An increasing proportion of patients who require extensive surgical resection of head and neck tumors have a history of treatment with radiation and chemotherapy. Chemoradiation-induced damage to the skin and soft tissues can cause complications following surgical reconstruction. Local rotational flaps can develop partial distal necrosis in the immediate postoperative period, with as much as 31% partial flap loss in pedicled musculocutaneous flaps used for head and neck, as well as breast reconstruction. Management of these complications generally requires either additional surgery or prolonged wound care, which can substantially delay further cancer treatment and increases the overall cost of treatment.

To develop new therapeutic options to prevent flap failure, it is imperative to understand flap physiologic characteristics. Three perfusion zones contribute to healthy skin flap condition and ultimate viability. Zone I is the pedicle to the flap, zone II is at the capillary level, and zone III is the interstitial space where the process of nutritional delivery takes place. In zones II and III of normal healthy tissue, the interstitial space is filled with proteoglycans and collagen with an evenly dispersed capillary network, unlike the state of irradiated tissue, where this space is replaced by fibrosis and the relative water content and capillary content are greatly diminished.2,3

Author Affiliations: Department of Otolaryngology–Head and Neck Surgery, Thomas Jefferson University, Philadelphia, Pennsylvania (Luginbuhl, Modest, Curry, Heffelfinger); Department of Radiation Oncology, Thomas Jefferson University, Philadelphia, Pennsylvania (Yan).

Corresponding Author: Adam Luginbuhl, MD, Department of Otolaryngology–Head and Neck Surgery, Thomas Jefferson University Hospital, 925 Chestnut St, Sixth Floor, Philadelphia, PA 19107 (aluginbuhl@gmail.com).
The published literature describes several different rat flaps ranging from random to free tissue models. In all of these studies, flaps are designed to demonstrate maximum necrosis in the control group with end points of improved flap survival over the control group. Multiple studies demonstrate necrosis in the control group.1-8 This stands in opposition to the primary goal of clinical flap design, which is to ensure 100% flap survival without necrosis. We thought it important that our model correspond to clinical practice, so in the present study, the nonirradiated rats (control group) had a 100% rate of flap survival from which we could test our hypothesis that radiation exposure would result in development of flap compromise and necrosis.

The primary objective of this study was to create a local rotational flap in the irradiated rodent model mimicking the rotational flaps used in head and neck reconstruction. Secondary objectives included evaluating whether irradiation of this model resulted in flap failure and necrosis and characterizing any changes in vascular density.

Methods

Animals

Fifteen male Sprague-Dawley rats weighing 400 to 800 g were used. The protocol was approved by the Thomas Jefferson University Institutional Animal Care and Use Committee. Animals were housed with a 12-hour light/dark cycle with food and water ad libitum. Animals were monitored daily following any irradiation or surgical procedure.

Radiation Protocol

Ten rats received 40-Gy electron beam irradiation to their ventral abdominal wall, and 5 rats received no radiation and served as a control. The ventral abdomen of all rats in both groups was shaved while they were under isoflurane inhalation anesthesia. After 7 days of acclimation, rats in the radiation group were anesthetized by means of subcutaneous injection of 80 mg/mL of ketamine hydrochloride, 1.6 mg/mL of acepromazine maleate, and 5 mg/mL of xylazine hydrochloride, with supplementation as needed. Once anesthesia was confirmed, 4 rats at a time were placed in a supine position on the irradiation table (Figure 1).

A 6-MeV electron beam accelerator (Elekta) was used to radiate the dermis and epidermis. To achieve a cumulative dose of 40 Gy, irradiation was administered over 8 days in 4 doses of 10 Gy given every other day. To deliver the intended dose to the dermis without delivering toxic radiation to the alimentary tract, the steep part of the dosimetry curve was used, with a rapid fall to 2 Gy at 2 cm below the epidermis (Figure 1A). This is achieved by delivering 20 Gy using 2.59 cm H2O–equivalent shielding to achieve a 50% dose (10 Gy) at the skin and a 10% dose (2 Gy) at the gut. A 25 × 25-cm cone was used, with the torso and head outside the beam and the genitals and lower limbs being shielded by the equivalent of 1 cm H2O (Figure 1B). Rats were given saline injections for rehydration following each dose of radiation, and their weights were monitored for 28 days following the last irradiation exposure.

Ventral Fasciocutaneous Rotational Flap Procedure

Rats were allowed to recover from the irradiation exposure for 1 month before undergoing the ventral fasciocutaneous rotational flap procedure. Isoflurane inhalation was used for all rats during the procedure, and a 0.25-mL dose of buprenorphine hydrochloride was administered prior to the first incision. First, the approximate location of the inferior epigastric artery was marked. Then a 3 × 8-cm flap was drawn with the medial part of the flap drawn down the midline of the abdomen 8 cm from the origin of the inferior epigastric artery. The lateral extent of the flap was 3 cm from the midline.

Using sterile technique, incisions were carried out along the medial, superior, and lateral sides, leaving the inferior part of the flap attached with the pedicle. The plane of dissection was superficial to the rectus abdominis fascia. All bridging vessels, as well as the superior epigastric vascular pedicle, were ligated. Dissection was carried out in this avascular plane until the inferior epigastric pedicle was visualized (Figure 2).

A defect was created on the left half of the abdomen. From the point of the right inferior epigastric pedicle, an incision 60° off the midline was carried for 8 cm to create a 3 × 8-cm defect. The flap was then rotated into this defect and sutured into place, and the donor defect was closed primarily using 4-0 Vic-
ryl buried mattress sutures followed by a running suture along the entire perimeter (Figure 2). Buprenorphine was administered for postoperative analgesia every 12 hours for 2 days.

We determined the appropriate flap and defect size by varying flap and defect dimensions in nonirradiated rats until necrosis occurred. Using a separate cohort of rats, the aforementioned procedure was performed with flaps 3 cm wide by 7, 8, and 9 cm long. The defects were also varied accordingly. Necrosis developed when the flaps and defects were longer than 8 cm. On the basis of these results, we established that the limits of this axial flap were a 3 × 8-cm flap rotated into a 3 × 8-cm defect. We then proceeded to test whether radiation exposure would lead to flap necrosis. Animals were monitored for 7 days postoperatively. Before the rats were killed, pictures were taken and the flaps were harvested for histological examination.

Evaluation of Flap Revascularization
Seven days after flap elevation, animals were reanesthetized as previously described and flap viability was evaluated by means of planimetry. Each flap was harvested and a standardized digital photograph was taken of each ventral flap (Nikon D60). These were then digitally processed, and a qualified blinded observer measured the amount of necrosis from the distal tip of the flap to the junction of viable tissue. Necrotic nonviable areas were defined as dry, hairless eschar, whereas healthy viable areas were defined as viable pink tissue.

Microvascular Density
Once photographed, the distal, middle, and proximal 1-cm sections of the flaps were harvested and then fixed with formalin and embedded in paraffin. These sections were then analyzed by the immunohistochemistry laboratory for the presence of von Willebrand factor. Vascularity was assessed by counting blood vessels marked with von Willebrand factor in 8 high-power fields per tissue section and then analyzed using a Wilcoxon rank sum test. Animals were excluded from the study if they developed a hematoma or self-mutilated their flap. X-ray paper vests were fashioned to prevent the rat from picking at the suture line.

Results
There was a significant difference in the survival of skin flaps between the rats that did not receive irradiation and those that did (40 Gy in 4 doses of 10 Gy). Six of the 10 rats in the irradiated group had necrosis of the distal flap ranging from 1 to 6 cm from the distal edge, compared with the control group, where no necrosis was seen (P < .001) (Figure 3). Radiated tissue outside the necrotic distal tip consistently presented with loss of nuclear detail, inflammatory infiltrates, thickened vascular walls, atrophy of skeletal muscle, and interstitial fibrosis. Histological analysis of the necrotic tissue in the irradiated animals demonstrated loss of epidermis, necrosis, and vessel thrombosis (Figure 4). In the control group, inflammatory infiltrates were observed in the distal tip region but without any evidence of necrosis.

Vascular density was significantly affected by radiation exposure. In the control group, the proximal, middle, and distal seg-
ments of the flap had a mean vascular density of 185, 209, and 162 vessels per 8 high-power fields, respectively, whereas those in the radiated rats had a mean vascular density of only 86, 85, and 79 vessels per 8 high-power fields (Figure 4). Wilcoxon testing of the distal, middle, and proximal segment vascular density resulted in \( P \) values of .004, .03, and .01, respectively (Figure 5).

**Discussion**

The objective of this study was to use a rodent model to simulate the effects of radiation treatment on normal tissue when stressed by surgical intervention. There is no current proactive or preventative solution to the well-established clinical problem of dramatic increases in wound complications seen in patients who have undergone radiation therapy. We hypothesized that the ability to revitalize irradiated tissue prior to surgery would decrease the rate of postoperative complications. Regardless of the complexity of reconstruction, whether primary closure or free tissue transfer, the quality of the local host tissue is integral to the success of the operation. The model in the current study was developed on the basis of the concepts and principles of the cervical facial rotational or advancement flap, in which tissue surrounding the resection site is undermined and rotated to close the defect. Developing models that mimic clinical experience is one of the initial steps in testing hypotheses that may alter this challenging clinical course.

With radiation dosing for primary cancer sites and nodal basins reaching 60 to 70 Gy, dosimetry predicts a dose of 40 to 50 Gy to the skin. During treatment, patients can experience deepithelialization with occasional ulceration of the skin. Once the acute phase of postradiation healing has occurred, the skin remodels with dense fibrosis, reduced peptidoglycans, and a loss of skin turgor, all leading to the classic “woody neck” appearance.² The abdominal skin in the radiated animals of the present study developed moderate epidermolysis with occasional ulceration at 2 weeks after radiation exposure. These areas were fully healed by 30 days after radiation exposure, which was the time of the flap creation. Because rats have a higher metabolic rate than humans, previous radiation studies in the rodent have used

---

**Figure 4. Histologic Analysis**

(A) Immunohistochemical staining for von Willebrand factor shows vessels outlined by dark staining (original magnification ×200) in nonradiated tissue (A) and radiated tissue (B). Note dense fibrosis. C, Radiated tissue necrosis of epidermal elements; no definable viable cells (hematoxylin-eosin, original magnification ×200). D, Fibrosis of proximal radiated tissue. Muscular layer is replaced by fibrosis (hematoxylin-eosin, original magnification ×50).
30 days after radiation as the time of flap creation.6 Angelos et al5 irradiated rats with either 23 or 40 Gy and found a significant delay in free flap revascularization with 40 Gy but not with 23 Gy, thus establishing that 40 Gy effectively demonstrates poor wound healing in the free myocutaneous gracilis flap model. Nall et al7 were unable to find a deleterious effect on wound healing in rats irradiated with 15 or 25 Gy.

Fibroblasts in healthy tissue play a pivotal role in reacting to tissue stressors and optimizing survival. At a fundamental level, the absolute loss of fibroblasts seen with radiation exposure, and their replacement with fibrosis, thwarts this survival process, and the demands of wound healing cannot be met. With the aid of a skin chamber model, Hori et al10 induced neovascularization in rodent skin using polyvinyl chloride. They subsequently irradiated the area with 10 Gy and discovered a dramatic decrease in overall vascular density over 96 hours, as well as a narrowing of normal vessel caliber. This and other studies demonstrate the devascularizing effect of radiation on existing blood vessels. Our model is consistent with the findings of Hori et al,10 in that a dramatic decrease in overall vascular density was observed in the irradiated group. The difference was seen throughout the length of the flap from the proximal, middle, and distal locations. Within the nonirradiated group, maximum vessel density was found within the middle portion of the flap, which can be explained by the relative ischemic environment induced by the creation of the flap, leading to a proangiogenic response (Figure 5).

Limitations of our study include the timing of surgery after radiation exposure and the small number of animals. Clinically, patients do not undergo surgical interventions 1 month after radiation treatment, yet because of the elevated metabolic rate of the rat, the complete resolution of all acute changes, and the histologic evidence of fibrosis, we believe that this model provides insight into the remodeling that takes place with radiation exposure. These changes ultimately affect the outcomes of surgical interventions, and this easily reproducible model will allow for further investigation into novel therapies to improve outcomes.

With the development of our model of the axial rotational flap in the irradiated rodent, future efforts will be aimed at increasing both capillary delivery and diffusion capacity through the interstitial space via a variety of therapeutic interventions. Unlike Schultz-Mosgau et al3 and others, who have looked at modifying tissue behavior at the time of radiation exposure, our focus will be on a period well after radiation exposure has taken place: 7 to 10 days prior to a planned surgical intervention. The irradiated rat model of an axial rotational flap is consistent with what is seen in patients who have previously undergone radiation therapy who develop skin necrosis after reconstruction with local rotational flaps (Figure 3). Based on these findings, efforts are now focused on the manipulation of growth factors and heat shock proteins and the exploitation of adipose stem cells to revitalize irradiated tissue in this novel flap model.

REFERENCES