Acute and Late Radiation Injury in Rhesus Monkey Parotid Glands

Evidence of Interphase Cell Death

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Acute and chronic salivary gland dysfunction are common sequelae of radiotherapy for head and neck cancer; but the associated morphologic changes, especially of the acute damage, have received relatively little study. For investigation of the morphologic characteristics of acute radiation injury to parotid glands, rhesus monkeys were studied 1–72 hours after parotid irradiation with single doses of 2.5–15.0 Gy. The acute damage from all doses was clearly expressed by 24 hours. Histologically, parotid glands irradiated with 2.5 or 5.0 Gy had random degeneration and necrosis of the serous acinar cells. Doses of 7.5–15.0 Gy produced widespread degeneration along with necrosis of whole acini. Serous cell damage was accompanied by neutrophilic inflammation that subsided after 24 hours to become replaced by plasma cell and lymphocytic infiltrates. Parotid glands receiving 7.5–15.0 Gy were atrophic at 16–22 weeks after irradiation and showed no recovery by 40 weeks. Although parotid acinar cells are well-differentiated nondividing cells, these observations show that they express lethal radiation injury in interphase within hours of receiving a radiation dose as low as 2.5 Gy. This is unlike most mammalian cells that express radiation injury during mitosis. Chronic atrophy is a consequence of this direct, irreversible, and early injury, rather than the result of radiation-induced changes in the vasculature. (Am J Pathol 1986, 124:469–478)

THE LUNAR ASTRONAUTS, in the novel *Space*, by James A. Michener, were exposed to sudden, intense sunspot activity while on the moon.[1] The astronauts quickly developed dryness of the mouth, which indicated to the NASA scientists on earth that a significant total body exposure to ionizing radiation had occurred. Although this is a fictitious incident, it illustrates accurately that alteration of salivary gland function is both a rapid and specific response to radiation exposure of the major salivary glands. Besides being a change that could make an individual aware of significant accidental exposure to ionizing radiation, reduced salivary gland secretion is a vexing late sequela of therapeutic radiation.

Radiotherapy is the most commonly used modality for treatment of cancer of the head and neck.[2] Because the sites of primary carcinomas of this area and the paths of lymphatic spread are in close proximity to the major salivary glands, by necessity, all or part of the salivary glands are often included in the radiation treatment fields. The most common complications of radiotherapy for head and neck cancer are those associated with dysfunction of the major salivary glands.[3–8] Many radiation therapy patients develop swelling and tenderness of the salivary glands within hours of receiving the first few treatments in a course of fractionated radiotherapy.[3,4,9–12] This early reaction is usually transient; but, depending upon the volume of salivary tissue that is irradiated, a significant proportion of patients will suffer permanent xerostomia.[3–5,9,10,13]

The reduced volume of saliva has increased viscosity and altered composition including decreased pH, lower content of protein and secretory antibodies,[6,14–16] increased salinity,[17] and increased numbers of cariogenic

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microorganisms.[18] The changes in quantity and quality of saliva alter taste acuity, create abnormal taste sensations, make swallowing difficult, and contribute to deterioration of oral hygiene.[3,6-8,19] In many persons with xerostomia, oral mucositis, rampant tooth decay, and osteoradionecrosis of the mandible develop subsequent to tooth extraction.[14,20-22] Even if radiotherapy succeeds in controlling the tumor, the oral complications associated with reduced salivary gland function may retard the patient's recovery and certainly degrade the quality of life.[3,8,9,15,22] Although salivary glands are nonvital organs, their adequate function is important for human health and well-being.

The morphology of late or chronic lesions in irradiated human salivary glands is well known,[14,23-25] but acute changes due to radiation have received little study. Biopsies from the parotid salivary glands of 10 patients taken at 24 hours after irradiation with single doses of 1000 or 2000 Roentgens constitute the largest morphologic study of acute radiation sialoadenitis in humans.[26] Radiation sialoadenitis has been studied in several species of animals. There are, however, some notable differences in anatomic, biochemical, physiologic, and morphologic characteristics between the salivary glands of rodents, lagomorphs, carnivores, and ruminants and those of human and nonhuman primates.[27-29] Rats and mice have been most widely used in the study of radiation sialoadenitis. However, both rat and mouse salivary glands are under endocrine control, and thus the effects of radiation differ between the sexes.[30-34] Some morphologic studies indicated that the rat parotid gland is more radioresistant than the human parotid gland,[34-37] and mouse salivary glands are even more radioresistant than those of rats.[33] In contrast to the condition in humans, acute inflammatory cell infiltration does not occur in irradiated rat salivary glands, and in these animals hyperamylasemia does not consistently develop after salivary gland irradiation.[7,34-38]

Although the salivary glands of monkeys and humans are most similar, we found only one report of a study of irradiated monkey salivary glands.[39] These investigators studied submandibular and sublingual glands from 6 rhesus monkeys at 10, 20, 30, or 60 days after irradiation with fractionated doses of orthovoltage radiation. The serous cell degranulation, degeneration, and necrosis, along with inflammation and fibrosis, described in these animals were comparable to those encountered in irradiated human salivary glands.[11,23,26]

Even with relatively low radiation doses, irradiation alters salivary gland function very quickly.[3-5,10] Conventional radiotherapy treatments generally are given in daily fractions of approximately 2.0 Gy, 5 days a week, for 5–7 weeks. Some patients experience xerostomia and dysgeusia during the first week of therapy.[3-5,10] When all of the parotid glands are irradiated, parotid saliva secretion may be reduced to near zero as quickly as the second day of treatment.[4] The acuteness of functional changes by irradiated parotid glands is widely recognized; yet circumstances have rarely permitted the study of the acute morphologic alterations. Limited morphologic studies of early radiation injury to human salivary glands indicate that serous acinar cells are more radiosensitive than mucous acinar cells.[26] To expand this premise, we used rhesus monkeys to compare lesions in the purely serous parotid gland with those in the mixed mucous/serous submandibular gland. We emphasized the heretofore unstudied evolution of the acute damage by taking sequential salivary gland biopsy specimens 1–72 hours after irradiation with single doses of 2.5–15 Gy. The observations made on the irradiated rhesus monkey parotid glands are the subject of this report, and a companion article describes our studies of irradiated submandibular glands from the same animals.[40]

Materials and Methods

The experiment is summarized in Table 1. Eighteen adult female rhesus monkeys, culled from a commercial breeding colony, were made available to us for short-term study. These animals were used in the acute phase of the study for biopsy of irradiated salivary glands at

Table 1—Cobalt-60 Gamma Irradiation of Rhesus Monkey Salivary Glands

Dose (Gy)	Times of biopsy	No. of monkeys
2.5	1, 3, 6, 9, 12, 24, 48, 72 hours	4*
	16–22 weeks	4†
	40 weeks	1‡
5.0	1, 3, 6, 9, 12, 24, 48, 72 hours	4
	16–22 weeks	4
	40 weeks	1
7.5	1, 3, 6, 9, 12, 24, 48, 72 hours	4
	16–22 weeks	4
	40 weeks	1
10.0	6, 12, 24, 48, hours	2
	16–22 weeks	2
	40 weeks	1
12.5	6, 12, 24, 48 hours	2
	16–22 weeks	2
	40 weeks	1
15.0	6, 12, 24, 48 hours	2
	16–22 weeks	2
	40 weeks	1

Footnotes apply to all doses.

* No salivary gland underwent biopsy more than once for study of the acute effects within hours of irradiation.

† The animals used for the acute study were killed 16–22 weeks after irradiation.

‡ A single monkey treated with one of the six doses underwent biopsy 40 weeks after irradiation.

eight time intervals, within 72 hours after irradiation. As Table 1 shows, six radiation doses were used. Three groups of 4 monkeys were treated with a single midline doses of 2.5, 5.0, or 7.5 Gy, and three pairs of monkeys received single midline doses of 10, 12.5, or 15.0 Gy. The 18 animals were sacrificed 16–22 weeks after irradiation. An additional 6 adult female rhesus monkeys received irradiation to the salivary glands with the same six doses. These latter animals had salivary gland biopsies taken at 40 weeks after irradiation, and the animals continue to be maintained for long-term observation. For comparison, biopsies of nonirradiated rhesus monkey parotid glands were taken from 6 monkeys that were being maintained in the same colony as the irradiated animals. All of the monkeys were housed in individual cages with water continually available from an automatic watering system. Their ration consisted of commercial pelleted primate diet (Purina Monkey Chow, Ralston Purina Co., St. Louis, Mo) supplemented twice weekly with fresh fruit.

Ketamine hydrochloride (Vetalar, Parke, Davis, Morris Plains, NJ), 10 mg/kg, was given intramuscularly for immobilization of the animals for radiation treatments. The radiation was delivered with a cobalt-60 gamma ray therapy unit with a dose rate of 1.5 Gy/min at a source–skin distance of 80 cm. Bilateral parallel opposed fields were used. The animals were irradiated in lateral recumbancy. The anterior and posterior field margins were the canine tooth and the transverse process of the first cervical vertebra, respectively. The superior margin was the top of the zygomatic arch, and the inferior margin was 2 cm below the body of the mandible.

The animals were fasted overnight before irradiation and salivary gland biopsy. To prepare the animals for biopsy, immobilization was accomplished with ketamine followed by endotracheal intubation and maintenance of anesthesia with halothane (0.25–1.0%) supplemented by a 2:1 ratio of oxygen to nitrous oxide given at a flow rate of 3 l/min. The animals were positioned in lateral recumbancy and the surgical field prepared in a routine fashion. Bilateral parotid gland biopsy specimens were taken, but no gland was used for biopsy more than once (Table 1). Biopsy specimens were taken from a standard location that permitted avoidance of the biopsy site when later samples were collected at necropsy. The specimens were collected from the midcaudal border of the parotid gland. Superficial sampling from this region avoids the facial nerve, major blood vessels, and the major parotid ducts. The gland was exposed through a 2.5–3.5-cm vertical incision that began about 2 cm beneath the external auditory meatus. At this level the parotid is covered only by the cervical extension of the thin platysma muscle and deep cervical fascia. Blunt dissection exposed the glandular tissue. It was unnecessary to incise the thin, transparent parotid capsule. Wedge biopsy specimens approximately 1–1.5 cu cm were taken by bluntly inserting two straight hemostatic forceps along natural planes of separation between caudal lobes of the parotid and underlying muscle and fascia. The forceps were applied at converging angles to a depth of 1–1.5 cm until the tips touched. The wedge was taken from between the forceps by cutting along the forcep edges with a scalpel. After the wedge was removed, hemostasis was achieved by applying electrocautery to the cut margins before releasing the forceps. Closure of the fascia, muscle, and subcutis was done with one or two layers of 2-0 chromic gut in a continuous or interrupted pattern. The skin was apposed with stainless steel sutures or staples.

The fresh wedge biopsy specimen was placed in phosphate-buffered 10% formalin for light microscopy, then processed by standard paraffin-embedding methods. Sections were cut at 2–6 μ, then stained with hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), or alcian blue (AB) at pH 2.5. A review of the gross and microscopic anatomy of rhesus monkey parotid glands, along with details of the technique for biopsy of these glands, has been reported.[41]

Results

Clinical, Surgical, and Necropsy Observations in Irradiated Monkeys

The monkeys that received single doses of 7.5–15.0 Gy had swelling of the cheeks, jaws, and/or upper cervical regions within 20 minutes to 4 hours of irradiation. By 24 hours after irradiation, the outward swelling had subsided in most animals. Some of the animals salivated excessively for 1 or 2 days after irradiation. Surgical exposure of the parotid region at 3–24 hours after irradiation revealed that the subcutis and fascia over the gland was edematous but not hyperemic. At surgery 48 and 72 hours after irradiation, slight edema of the parotid region was still evident in monkeys irradiated with doses higher than 7.5 Gy but not in those exposed to lower doses. In the monkeys that received doses of 12.5 or 15.0 Gy and were maintained beyond 22 weeks, firm, persistent swelling of the face and cheeks developed at about 30 weeks after irradiation. At this writing, about 80 weeks after irradiation, the swelling has persisted. Both animals have mucinous saliva, and they exhibited some difficulty with chewing, which was overcome by learning to moisten their feed biscuits.

The parotid gland biopsy specimens were taken from a standard location along the midcaudal border of the gland. This permitted evaluation of gross alteration of the parotid glands and avoidance of the biopsy sites

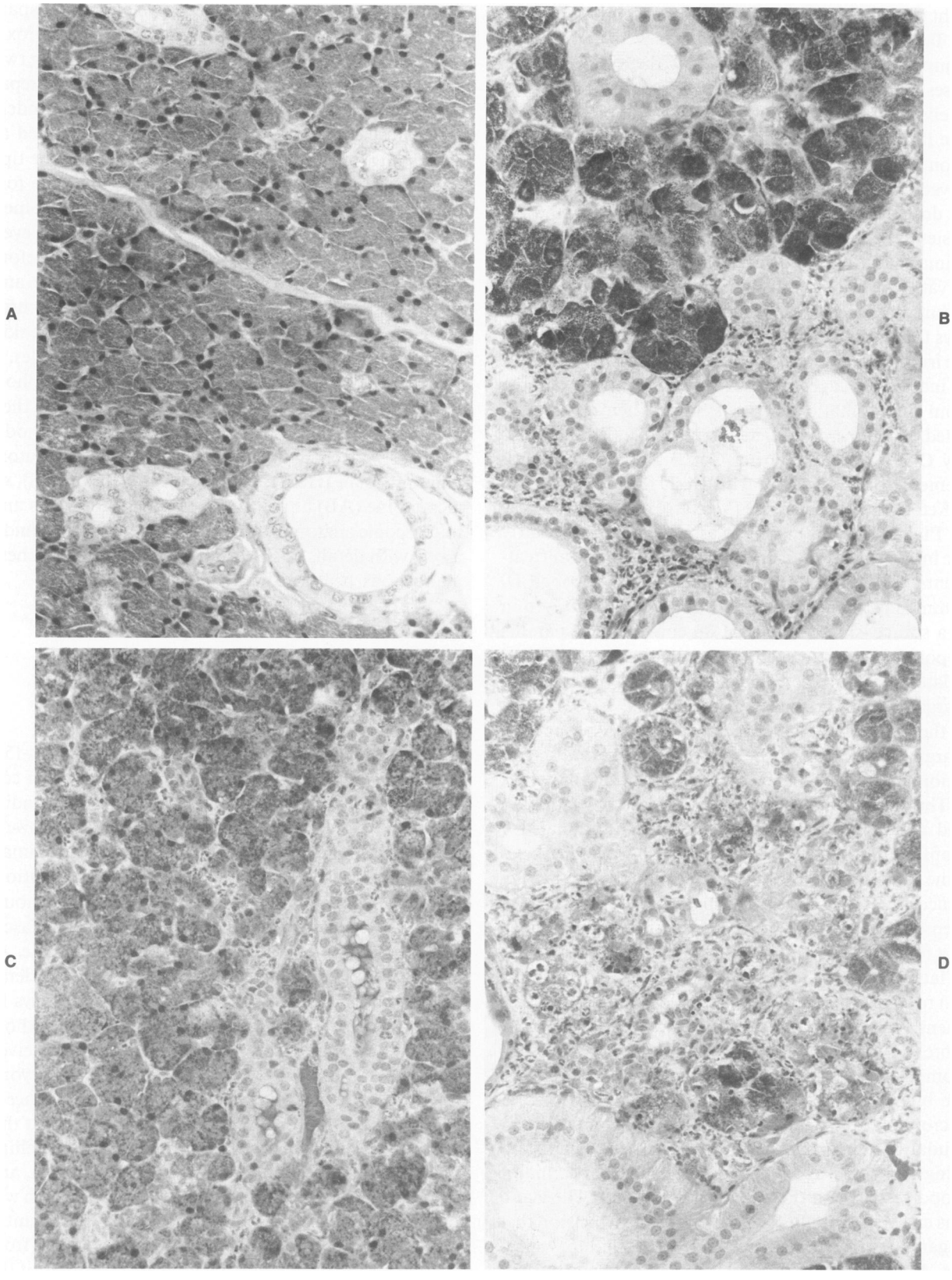

Figure 1—Progression of acinar alterations and inflammation in parotid glands irradiated with 7.5 Gy. **A**—Nonirradiated. Serous cell cytoplasmic granularity and location, shape, size, and staining of nuclei are uniform. **B**—Three hours. Serous cells exhibit swelling and variability of granularity, and some nuclei are pyknotic. Neutrophils fill vessels. **C**—Six hours. Increased variability of serous cell granularity and nuclei. Neutrophils among acini. **D**—Twenty-four hours. Necrosis of whole acini. Neutrophils infiltrate acini in the necrotic area. (H & E, ×250)

when samples were collected at necropsy of monkeys killed at 16–22 weeks after irradiation. The parotid glands exposed to doses greater than 7.5 Gy were paler, firmer, and smaller than parotid glands in monkeys that had not been irradiated. The reduction in size was subjectively greater with increasing dose. Atrophy and firm texture were noted during biopsy procedures at 40 weeks after irradiation, but again, only in those monkeys exposed to doses of 7.5 Gy and greater.

Morphologic Findings

Acute Effects

Major morphologic alterations of individual serous cells were similar after all radiation doses, but the numbers of degenerating and necrotic serous cells increased with time and became more numerous with increasing dose. Although individual cells in all stages of degeneration and necrosis could be found in specimens taken at all times and exposed to all of the doses, the relative numbers of cells with particular features suggested a sequence of alterations (Figure 1A–D). In the earliest biopsies (1–6 hours), the initial change appeared to be enlargement of nuclei and peripheral clumping of chromatin, which resulted in nucleoli becoming visible in some serous cell nuclei. The nuclei tended to move from the base of the cells to a more central location. Concurrent with the nuclear changes, the cytoplasmic granules enlarged and the serous cells became more lightly stained, exhibiting a grayish tint, rather than being uniformly dark purple with H&E and PAS staining. In the 6-hour samples, clumps of enlarged serous cell granules had formed, and partially or totally degranulated cells became numerous. Chromatin clumping became more prominent, and nuclear outines became irregular and angular. Serous cells became separated, which resulted in loss of contact between adjacent cells. Associated with the individualization of serous acinar cells, the basement membranes of acini became visible. Myoepithelial cell nuclei were enlarged, and their boundaries were sometimes prominent, because of reduced acinar size and hypertrophy. Subsequent 12- and 24-hour samples contained increased numbers of serous cells with darkly stained, crescent-shaped nuclei and nuclei that exhibited pyknosis, karyorrhexis, and karyolysis. With doses less than 7.5 Gy, necrosis involved only individual cells (Figure 2); whereas the higher doses resulted in necrosis involving all cells comprising some acini in addition to the death of individual cells (Fig-

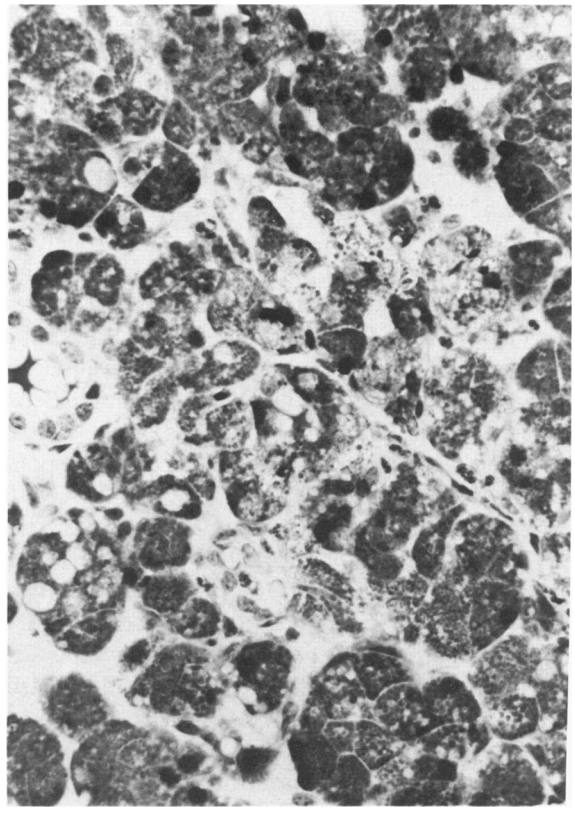

Figure 3—Twenty-four hours after irradiation, 15.0 Gy. Necrosis of all cells comprising multiple acini. Some serous cells are vacuolated. (H & E, ×400)

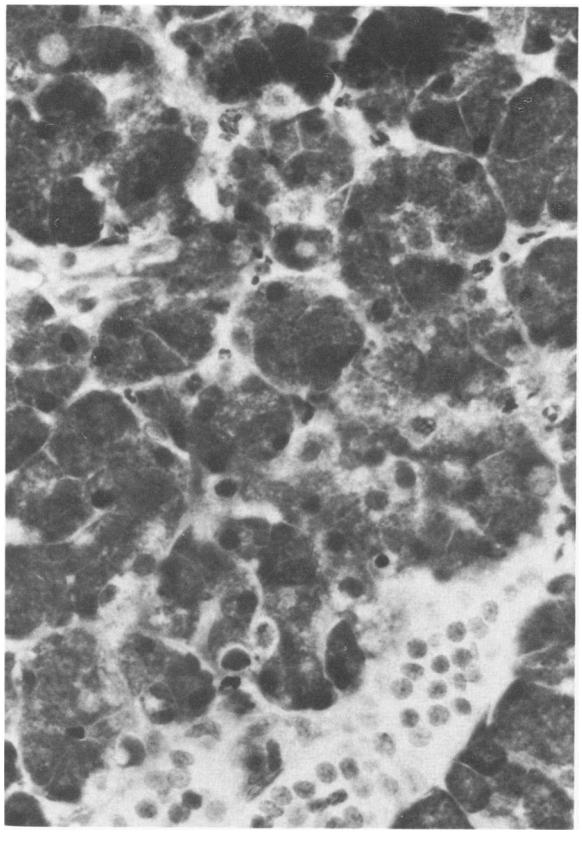

Figure 2—Twenty-four hours after irradiation, 2.5 Gy. Generalized serous cell enlargement and necrosis of widely scattered cells. (H & E, ×400)

ure 3). In the parotid glands exposed to doses of 12.5 or 15.0 Gy, some regions had lobules devoid of acini, which indicated that there had been necrosis and total loss of multiple acini in selected areas. The higher doses also resulted in vacuolation of serous cells that seemed to rupture some cells and cleave them from the basement membrane, leaving an empty basilar space. Part of the circumference of some acini was empty, which was attributed to total loss of individual serous cells. Many cells had large, discrete, possibly membrane-bound areas in the cytoplasm with very fine, eosinophilic granularity. Other cells contained homogeneous hyaline and PAS-positive droplets similar in size to the nucleus.

In the sequential biopsy specimens of the parotid glands there was a classical acute inflammatory response (Figure 1A–D). With doses of 2.5, 5.0, and 7.5 Gy, the inflammation seemed to increase in a dose-related fashion. The exudation of neutrophils followed the typical course of concentration in the vasculature and margination at 1–3 hours. In the 6- and 9-hour specimens, neutrophils were most numerous in the periductular stroma and among the acini. There was invasion of acini by neutrophils in the 12- and 24-hour specimens. At 48 and 72 hours, the number of neutrophils was reduced. Associated with declining numbers of neutrophils, there were increasing numbers of lymphoid cells. A few lymphocytes and plasma cells were observed in the periductular connective tissue of control parotid glands. Likewise, insignificant numbers of these cells were in specimens taken through 24 hours after irradiation. The 48- and 72-hour samples contained increased numbers of plasma cells and lymphocytes in the periductular stroma as well as infiltrating among the acini.

The first biopsy specimens of the parotid glands exposed to 10.0, 12.5, and 15.0 Gy were obtained at 6 hours after irradiation. The intensity of the diffuse neutrophilic exudation was consistently less than that observed in the specimens exposed to the lower doses. Although the numbers of neutrophils were reduced in the specimens exposed to the higher doses, their location at 6 hours, ie, periductular and among acini, was the same. In subsequent 12-, 24-, and 48-hour samples, the locations of the neutrophils were also similar to what was observed with the lower doses, and at 48 hours there were increased numbers of plasma cells and lymphocytes. This indicates that the temporal course of the diffuse inflammatory response was comparable at all doses. The parotid glands treated with doses higher than 7.5 Gy had multifocal persistence of neutrophils through 48 hours after irradiation in regions of extensive necrosis. The time course of edema accumulation and eventual subsidence correlated with the rise and fall of diffuse neutrophilic infiltration in parotid glands exposed to 7.5 Gy and below. Although fewer neutrophils were present in the specimens irradiated with doses greater than 7.5 Gy, the edema tended to be greater and persist through 48 hours in parotid glands exposed to 10.0, 12.5, and 15.0 Gy. Above 7.5 Gy, the stroma, in addition to being edematous, appeared hyalinized.

Beginning in the 24-hour samples, the epithelia of excretory and striated ducts were sometimes infiltrated

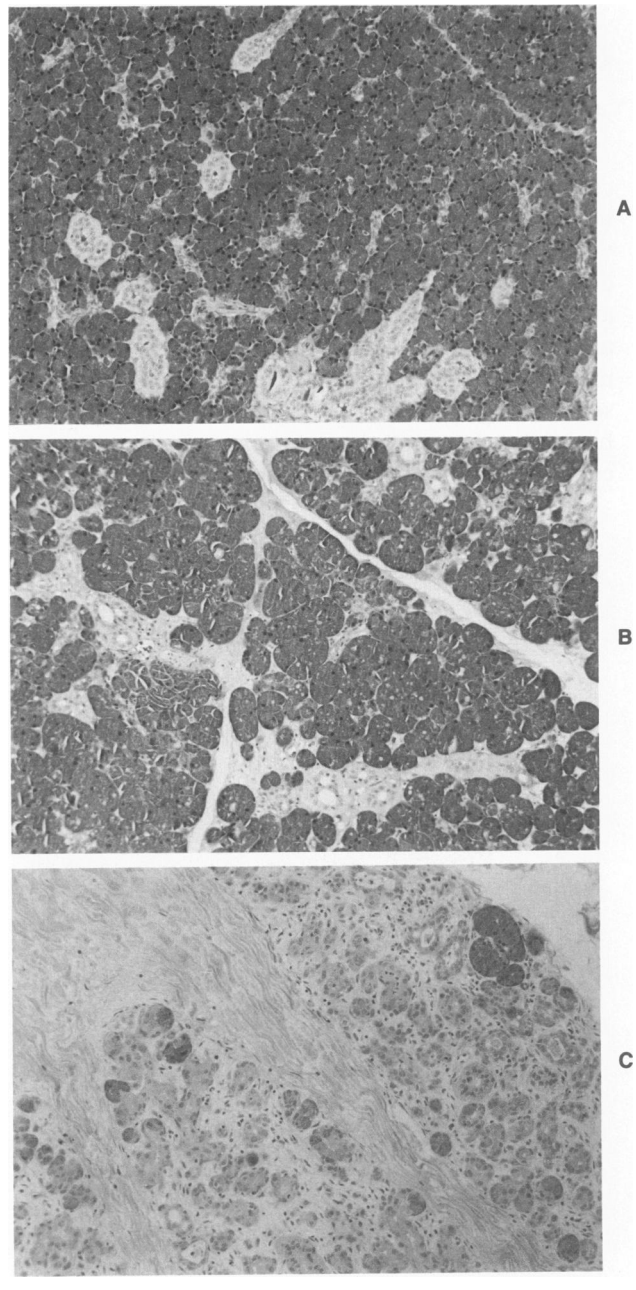

Figure 4—Atrophy or irradiated parotid glands. **A**—Nonirradiated for comparison of lobule size and amount of stroma. **B**—At a dose of 7.5 Gy 20 weeks after irradiation. Lobule size is reduced, and periductal and interlobular connective tissue is increased. **C**—At a dose of 15.0 Gy 20 weeks after irradiation. Marked reduction in lobule size and number of acini. Abundant sclerosis. (H & E, ×100)

with neutrophils. No inflammation directly involved the ducts in the parotid glands exposed to doses lower than 7.5 Gy. In addition to neutrophilic infiltration of the ducts at the doses of 10.0, 12.5, and 15.0 Gy, there was necrosis of ductal epithelium, and the lumens often contained sloughed epithelium and neutrophils. This involvement of the ducts was most evident in glands exposed to 12.5 and 15.0 Gy. Some blood vessels in glands exposed to doses greater than 7.5 Gy had endothelial cell nuclei that were hyperchromatic and greatly enlarged. The intima had relatively acellular, lightly stained widening, and the smooth muscle nuclei of the tunica media were hypertrophied.

Chronic Effects at 16–22 Weeks After Irradiation

The monkeys treated with single doses of 2.5 to 15.0 Gy from which salivary gland biopsy specimens were taken within hours of irradiation were sacrificed 16–22 weeks after irradiation. Postmortem examination showed that parotid glands exposed to 2.5 Gy resembled those of control monkeys, which indicated that there was no residual irradiation-induced damage. In the majority of serous cells in the parotid glands exposed to 5.0 Gy, the number of granules was reduced, and both granule size and staining intensity varied considerably even within individual cells. Nuclei were enlarged and surrounded by an agranular gray zone. Small numbers of plasma cells, along with lesser numbers of lymphocytes, infiltrated the periductal stroma and among the acini. The lobules were of the expected size, and the duct system and its supporting stroma appeared normal. Mild lobular atrophy seemed to have occurred in the specimens exposed to 7.5 Gy (Figure 4B). The connective tissue was increased between lobules and surrounding intralobular ducts. Numerous plasma cells infiltrated among the acini, and aggregates of lymphocytes were in the periductal connective tissue. Nuclei were only mildly enlarged, but nuclear outlines were consistently irregular. Large numbers of serous cells had empty intracytoplasmic vacuoles. There was increased AB-positive staining of intercalated ducts, and the lumens of some acini contained AB-positive material.

The parotid glands exposed to 10.0 Gy were atrophic. In all lobules, only a few intact acini remained. Single or groups of two or three serous cells were common. Surviving serous cells generally had uniform granularity, and the nuclei exhibited only occasional irregularity of shape and chromatin distribution. The parotid lobules were mainly composed of ducts and plump stromal cell nuclei admixed with large numbers of plasma cells and lymphocytes. The striated ducts were in relatively good condition, whereas the intercalated ducts were mildly dilated and their epithelial cells varied so much in shape and size that cytologic atypia was recorded. PAS- and AB-positive staining of ductal epithelium increased substantially. Some ducts were almost completely lined by cells that were strongly positive for both mucosubstances. AB-positive material commonly filled ducts and acini. The stroma between lobules was densely collagenous, and the tunica media of some intraglandular arteries was hypertrophied.

Atrophy, interlobular sclerosis, and plasmacytic infiltration were increased in the parotid glands exposed to 12.5 Gy. The surviving serous acini and isolated serous cells were generally sparsely granulated and sometimes vacuolated. Compared with the 10.0 Gy specimens, dilation and epithelial alterations, including atypia of intercalated ducts, were more prominent. The excretory ducts had either increased numbers of large cells that stained positively for mucosubstances or they were lined by epithelium that exhibited squamous metaplasia. Stromal cell nuclei within lobules were frequently very large and contained prominent nucleoli. Some stromal cells and intercalated duct epithelial cells contained homogeneous light yellow material, which, because of its refractile appearance, was interpreted to be crystalline. The severity of all the changes seen in the parotids exposed to 12.5 Gy was substantially increased in those irradiated with 15.0 Gy (Figure 4C). In contrast to the specimens irradiated with lower doses, there was considerable loss of polarity of the atypical epithelium lining intercalated ducts. Some of these cells were necrotic, and lumens of ducts contained sloughed cells and neutrophils. These latter changes might be related to infection that had ascended the duct system from the grossly infected oral mucosa.

Chronic Effects at 40 Weeks After Irradiation

The biopsy specimens taken at 40 weeks after irradiation were from a group of 6 monkeys that are being held for long-term observation. The parotid gland exposed to 2.5 Gy showed no abnormalities. In the ones exposed to 5.0 or 7.5 Gy, the amount of interlobular connective tissue was mildly increased, and a few lymphocytes were among the acini. Mild lobular atrophy was suspected in the parotid tissue irradiated with 7.5 Gy. In samples irradiated with 10.0, 12.5, or 15.0 Gy, the tissue was obviously atrophic with dense collagenous stroma between the lobules and prominent concentric collagenous thickening of the adventitia of arteries. Some lobules were composed exclusively of ducts. The epithelium lining intercalated ducts exhibited extreme variability of shape, size, and polarity. Intercalated ducts were ectatic, and excretory ductal epithelium exhibited squamous metaplasia. Individual epithelial cells of striated and excretory ducts were necrotic in the glands exposed 12.5 or 15.0 Gy. The lumens of these ducts often contained neutrophils. Atypical nuclei were

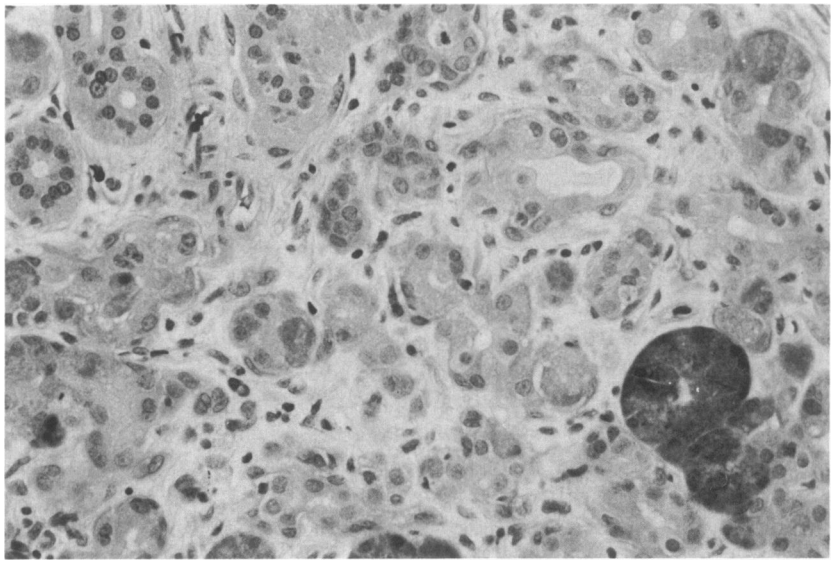

Figure 5—Atrophy at 40 weeks after irradiation, 12.5 Gy. Variability of shape and size of nuclei in ducts, stroma, and surviving acini. Infiltration by lymphocytes. (H & E, ×250)

observed in the epithelium lining ducts and in the stroma (Figure 5). In contrast to the predominance of plasma cells over lymphocytes in the specimens examined at 16–22 weeks after irradiation, these specimens at 40 weeks contained more lymphocytes than plasma cells. Although there was a shift to lymphocyte predominance, the stromal infiltration of mononuclear leukocytes was consistently less in the 40-week specimens, as compared with the 16–22-week samples. The atrophic parotid glands irradiated with the three highest doses contained increased numbers of mucopolysaccharide-positive cells. The increase of acidic mucopolysaccharides (AB-positive) was particularly prominent. These cells lined altered ducts and formed structures in some lobules that were believed to be mucuslike acini.

Discussion

It is an axiom of radiobiology that the rate of turnover of a tissue is the major factor determining perceived clinical radioresponsiveness and whether the tissue will exhibit an acute or late reaction to radiation.[42–45] By definition, acute reactions occur during or soon after irradiation, whereas the onset of late reactions is delayed for months or years. Tissues that react acutely are typically those whose stem cells are rapidly proliferating, eg, bone marrow, mucous membranes, and skin. Conversely, late reacting tissues are composed of parenchymal or supporting cells that are slowly proliferating, eg, the central nervous system, liver, and kidney. Salivary acini are composed of highly differentiated cells that display a low rate of mitotic activity, because most cells are in a protracted Gl phase of the division cycle or interphase. However, they can be stimulated to divide, and have been classified as postmitotic reverting cells.[11,44,46] Because of these characteristics, these cells would be expected to be late responders to radiation. Yet early changes of saliva volume and composition that occur soon after salivary gland irradiation indicate that the tissue is acutely radioresponsive, at least in a functional context. Some researchers have attributed the acute and chronic changes in irradiated salivary glands to ischemic injury caused by damaged to the fine vasculature.[46] Enhancement of the acute damage has been ascribed to inflammation, and because of lymphoplasmacytic infiltrations detected in specimens at later times after irradiation, a contribution of autoimmunity to the damage has been considered.[46] Finally, some investigators have concluded that radiation directly and selectively damages the serous cells.[23] This latter concept is reasonable in that it explains the specificity and rapidity of functional alteration of the acinar cells.

Our goal in this study was to document the evolution of early radiation injury in salivary glands of nonhuman primates as the best model of the human situation. Biopsy specimens of rhesus monkey parotid glands were taken within 1–72 hours of irradiation with single doses of 2.5–15.0 Gy. We found degranulation, degeneration, and necrosis of serous acinar cells as early as 1 hour after treatment with a dose as low as 2.5 Gy. Numbers of altered serous cells increased both with time and higher radiation doses, but the morphologic features of the damage of individual serous cells were qualitatively the same with all doses. At 6 hours after irradiation, parotid glands treated with doses of 2.5–7.5 Gy showed necrosis of individual serous cells, whereas with doses of 10.0–15.0 Gy, whole acini were lost. Compared with the acinar cell damage, alteration of blood vessels and ducts was either nonexistent or minimal. Numbers of neutrophils infiltrating the irradiated tissue did not

correlate with the severity of damage and consequently did not rise proportionally with increasing radiation dose. The exudation of neutrophils appeared to begin to subside by 24 hours after irradiation, but serous cells in all stages of degeneration and necrosis were still present. Although neutrophils showed a tendency to accumulate in foci of greatest damage and sometimes to persist in these locations later than 24 hours after irradiation, some foci with acinar damage of equal severity contained few or no neutrophils. The parotid glands treated with doses greater than 7.5 Gy and examined 16–22 weeks and 40 weeks after irradiation exhibited comparable degrees of atrophy. This indicates that if damaged serious acini had been replaced, this had occurred before sampling at 16–22 weeks, and the severity of the atrophy would indicate that the ability to overcome the acute injury was limited.

Changes in parotid serous cells similar to those we describe have been reported in rat parotid glands exposed to much higher radiation doses.[38,47,48] Some studies with doses of 16 or 64 Gy showed radiation damage as early as 2 hours after irradiation.[38,48] The damage was qualitatively similar with their low and high doses. Careful, specific evaluation of the microvasculature failed to reveal lesions.[38] In contrast to those of humans and monkeys, the irradiated rat parotid glands did not contain neutrophils. The fact that the serous cell lesions were similar in the three species with or without neutrophils favors the conclusion that inflammation in the parotid is a nonspecific radiation response. Furthermore, it has been shown that exudation of neutrophils may be a transient response to chemotactic factors released from irradiated endothelium.[49] The investigators who used the rat model concluded that the serous acinar cells were directly damaged by radiation because of the lack of vascular damage and any participation by inflammatory cells.[38] Because acinar cells in the rat parotid turn over about every 40 days,[50] the rapidity of the damage indicated that these slowly dividing cells were susceptible to lethal damage while in interphase.[38,47]

From our observations made on sequential biopsy specimens of irradiated nonhuman primate parotid glands within hours of irradiation with a range of doses, we concluded that acinar cell damage is the primary mechanism of radiation-induced injury to this tissue, and that acinar cells are susceptible to interphase death. In addition to overt necrosis, some of the cytologic changes observed by us in irradiated rhesus parotids and by others in irradiated rat parotids[38,48] resemble changes characteristic of cell loss by the process of shrinkage necrosis or apoptosis, which is a mode of interphase death.[38,48,51,52] The nuclear changes associated with death of serous acinar cells may be nonspecific indications of lethal injury. It is still not possible to determine whether nuclear changes that culminate in cell death are caused directly by radiation or indirectly by other mechanisms such as alteration of cell membranes or release of lysosomal enzymes, or both. Additional studies to clarify the precise subcellular mechanism of injury are in progress.

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