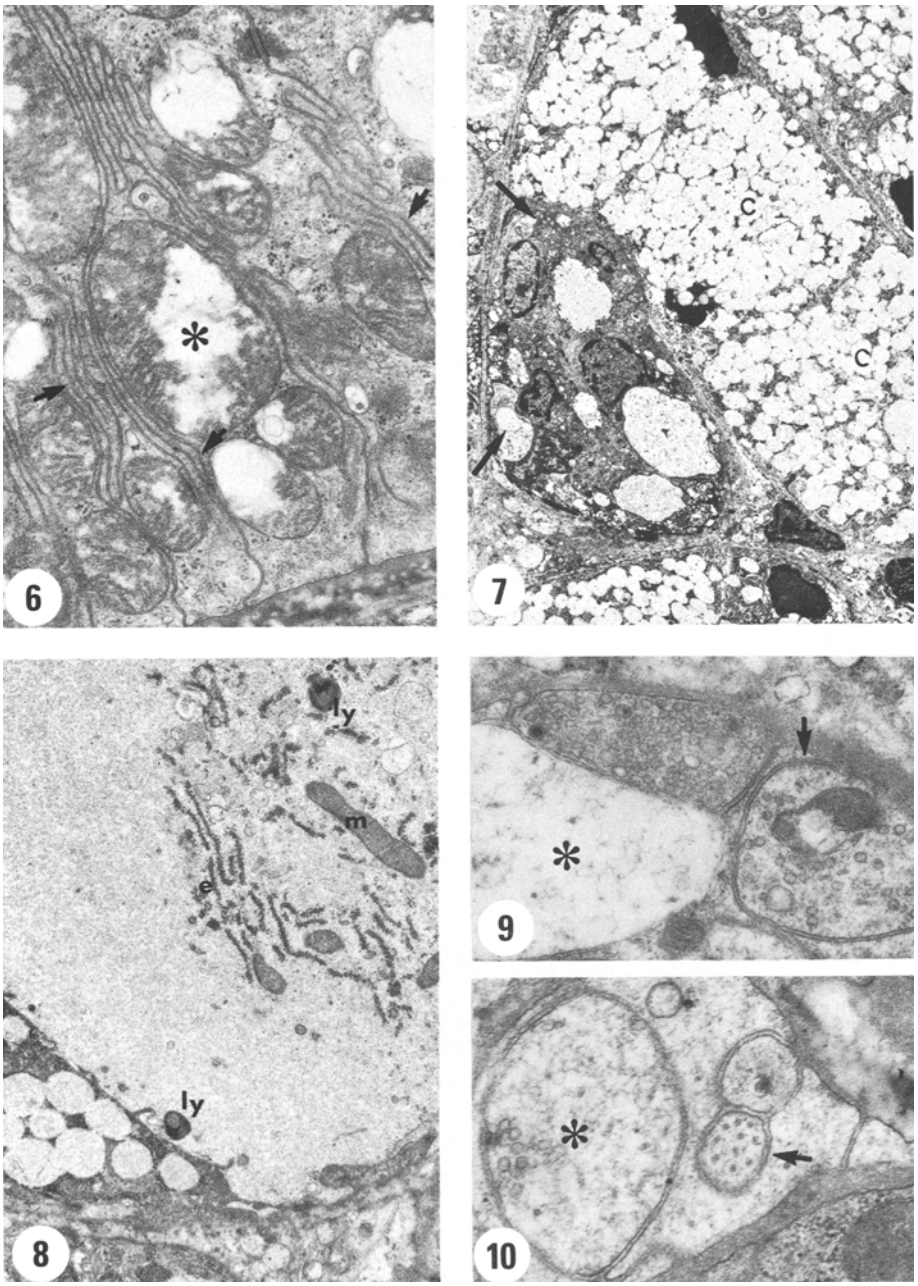

Fig. 6. Rat submandibular gland 45 days after irradiation. Note the ductal regeneration, infoldings of the plasma membrane (➡) and oedematous and vacuolated mitochondria (*). × 13,000

Fig. 7. Rat submandibular gland 75 days after irradiation showing a cell (*C*) engorged with secretory vacuoles forcing the nucleus to the base of the cell. Persistence of cell regeneration in the intercalated ducts (➡). × 4,000

Fig. 8. Rat submandibular gland 70 days after irradiation showing a necrotic cell engorged with secretions. Note the fragmentation of intracellular structures [ergastoplasm (*e*), mitochondria (*m*), lysosome (*ly*)]. × 7,000

Figs. 9, 10. Nerve alterations showing the coexistence of subnormal axones (➡) containing numerous vesicles and edematous axones (*) lacking their normal organelles (disappearance of vesicles). × 18,000

the ergastoplasm. The vacuoles appeared to be eliminated with difficulty and overabundant (Fig. 7). Accumulated within the cell, they appeared to force the nucleus to the base. Some particularly engorged cells were undergoing necrobiosis (Nuclear pyknosis, ergastoplasmic fragmentation) and this no doubt explained persistent signs of regeneration. In the interstitial tissue, while myoepithelial cells and vessels were normal, nerve endings appeared swollen, clear and contained abnormally few neurofilaments. Synaptic vesicles were sparse and most of these contained no dense material.

On the 70th post irradiation day, apart from a few vacuolated mitochondria and an abnormally high number of lysosomal bodies, the striated ducts had recovered their normal structure with reappearance of basal infoldings. In the acini, however, secretory engorgement and cytolysis persisted (Fig. 8). The moderate nerve ending abnormalities were still observable at this time (Fig. 9), 10).

In brief, while rapid regeneration of initial cytolytic lesions occured, residual secretory abnormalities could still be seen several weeks later and appeared to correlate with persistent alterations of nerve endings.

Discussion

Numerous studies have previously been carried out on early light (Shafter 1953; English 1954; Kashima et al. 1965; Santangelo and Toto 1965; Seifert and Geier 1971; Sinn et al. 1972) and electron (Pratt and Sodicoff 1972; Sholley et al. 1974; Stern et al. 1976) microscopic changes subsequent to experimental irradiation of salivary glands, particularly the submandibular glands in rodents. Early light microscopic changes are not very striking (small foci of necrosis and a mild interstitial inflammatory reaction. Electron microscopic examination reveals that as early as 16 h after exposure, highly osmiophilic and granular karyolytic bodies can be seen. Forty-eight hours post irradiation, autophagosomes and ductal mitochondrial changes (swelling, cristal lysis) are visible together with disappearance of basement membrane infoldings. All of these alterations reflect the direct effect of radiation on cells; indeed, vascular lesions are negligible (Sinn et al. 1972; Sholley et al. 1974; Stern et al. 1976). Six days after exposure, both active regeneration (increased nuclear volume and ergastoplasmic proliferation in acinar cells) and true regeneration by cell multiplication in the intercalated ducts take place.

Our findings were largely consistent with these observations. Slight differences in the degree and time of onset of cell damage may be attributed to the type of irradiation used (cobalt 60). The maximum intensity of the lesions induced was observed a little later on day 6. These lesions were predominantly autophagosomes, karyolytic bodies being visible in large numbers only on days 9 and 14. On the whole, however, the alterations observed were virtually identical to those described by other authors and the main changes (appearance of autophagosomes, mitochondrial modifications and later active ergastoplasmic regeneration) have evident consequences on enzyme-histochemical tests: increased hydrolase activity and irregular ductal oxidative activity followed by increased intensity of Brachet's test in acinar cells. In fact, it must be stressed that most

of these alterations are not specific for radiation or even for radiomimetic agents (Adkins 1974; Harrison and Garrett 1976) and are identical to changes seen following experimental ductal ligation (Seifert 1962; Shiba et al. 1972; Donath and Seifert 1975; Cutler et al. 1979). These alterations must therefore be viewed as a common mode of reaction of the salivary glands to a wide variety of types of injury.

Our observations prove that ultrastructural and functional alterations persist several weeks after irradiation (Phillips 1970). Forty-five days after exposure, neither the striated ducts nor the acini had recovered their normal structure. In the ductal cells, the mitochondria remained vacuolated and the normal infoldings of the basement membrane had not reappeared. Moreover, evidence of active cell damage persisted in the form of karyolytic bodies in the interstitial tissue and the lumen of lymphatics. All these changes had a lasting effect on enzyme reactions. At this time, regeneration always took place in the region of the intercalated ducts – it should be noted that in our cases regeneration did not result in the formation of adenomas (Cherry and Glucksmann 1959; Elzay et al. 1969). The most important finding, however, was that at 45 and 70 days after exposure, dyscrinism due to excretory deficiency of acinar cells persisted. Most of the cells were engorged with secretions which were not evacuated into the ductal lumen. A few cells were undergoing cytolysis. The reason for this morphological abnormality is not clear and it is remarkable to note the similarities with human (Donath and Seifert 1975) and experimental (Seifert 1962a, b; Donath et al. 1974; Cutler et al. 1979) sialadenoses. Moreover, although intralobular connective tissue was hardly affected, nerve endings showed changes including swelling of axones, lysis of neurofilaments and a decrease in the number and density of synaptic vesicles. Thus, persistent functional disturbances following irradiation may be due to neurosecretory dysfunction.

References

Adkins KF (1974) Ultrastructural changes induced by actinomycin D in acinar cells in submandibular glands of rats. Arch Oral Biol 19:859–863

Cherry CP, Glucksmann A (1959) Injury and repair following irradiation of salivary glands in male rats. Br J Radiol 32:596–608

Cutler LS, Pinney HE, Christian C, Russotto SB (1979) Ultrastructural studies of the rat submandibular gland in streptozotocin induced diabetes mellitus. Virchows Arch [Pathol Anat] 382:301–311

Donath K, Jirsch-Hoggmann HU, Seifert G (1973) Zur Pathogenese der Parotisatrophie nach experimenteller Gangunterbindung. Ultrastrukturelle Befunde am Drüsenparenchym der Rattenparotis. Virchows Arch [Pathol Anat] 359:31–48

Donath K, Spillner M, Seifert G (1974) The influence of the autonomic nervous system on the ultrastructure of the parotid acinar cells. Experimental contribution to the neurohormonal sialadenosis. Virchows Arch [Pathol Anat] 364:15–33

Donath K, Seifert G (1975) Ultrastructural studies of the parotid glands in sialadenosis. Virchows Arch [Pathol Anat] 365:119–135

Elzay RP, Levitt SH, Sweeney WT (1969) Histologic effect of fractionated doses of selectively applied megavoltage irradiation on the major salivary glands of the Albino rat. Radiology 93:146–152

English JA (1954) Morphologic effects of irradiation on the salivary glands of rats. J Dent Res 34:4–11

English JA, Wheatcroft MG, Lyon HW, Miller C (1955) Long-term observations of radiation changes in salivary glands and the general effects of 1,000 r to 1,750 r of X-ray radiation locally administered to the heads of dogs. J Oral Surg 8:87–99

Harrison JD (1974) Salivary glands of the cat: a histochemical study. Histochem J 6:649–664

Harrison JD, Garrett JR (1976) The effects of ductal ligation on the parenchyma of salivary glands of cat studied by enzymehistochemical methods. Histochem J 8:35–44

Hayashi M (1967) Comparative histochemical localisation of lysosomal enzymes in rat tissues. J Histochem Cytochem 15:83–92

Kashima HK, Kirkham WR, Andrews JR (1965) Postirradiation sialadenitis. A study of the clinical features, histopathologic changes and serum enzyme variations following irradiation of human salivary glands. Am J Roentgenol Radiumther Nucl Med 94:271–291

Pearse AGE (1972) Histochemistry, theoretical and applied, 3rd ed. Churchill Livingstone, London

Phillips RM (1970) X-ray induced changes in function and structure of the rat parotid gland. J Oral Surg 28:432–437

Pratt NE, Sodicoff M (1972) Ultrastructural injury following X-irradiation of rat parotid gland acinar cells. Arch Oral Biol 17:1177–1186

Santangelo MV, Toto PD (1965) Radiation effects on mouse submandibular gland. J Dent Res 44:1291–1298

Seifert G (1962a) Experimentelle Speicheldrüsenvergrößerungen nach Einwirkung von Noradrenalin. Beitr Pathol Anat 126:321–351

Seifert G (1962b) Elektronen mikroskopische Befunde an den Speicheldrüsenacini nach Einwirkung von Noradrenalin. Beitr Pathol Anat 127:111–136

Seifert G, Geier W (1971) Zur Pathologie der Strahlen-Sialadenitis. Z Laryngol-Rhinol-Otol 5:376–388

Shafter WG (1953) The effect of single and fractionated doses of selectively applied X-ray irradiation on the histologic structure of the major salivary glands of the rat. J Dent Res 32:796–806

Shiba R, HAmada T, Kawakatsu K (1972) Histochemical and electron microscopical studies on the effect of duct ligation of rat salivary glands. Arch Oral Biol 17:299–309

Sholley MM, Sodicoff M, Pratt ME (1974) Early radiation injury in the rat parotid gland. Reaction of acinar cells and vascular endothelium. Lab Invest 31:340–354

Simson JAV, Hall BJ, Spicer SS (1973) Histochemical evidence for lipoidal material in secretory granules of rat salivary glands. Histochem J 5:239–254

Sinn DP, Stoker NG, Epker BN (1972) Effects of fractionated doses of Cobalt 60 irradiation on rabbit submandibular glands: light microscopic studies. J Oral Sug 30:277–283

Stern MH, Turner JE, Jett LS, Mincer H, Mc Ginnis JP (1976) Electron microscopic changes in rat parotid and submandibular glands subsequent to total body irradiation with fast neutrons J Oral Surg 42:620–630

Toner PG, Carr KE, Wyburn GM (1971) The digestive system and ultrastructural atlas and review. Butterworth London

Accepted January 16, 1981