THE EFFECTS OF VITAMIN E ON WOUNDS RECEIVING PREOPERATIVE RADIATION

by

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STATEMENT BY AUTHOR

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ABSTRACT

Radiation inhibits the infiltration of leukocytes into the wound area. Various steps of wound healing parameters such as epithelialization, contraction, collagen metabolism, and the gain in the breaking strength of wounds are depressed because of ionizing radiation. A hypothesis is presented that describes how radiation initiates lipid peroxidation which, in turn, causes damage to the cells involved in wound healing. It was the purpose of this study to find out if the prophylactic administration of Vitamin E, an antioxidant, would help normalize the healing of wounds receiving preoperative radiation.

Vitamin E was given intravenously every other day to rats starting six days before 700 rads of local radiation was delivered. Five centimeter incisions were made in the area of radiation two hours following the radiation treatment. With increasing levels of Vitamin E, there was an increase in the breaking strength of wounds. However, wounds treated with the highest amount of Vitamin E did not demonstrate a significant decrease in lipid peroxidation. Biochemical studies did not demonstrate an increase in the amount of collagen synthesized 14 days postoperatively with Vitamin E supplements. A relation between the amount of collagen synthesized and the gain in wound strength was not correlated, although other studies have shown such a relationship. The propensity of semiquantitative histological scores illustrated a slight increase in collagen synthesis, but no changes in cellular infiltration and epithelialization 14 days postoperatively.
CHAPTER 1

INTRODUCTION

Wound healing is the process of repairing injured tissue. Tissue injury may be induced by various modes including chemicals, radiation, burns, accidental trauma, and the surgeon's scalpel. Wound healing is constantly in a dynamic state. There is a continuous turn-over of cells and ground substance in scar tissue.

The healing process may be separated and studied as morphologic, chemical, and physical events. These events are present during wound healing simultaneously. For the purpose of this paper, wounds and wound healing will refer to the traumatization of mammalian skin either by accident or by the surgeon's scalpel and the healing process that occurs.

Ionizing radiation delivered to tissue either before, during, or after wounding causes the early stages of wound healing to be inhibited. It was the purpose of the experiments presented herein to study one possible mechanism of radiation induced wound impairment and one prophylactic measure. A system that I hypothesize as being implicated can be stated as follows: The radiation delivered to the tissue initiates lipid peroxidation at a rate that is not suppressed by the available antioxidants. The free radicals, peroxides, and other products of lipid peroxidation in turn cause intracellular damage. The damage to organelal membranes leads to the malfunction or death of cells involved in wound healing. If the ability to neutralize lipid peroxidation is increased, then the function of cells involved with wound healing will be normalized.
The following sections describe 1) the wound healing process, 2) radiation's effect on wound healing, 3) lipid peroxidation and its effect on cellular metabolism, and 4) the relation between radiation, lipid peroxidation and antioxidants, including Vitamin E.

**Wound Healing**

**Morphologic Events**

The initial physiological response to wounds that sever capillaries is contraction of blood vessels through local myogenic stimulation. The vascular walls are able to remain contracted for 30 minutes followed by local dilatation. The wound edges retract and thicken. During this time, blood clotting factors are activated through a cascade effect and a fibrin matrix is laid down to help maintain blood vascular homeostasis. The proliferation of capillaries starts within one day.

There is no epidermal growth for the first 12 hours (Odland and Ross, 1968). By 24 hours, the migration of epidermis is well underway. Epithelialization starts at the wound edge and moves toward the center of the wound. The regenerating epithelium have ruffled membranes that are adhesive. The cells are characterized by increased stratification and a large number of filaments before they become squamous cells (Odland and Ross, 1968).

Within three hours after wounding, there is a significant number of leukocytes in the wound area (Ross and Odland, 1968). Initially, the predominant cells are polymorphonuclear leukocytes (PMNs). These cells dominate in number until 72 hours after traumatization. The PMNs are surrounded by a network of fibrin and serum proteins. PMNs appear
to have two functions in non-infected wounds. First, they phagocytize fibrin and serum proteins within the fibrin matrix. Secondly, PMNs release endocellular enzymes upon lysis that aid in fibrolysis (Ross and Odland, 1968).

There are few macrophages present in the wound within the first 24 hours (Ross and Odland, 1968). The number of macrophages increase in the wound area after two to three days. Macrophages are the predominant leukocyte in five day old wounds. Initially, macrophages have little phagocytic activity. By five days, there is an increase in the number of membrane bound structures and intracellular vacuoles within macrophages that contain particles of various sizes and density. The increase in intracellular particles parallel the decrease in extracellular material, mainly fibrin and other serum proteins (Ross and Odland, 1968).

By the second or third day, some mesenchymal cells proximal to the wound start to differentiate (Grillo, 1963). The active cells that differentiate located in perivascular connective tissue are termed "fibrocytes". Fibrocytes mature into fibroblasts. Myofibrocytes are transient cells that precede the evolution of fibroblasts.

Myofibroblasts can be differentiated from fibroblasts by their long intracytoplasmic microfilaments usually running parallel to the long axis of the cell (Grillo, 1963). The myofibroblasts frequently have intercellular connections with each other and with epidermal cells along the wound edge. The microfilaments in the myofibroblasts are related to the actin-myosin filaments found in smooth muscle cells. The cytoplasm of the myofibroblasts have antigenic determinate sites that react with smooth muscle antibodies as demonstrated by immunofluorescent labeling (Ryan, et al, 1974).
Both myofibroblasts and fibroblasts have nuclear deformations present that are expressed as multiple indentations and deep folds of the nuclear membrane (Ryan, et al, 1974). Microfilaments are present in fibroblast, but at a considerable lower quantity. Fibroblasts contain an extensively developed rough endoplasmic reticulum whose cisternae is present throughout the cytoplasm. Numerous aggregates of ribosomes are attached to the membrane of the endoplasmic reticulum. Also present in the mature fibroblasts are large mitochondria and a well developed Golgi apparatus.

The greatest number of fibroblasts are present in the wound during the second and third week. By the fourth or fifth week, there is a decrease in the number of fibroblasts.

Collagen fibers are present in four and five day old wounds. The fibers enlarge and become dense bundles. After 28 days when less fibroblasts are present, collagen become the predominant feature in wound tissue.

Chemical Events

The inflammatory response of tissue is characterized by dilated capillaries and the migration of leukocytes to the wound area. Histamine released from mast cells, leukocytes and platelets may play a role in vasodilation shortly after wounding. Plasma peptides classified as kinins are also implicated in the vascular response.

Chemotaxins are substances that attract leukocytes to the wound area. Chemotaxins may be peptides of enzymatically degraded albumin from wound tissue. Certain fractions of the complement system also cause chemotaxis.
The synthesis of ground substances is the primary way wounds are repaired. All scar tissue is basically composed of similar components such as fibrous proteins, collagen, and glycosaminoglycans.

The primary compound responsible for the recovery of strength in a wound is collagen. Before discussing the rate of collagen synthesis and the collagen content of wounds, the basic chemistry of collagen and the mechanism for collagen synthesis should be reviewed. An extensive review has been completed by Peacock and VanWinkle (1976). Mature collagen is a glycoprotein that consists of three linear peptides. Its amino acid sequence has every third amino acid as glycine (Gly). The presence of hydroxyproline (Hyp) and hydroxylysine (Hys) adds to the uniqueness of collagen. Hyp and Hys, when present, always precede Gly. Additionally, some of the Hys side chains contain substituted o-galactosyl or o-galactosyl-β glycosyl residues. These side chains make collagen the glycoprotein. Presently there are four types of collagen reported (Types I-IV).

The individual peptides are twisted in a right handed helix. When first synthesized, the three chains are held together by weak electrostatic forces. The protein at this point is referred to as tropocollagen. The three chains are then twisted into a left handed superhelix and held together by hydrogen bonds. As collagen matures, the tropocollagen strands polymerize into fibrils. The formation of intramolecular and intermolecular covalent crosslinks make collagen insoluble except in solutions capable of causing hydrolysis.

The bonds that form the covalent crosslinks are derived from the oxidative deamination of lysine (Lys) and Hys by lysyl oxidase. Crosslinks are formed by aldol condensation. After deamination of Lys or
Hys, a Schiff base is formed with a NH$_2$ group of the other side chains in the molecule. The Schiff base in turn, is reduced forming a permanent covalent crosslink (Tanzer, 1973).

As in the synthesis of other proteins, the nucleus of the fibroblast contains the blueprint for the collagen molecule. What activates the synthetic process is still questionable. Possibly an extracellular product from the wound blocks a substance that represses collagen synthesis. Once blocked, the nucleus releases messenger RNA (mRNA) into the cytoplasm. mRNA enters the ribosome where the nucleotide sequence is translated into the amino acid sequence by attaching to transfer RNA. The synthesis of the collagen peptide starts at the amino end. Hydroxylation of proline (Pro) occurs at the ribosome. Pro is first attached to the peptide and then hydroxylated (Peterkofsky and Underfriend, 1963). Hyp is not directly incorporated into the amino acid sequence. Hydroxylation occurs only when the peptide has reached a minimum length. During a high rate of collagen synthesis, the optimum amount of hydroxylation may not occur. The procollagen moves to the cisternae of the endoplasmic reticulum. The procollagen then passes to the Golgi apparatus where glycosylation occurs. From the Golgi apparatus, the compound is secreted into the extracellular space by the process of vesicle formation, migration, and fusion with the cell membrane.

Abercrombie, et al. (1954) measured the collagen content of a rectangular wound. At 25 days postoperative, the collagen content in the area of the wound was 40% of unwounded skin. More recently, Madden and Peacock (1968) used H$^3$Pro to measure active collagen synthesis until 70 days postoperative. The greatest activity of Pro hydroxylation was
between seven and 14 days postoperative. The high rate of collagen synthesis at this time corresponds with the increased number of ribosomes present in fibroblasts during the same time period as reported by Ross and Odland (1968). Initially, there is a lag phase before active collagen synthesis occurs. The delay is due to the time it takes the mesenchymal cells to differentiate, migrate, and to initiate fibrogenesis. Levenson, et al. (1965) also demonstrated that collagen turnover is continual in the wound area for 6-7 weeks and not just the first 2-4 weeks postoperatively.

Physical Events

The physical events that occur during wound healing include the gain in wound strength and wound contraction. Contraction has a greater role of importance in open wounds compared to closed wounds.

After two days, incised wounds start to gain in strength. The specific activity of $^3$Hyp parallels the breaking strength of wounds for the first 21 postoperative days. At this time, the strength of the wound continues to increase, while the accumulation of collagen levels off. The increase in the intermolecular and intramolecular crosslinks and orientation of the collagen fibers seem to be the most influential factor for determining the final strength of wounds. Wounds continue to gain in strength at a rapid rate for 56 days. The strength gain continues at a slower rate for up to one year. However, even after one year, wounded tissue is not equal to the strength of uninjured tissue (Levenson, et al, 1965).

Contraction is the centripetal inward movement of the wound edge that occurs early in wound healing (Van den Brenk, et al, 1974). This
process is distinctly different than epithelialization. In open wounds, contraction has a more important role than in closed wounds due to the increased distance between the wound edges. Contraction is not caused by the wound edge, but due to the wound content (Zahir, 1964). Several theories for the mechanism of contraction have been developed. Currently, the pull theory has the most supportive data. During contraction, inward tension develops within the wound area and the surrounding tissue. In guinea pigs, when the content of the wound is dissolved with trypsin, the tension that has developed is released and there is expansion of the wound edges (Zahir, 1964). The expansion of the wound edge demonstrates that the contraction was associated with a protein substance within the wound. The tension that develops pulls the surrounding tissue inward. This process continues until approximately ten days after wounding in 25 cm² excised wounds (Abercrombie, et al, 1954).

Abercrombie, et al. (1954) provided evidence that contraction was not due to the shortening of collagen fibrils. Contraction starts before there is a significant amount of collagen synthesized and stops, while collagen synthesis continues (Abercrombie, et al, 1954). Additionally, in scorbutic rats in which collagen synthesis is decreased, contraction is not inhibited. It is now known that contraction is cellular in nature with myofibroblasts that originate from mesenchymal cells being responsible for the contraction (Ryan, et al, 1974; Van den Brenk, et al, 1974; Grillo, 1963). The initial decrease in the wound area is due to contraction of myofibroblasts within the wound that pull the wound edges together through cell-to-cell connections (Ryan, et al, 1974).
Radiation and Wound Healing

All parameters of wound healing have been studied in respect to the effects of radiation. The protocols that have been used include different types and sources of radiation (x-rays, gamma rays, β-rays, electrons, orthovoltage, and cobalt). The radiation effect depends on the time the tissue is exposed and on the dose of radiation.

Morphologic Events

Stajic and Milovanovic (1971) reported that 700 rads of $^{60}$Co gamma radiation given two hours before wounding reduced the infiltration of leukocytes. In non-irradiated rats, there is an abundance of cellular infiltration in wounds within 24 hours. Preoperative exposure to radiation delayed infiltration until three days postoperatively. Grillo (1963) delivered 750 rads of x-rays to rats 20 minutes after wounding and reported that there was no difference in the leukocytic infiltration for the first six hours. However, by 28 hours postoperatively, there was a decrease in the cellular infiltration in the rats receiving radiation compared to a non-irradiated group of rats. When local radiation was delivered 24 hours postoperatively, fewer macrophages were present in five day old wounds. When radiation is delivered during the proliferative stage, then those cells that are migrating into the wound area are decreased. This is the reason why radiation delivered just prior to wounding or shortly afterwards decrease the number of PMNs, while radiation delivered after 24 hours decrease the number of monocytes present (Grillo, 1963).
Histological studies have also demonstrated anomalies in the epithelialization of wounds. X-ray delivered two hours prior to wounding results in a lack of active cellular division in the basal layer of skin wounds (Stajic and Milovanovic, 1971). At seven days postoperatively, epithelialization covers two-thirds of the wound, while in irradiated wounds epithelialization covers only one-third of the wound area. By ten days postoperatively, control wounds are completely covered, while irradiated wounds, although more advanced than at seven days, are not completely covered. Ten thousand rads of $\beta$-radiation administered immediately after closing a wound results in the number of cells in the epithelial layer to be reduced by approximately one-third three weeks postoperatively (Morrison, et al, 1971). The cytoplasm of corneal epithelium of irradiated wounds contain abnormal organelles (Morrison, et al, 1971). On the other hand, Blythe, et al. (1975) reported that radiation had little or no effect on epithelium when delivered the day of wounding or three or five days after wounding.

The microcirculation surrounding the eschar supply nutrients to the wounded skin. Irradiation causes several changes in the vascular bed. The endothelial lining of the vascular bed develops an occlusive thickening with radiation damage (Dimitrovich, et al, 1977). These changes in the microcirculation passively alter the normal healing process of wounds.

When a low dose of radiation (less than 1,000 rads) is delivered, the effect on vascularization appears minimal (Stajic and Milovanovic, 1971). When 1,000 rads of local radiation is delivered postoperatively, neovascularity is reduced (Blythe, et al, 1975). When 1,500
rads is given immediately following wounding, the sprouts of migratory vessels are haphazardly oriented compared to the polarized distribution of dense capillaries organized radially to the open surface in non-irradiated wounds (Van den Brenk, et al, 1974). When 6,000 rads is given, either zero, three or six days postoperatively, there is also an initial reduction in vascularization. However, in the later stages of healing, days 14 and 21, there is hypervascularization. By 75 days postoperatively, vascularization is the same in irradiated and non-irradiated wounds (Dotto, et al, 1970).

When an irradiated wound includes the panniculus carnosus, the muscle layer fails to regenerate as a complete layer. The panniculus carnosus is increasingly more complete and uniform when radiation is delivered to a wound from zero to five days postoperatively (Blythe, 1975).

Grillo (1963) used an autoradiographic study to determine the effect of radiation on fibroblasts. Seven hundred and fifty rads of x-ray were delivered to tissue either 20 minutes prior to wounding, 20 minutes postoperatively, or 28 hours postoperatively. Irradiation 20 minutes prior to surgery slightly decreased cellular proliferation when measured five days postoperatively. When radiation was delivered 20 minutes postoperatively, cellular proliferation was decreased even more. The proliferation of fibroblasts was reduced by greater than 50% in the guinea pig wounds when the radiation was delivered 28 hours postoperatively.

Chemical Events

When 700 rads of radiation is delivered two hours prior to wounding, the result at seven days postoperatively is less collagen present than in non-irradiated wounds. By the 14th postoperative day, the amount
of collagen present has increased in the wounds receiving preoperative irradiation, but not as extensively as in control wounds (Stajic and Jovanovic, 1969).

Chemical studies have reported that the collagen synthesis by fibroblasts is also impaired by radiation. The hydroxylation of Pro in wounds that immediately receive 750 rads of radiation is depressed for four days, but the rate of hydroxylation returns to control values thereafter. When 1500 rads was delivered to wounds, hydroxylation was depressed for 21 days (Archer, et al, 1970). Stajic and Jovanovic (1969) measured the collagen content in circular excised wounds 5 mm in diameter that received 700 rads two hours preoperatively. The amount of deposited collagen in the wound area was delayed by three days. The collagen content thereafter followed a sigmoid curve similar to control wounds. The greatest difference in collagen content between control wounds and wounds receiving preoperative radiation was at 14 days postoperatively.

Physical Events

Archer, et al. (1970) measured a marked reduction in the gain in tensile strength of linear wounds that immediately received 750 or 1500 rads. The strength of the irradiated wounds were equal to the control wounds at three days postoperatively, but only 40% of control at five days and remained subnormal 21 days postoperative. When 700 rads preceded wounding by two hours, it was not possible for Stajic and Milovanovic (1970) to measure the tensile strength at five days postoperatively, while control wounds had a strength of greater than 100 grams.
per 5 mm length of wound. After five days, the wounds receiving pre-
operative radiation gained in tensile strength, but remained signifi-
cantly below control levels for 28 days. Once again, the maximal dif-
ference was at 14 days postoperatively (Stajic and Milovanovic, 1970;
Stajic and Jovanovic, 1969). Even the strength of corneal wounds of
rabbits that received radiation three months prior to surgery were sig-
nificantly lower 20 days postoperatively (Morrison, et al, 1971).

Grillo and Potsaid (1961) reported that the delay in contraction
was less when radiation was delivered either 24 hours prior to wounding
or when it was delayed until the fifth to seventh day postoperatively.
The maximum amount of inhibition resulted when radiation was delivered
36 hours postoperatively. The wound area in irradiated wounds was still
25% of the initial area compared to less than 10% in non-irradiated
wounds 14 days postoperatively. Grillo and Potsaid (1961) concluded
that when radiation is delivered after contraction has gained momentum;
i.e., five days postoperatively; there is less inhibition than when ra-
diation is delivered closer to the time of wounding. When radiation is
delivered before contraction has been initiated, then the result is a
delay in the start of contraction. Once contraction starts after the
initial delay, it proceeds at a normal rate. Van den Brenk, et al.
(1974) studied the effect of various doses of radiation on contraction.
In wounds 3.5 cm in diameter, contraction in unirradiated rats was mea-
surable three to five days postoperatively; by 12-15 days postoperative-
ly, the mean diameter had decreased about 50%. When 800 rads was de-
ivered five minutes before wounding, there was not a delay in the start
of contraction, but a slight decrease in the rate resulting in the
diameter decreasing by approximately 43% by 12-15 days postoperatively. When 1600 rads was delivered five minutes prior to wounding, there was both a delay in the initiation of contraction and an initial decrease in the rate (Van den Brenk, et al, 1974). With 1600 rads, the maximum rate of contraction did not occur until 14-21 days postoperatively, compared to 12-15 days in non-irradiated rats. The degree that contraction progressed was also inversely proportional to the amount of radiation delivered when increased in a step-wise fashion from 800 rads to 1800 rads. In the same report, delaying wounding progressively from 0 to 14 days after tissue has been exposed to 1500 rads benefitted contraction. However, even when radiation was delivered 14 days prior to wounding, the wound diameter was still greater than in non-irradiated wounds 32 days postoperatively.

Complications

Mild or severe anastomotic stenosis was present in all cases of dogs that received 500 rads over a one week period, three weeks prior to the segmental resection of the bronchus or trachea (Tsubota, et al, 1975). In Rhesus monkeys, orthovoltage radiation delivered to the jaw area prior, during, or after molar extraction caused osteoblastic activity at the base of the socket to be reduced (Zach, et al, 1973). The sockets became filled with immature connective tissue. The reduction in osteoblastic activity resulted even when radiation preceded surgery by three months. Meyer, et al. (1966) reported that with cobalt radiation, gingival healing is closer to normal than with orthovoltage.

Finally, surgical complications are increased in cancer patients that receive preoperative irradiation. Yarington, et al. (1976) report
almost a 300\% increase in the number of complications. Daly, et al. (1978) reported full scale necrotizing infections in patients that received preoperative radiotherapy. The infections described consisted of Bacteroides species and gram negative bacillus and/or gram positive cocci. The full scale infections had only been seen by the investigator in patients that received preoperative radiotherapy.

Lipid Peroxidation

Lipid peroxidation starts with the extraction of a hydrogen atom from a polyunsaturated fatty acid (PUFA). This is followed by the rearrangement of a double bond to form a conjugated diene. The addition of molecular oxygen to the dienes results in a peroxy radical. The peroxy radical can decompose in two ways. First, the oxygens may rearrange to form an endoperoxide which breaks down to malendialdehyde (MDA). Subsequently, fluorescent, ceroid, and lipofuscin pigments are formed by MDA crosslinking with amino acids of proteins and nucleic acids. The structure responsible for the fluorescence is a Schiff-base product, \( RN = CH=CH-NR-R \) (Tapple, 1972). The second possibility is that the lipid peroxy radical can extract a hydrogen atom from another PUFA to become a lipid hydroperoxide, while the second PUFA changes to a free radical to continue the chain reaction of lipid peroxidation. Propagation of the reaction continues until free radical scavengers neutralize the reactive radicals (Buege and Aust, 1978). Recently, ethane and pentane have been measured as end products from the peroxidation of linolenic and linoleic acids respectively (Hafeman and Hoekstra, 1977a; 1977b).
There are numerous oxidants and enzyme systems implicated with causing lipid peroxidation. Lipid peroxidation occurs naturally within cells and can be accelerated by drugs, dietary deficiencies, and radiation (Pederson, 1975; Masugi and Nakamura, 1976; Utsumi, et al, 1966, respectively). The free radicals formed by the various methods are similar in nature. Oxidants involved with lipid peroxidation include hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻), and free radicals (R·), more specifically, peroxyl radicals (ROO·), alkoxy radicals (RO·), and hydroxy radicals (-OH) (Tapple, 1978). Carbon tetrachloride is used experimentally to promote lipid peroxidation. The compound forms a chloride radical (Cl·) that will extract a hydrogen atom from PUFAs (Riely, et al, 1974).

Non-enzymatic lipid peroxidation is promoted by iron and ascorbate (Tapple, 1972; Pederson, 1975). Ferrous and ferric iron are part of several reactions that produce free radicals. One system postulated by Lai and Piette (1977) includes the reduction of Fe²⁺ accompanied by the decomposition of a lipid peroxide to an alkoxy radical. Lai and Piette (1977) have also proposed that the Fenton reaction may produce hydroxy radicals involved with lipid peroxidation (Fe²⁺ + H₂O₂ → Fe³⁺ + OH⁻ + ·OH). Vladimirov, et al. (1973) have described a chain of reactions involving iron that produce superoxide anions, lipid hydroxides, and lipid peroxy radicals. The possible sequence of reactions is:

   a) RH + Fe³⁺ + O₂ → Fe²⁺ + RO · + H⁺  
   b) RO₂ · + ROOH → RO₂ + ROOH  
   c) ROOH + Fe²⁺ → Fe³⁺ + OH⁻ + RO₂ ·  
   d) RO₂ · + Fe²⁺ → RO₂ · + InH (compound stopping chain reaction)
Additionally, reduced iron can decompose hydroperoxides to form peroxo radicals (ROOH + Fe^{3+} \rightarrow ROO^- + H^+ + Fe^{2+}) (Tapple, 1972).

Biological systems promoting lipid peroxidation are NADPH - cytochrome reductase, xanthine oxidase, and oxidative phosphorylation (Pederson, 1975).

Since lipid peroxidation occurs with unsaturated lipids, the more unsaturated lipids are more susceptible to peroxidation. Mitochondrial and microsomal membranes have a large amount of PUFA. Additionally, tissue exposed to higher concentrations of oxidants also have an increased amount of oxidation. For these reasons, the primary sites for lipid peroxidation are mitochondria, microsomes, and phagolysomes where both large amounts of PUFAs and oxidants are present (Dillard and Tapple, 1971; Serfass and Ganther, 1975). When lipid peroxidation continues, erythrocytes are more susceptible to lysis (Mengel, 1972; Roders, et al., 1978). There is an increase in peroxides and MDA before lysis (Roders, et al., 1978). Additionally, with an increased amount of lipid peroxides within the erythrocytes, after hemolysis, peroxides are released into the plasma (Mengel, 1972). This release of peroxides may cause damage at a distant locus. When erythrocytes and peroxidizing microsomes are separated by a dialysis membrane, MDA accumulates on both sides of the membrane before hemolysis. Benedetti, et al. (1977) propose that toxic products of lipid peroxidation crossed the membrane to cause the hemolysis. They concluded it unlikely that free radicals would be the culprit because of their short half-life and high reactivity.
Antioxidative Systems

Protective systems are present within organelles, membranes, and the cytosol to limit lipid peroxidation. Another factor that suppresses lipid peroxidation is the exposure of lipids are physically protected from high concentration of oxygen and iron compound catalysis (Tapple, 1972).

Catalase, heme peroxidase, and reducing compounds protect cells from \( \text{H}_2\text{O}_2 \) produced by microsomal and mitochondrial electron transport (Loschen and Azzi, 1975; Kameda, 1977). Superoxide anion is neutralized by superoxide dismutase located in the cytosol and mitochondrial matrix (Johnston and DeChatelet, 1974). The antioxidants that may be influenced the most pharmacologically are Vitamin E, Selenium-Glutathione peroxidase (Se-GSHpx), and glutathione. Vitamin E is present in the membranous parts of cells and neutralizes free radicals. Se-GSHpx and glutathione prevents lipid peroxides from forming free radicals and neutralizes \( \text{H}_2\text{O}_2 \) in the cytosol and mitochondrial matrix (Hoekstra, 1975).

Superoxide anions are generated by NADPH-cytochrome C, flavin oxidation, and xanthine oxidase in rat liver (Tapple, 1978). Superoxide dismutase is unchanged when neutralizing superoxide anions. The enzyme will slow down lipid peroxidation by producing \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \) from two superoxide anions. This may divert the anion from extracting a hydrogen atom from an unsaturated fatty acid. Superoxide dismutase protects phagocytes from superoxide anions that are produced in phagolysosomes and cross into the cytosol (Serfass and Ganther, 1976).

The hemoprotein catalase uses one molecule of \( \text{H}_2\text{O}_2 \) as an electron donor and another molecule of \( \text{H}_2\text{O}_2 \) as an oxidant to produce two molecules of water and a molecule of oxygen. On the other hand, it
takes two Se-GSHpxs to neutralize a molecule of $H_2O_2$. The resultant products are two molecules of water and molecular oxygen (Tapple, 1972, 1978). Another mode of action of Se-GHSpx is the conversion of lipid peroxides to hydroxy fatty acids (Hoekstra, 1975).

Vitamin E and Lipid Peroxidation

Vitamin E is transported in the body bound to $\alpha_2$ globulins within low density lipoproteins. However, it is not used as an antioxidant in the plasma (Vidlakova, et al, 1972). Vitamin E does act as a chain breaking antioxidant by donating hydrogen atoms in order to neutralize free radicals present in organal membranes (Tapple, 1972). During oxidation within membranes, Vitamin E will be consumed before unsaturated fats are peroxidized (McCay, et al, 1972). There is an inverse relation to cellular oxygen uptake and MDA production with plasma levels of Vitamin E (Dillard and Tapple, 1971; Coombs and Scott, 1974).

In the sarcoplasmic reticulum of muscle cells, there is an accumulation of products derived from the free radical oxidation of phospholipids accompanied by a loss of the calcium transporting ability with avitaminosis E (Arkhipenko, 1976). With Vitamin E supplementation, there is a stabilization of the membrane lipid bilayer and a decrease in the passive permeability of calcium ions. There is more lipid peroxidation in microsomes compared to the mitochondria in rats fed a Vitamin E free diet. Microsomes have five times the oxygen uptake and three times the fluorescence of mitochondria (Dillard and Tapple, 1971). When the diet of rats is increased in regards to Vitamin E, there is both a decrease in the oxygen uptake and fluorescence in mitochondria and microsomes. Mengel (1972), studying oxygen toxicity, determined that red
blood cells from Vitamin E deficient rats produced considerably more H$_2$O$_2$ than chow fed rats. Additionally, red blood cells from deficient rats had three and five times more lysis when exposed to H$_2$O$_2$ compared to red blood cells from chow fed and Vitamin E supplemented rats respectively. With the dialuric acid hemolysis test, gamma tocopherol has only 38% of the effectiveness compared to alpha tocopherol in preventing hemolysis (Bieri, et al, 1976).

Vitamin E also has a structural role within the lipid bilayer of membranes. Vitamin E interacts with phospholipids at a lipid-water interface (Diplock, et al, 1977). Through molecular model building, Vitamin E may act as a filler to increase the stability of phospholipids. This is accomplished by having the methyl groups of Vitamin E fit into pockets created by cis double bonds. The presence of Vitamin E at this location possibly prevents the destruction of phospholipids by membrane bound phospholipases (Lucy, 1972).

Vitamin E deficient rats have a decrease in the synthesis of nuclear RNA in liver cells (Hauswirth and Nair, 1972). Protein synthesis, in turn, is inhibited as measured by the incorporation of leucine-$_{14}$C. When $^3$H Vitamin E is given to deficient rats, the chromatin followed by the mitochondria and microsomes are the locations where the greatest amount of Vitamin E uptake is present. Returning Vitamin E to the diet also increases protein synthesis.

The roles of Vitamin E and Se-GSHpx are closely related. Several studies have reported that during Vitamin E deficiency, there is an increase in the activity of Se-GSHpx (Chow, et al, 1973; Kameda, et al, 1977). The increase in the enzyme activity is in response to the increase
in lipid peroxidation. On the other hand, Masugi and Nakamura (1976) reported no change in the status of Se-GHSpx in rats fed Vitamin E deficient diets. In chickens, dietary Selenium affects plasma Vitamin E levels. When dietary Selenium is increased linearly from 0 ppm to 0.10 ppm, there is a linear increase in plasma Vitamin E levels from 1.6 mg/dl to 3.96 mg/dl (Coombs and Scott, 1974). The increase in the Vitamin E level provides evidence that the two nutrients are complementary. That is, when Selenium is present in the diet, Vitamin E is spared.

When an adequate amount of Selenium is present in the diet, the optimal activity of Se-GSHpx is not with Vitamin E deficiency, but when the diet consists of 25-250 IU of Vitamin E/kg feed for the rat. The Se-GHSpx activity is greatly lowered when the diet consists of 25,000 IU of Vitamin E/kg feed (Yang, et al, 1976). When rats are deprived of Vitamin E and Selenium in their diet for 4-6 weeks, the Se-GSHpx activity is one-eighth the activity in alveolar and peritoneal leukocytes compared to rats deprived only of Vitamin E (Serfass and Ganther, 1976). These antioxidants work together in stopping lipid peroxidation. Vitamin E neutralizes free radicals at the site of their formation, while Se-GSHpx is active in the plasma and in the cytosol of cells.

**Radiation and Antioxidants**

Ionizing radiation has several effects on cellular metabolism. Lipid peroxidation is the causative factor for some of the cellular damage (Gutteridge and Scott, 1976). One to three day old rats have three times the amount of fluorescent products present in their skin shortly after ten minimum erythema doses of UV radiation is delivered
compared to non-irradiated rats (Meffert, et al, 1976). Additionally, there is greater fluorescence in forehead epidermis of humans compared to abdomen epidermis. It can be assumed that the greater fluorescence from the forehead epidermis is due to chronic exposure to UV rays (Meffert, et al, 1976). Meffert and Reichman (1972) have reported an increase in MDA production from human skin surface exposed to ionizing radiation, with a decrease in the fatty acid composition. Lipid peroxidation is greatest in adipose tissue when radiation is delivered at high doses and a slow rate (Glavind, 1965).

When $3 \times 10^5$ and $3 \times 10^6$ rads are delivered to rats, there is a significant decrease in the non-protein antioxidants located in the spleen, liver, cerebrum, kidneys, and adipose tissue (Glavind, 1965). Utsumi, et al. (1966) reported a decrease in the lag phase of Fe$_{2+}$ induced lipid peroxidation in liver microsomes when 1000 rads of whole body x-ray radiation is delivered to rats. The time before lipid peroxidation is measurable, is decreased for 22 hours and does not return to normal until 48 hours after irradiation.

It has been inferred that Vitamin E and other antioxidants are radioprotective (Kawasaki, et al, 1979; Hruba, et al, 1976). Cultured mammalian L-5 cells exposed to 200 rads during the exponential growth phase results in inhibiting mitosis. When the cells are treated during radiation with Glutathione or Cysteamine, both antioxidants, the recovery of the mitotic index is improved, however, the time for the initiation of mitosis following radiation is not shortened (Kawasaki, 1979).

Esterified forms of Vitamin E increase the survival time of mice receiving 700 rads of whole body radiation. The gain in body weight of
irradiated rats is also increased with Vitamin E supplementation (Shinoda, et al, 1968). On the contrary, Fritz-Niggli (1961) did not demonstrate protection from radiation with Vitamin E in regards to survival time. There was also no sensitization to radiation when a Vitamin E deficient diet is fed 2-3 weeks prior to radiation.

The in vivo studies on the dynamics of Vitamin E during radiation therapy in humans and animals give more light to the radioprotective properties of the vitamin (Hruba, et al, 1976). When 3600 rads, 25 K-rads, or 80 K-rads of whole body radiation is delivered to rats, there is a decrease in the plasma levels of Vitamin E within one hour. The higher the dose, the greater the drop in the plasma level. This pattern was also true in human patients receiving therapeutic radiation. The decrease plasma level may indicate an increase in the consumption of Vitamin E by tissue. Hruba, et al. (1976) also reported that Vitamin E given orally seems to be more protective than when given intramuscularly. The radioprotective property of Vitamin E is also more noticeable in younger animals.

In summary, there is a relation between the surgical wound, radiation, lipid peroxidation, antioxidants, and wound healing. Following surgery, there is an increase in the rate of metabolism (Schneider, et al, 1977; pp 485-499). The Vitamin E pool also has an increased turnover with an increase rate of metabolism (Utsumi, et al, 1966). Radiation increases lipid peroxidation and decreases the availability of antioxidants including Vitamin E (Gutteridge and Scott, 1976; Glavind, et al, 1965). The increase in lipid peroxidation inhibits the respiration of fibroblasts (Meffert and Lohrisch, 1971; Meffert, et al, 1972).
The respiration is inhibited proportionally to the amount of MDA formed (Meffert and Lohrisch, 1971). It is therefore reasonable to think that an increase in lipid peroxidation and an increase turnover of Vitamin E may cause cells involved with wound healing to be less efficient.
CHAPTER 2

MATERIALS AND METHODS

Three experiments were designed to determine if Vitamin E has an effect on wounds receiving preoperative radiation. Breaking strength, collagen deposition, and histology were the studies performed as part of Experiment 1. After the results of Experiment 1 were analyzed, it was decided that breaking strength would be part of Experiment 2. The second experiment also studied the relation between radiation and lipid peroxidation measured by fluorescent products as affected by Vitamin E. Experiment 3 was another attempt to study the relation between radiation, lipid peroxidation, and Vitamin E using a recently developed gas chromatographic method that measures ethane production. However, Experiment 3 did not get past setting up the apparatus and running a few test trials because of technical difficulties.

Experiment 1

In this experiment, there were 36 male Sprague-Dawley rats (mean body weight ± 1 standard deviation (SD) = 256 ± 27 grams) randomly divided into six groups. The animals were individually housed in wire mesh cages. A standard rat feed (Wayne Lab Blox) and water was available ad libitum. The feed, in part, included 24.5% protein, 4.1% fat, 3.6% fiber, 7.7% ash, and 50.2% nitrogen complete extract. It contained all the known minerals and vitamins at concentrations that provide for adequate growth. The concentration of Vitamin E in the form of tocopheryl acetate was 35 ppm.
Three experimental groups were pretreated with additional DL-\(\alpha\)-tocopheryl acetate prior to a single irradiation treatment. An irradiated group received no Vitamin E, but did get pretreated with laboratory grade sesame oil which was used as the carrier for the DL-\(\alpha\)-tocopheryl acetate. The two other control groups did not receive sesame oil; one group was irradiated, while the other was the non-irradiated control. All the animals were wounded two hours after the irradiation treatments. The animals were sacrificed 14 days postoperatively and the breaking strengths of wounds were measured. Tissue slices were taken from eschars for histological examination. Tissue from the eschar was also frozen for the biochemical assay of collagen.

Nutritional Treatment

DL-\(\alpha\)-tocopheryl acetate (General Biochemicals) was dissolved in laboratory grade sesame oil (Fischer Scientific Co.) for intraperitoneal injections. Groups D, E, and F received 10, 20, and 40 IU of the vitamin E supplement in 0.2 ml of sesame oil, respectively, starting on day one of the experiment. Group C received only 0.2 ml of sesame oil. Groups A and B did not have any injections. On days three and five, rats in each group received their respective dose of Vitamin E or sesame oil. Therefore, groups D, E, and F received a total dosage of 30 IU, 60 IU, and 120 IU, respectively, and group C received 0.6 ml of sesame oil.

Irradiation Treatment and Wounds

On day seven, rats in groups B, C, D, E, and F were treated with local irradiation along the midline of their dorsal side while under ether anesthesia. The irradiated area measured 12.5 cm x 1.0 cm. A
lead shield was used to protect the rest of the animals from irradiation (Figure 1). A dose of 600 rads was delivered to the animals from a Clinac 18 (Varion and Associates), utilizing a 6 Mev electron beam. The calometer had a setting of 20 x 20 cm with a 15 x 15 cm cone. The source to the skin distance was 100 cm. During the time-of-the irradiation treatments, the rats in Group A were anesthetized, but did not receive irradiation.
Figure 1. Area of Incision and Local Irradiation

The lead shield used to limit the area exposed to radiation prior to surgery surrounds the sutured wounds.
Within two hours after the irradiation, the animals were anesthetized with innovar-vet (Pittman-Moore, Inc.) intramuscularly at a dose of 0.02 cc/100 grams body weight. The hair on the dorsal side was removed with electric clippers. The skin was washed with 75% alcohol and then liberally swabbed with an iodine solution. Starting in the area at the base of the head, two 5 cm incisions, in line with each other, 2 cm apart, were made down the midline of the animal's dorsal side using a #15 scalpel blade. The depth of the incisions were down to the panniculus carnosus. Any capillary bleeding was stopped prior to the closing of the wound. Four interrupted sutures of 4-0 prolene with a V-4 taper cut needle (Ethicon, Inc.) was used to close the wound (Figure 2).
Figure 2. Sutured Wounds 14 Days Postoperative
Termination of the Animals

Fourteen days postoperatively the rats were anesthetized with innovar-vet and then sacrificed by introducing an air embolus via the tail vein. The preceding protocol is illustrated in Table I.

Breaking Strength

The anterior wound was used to measure the breaking strength. The wound and the skin surrounding the eschar was excised. The sutures were removed. By pinning down the skin and exposing the subcutaneous side, the fascia was removed using iris scissors and small thumb forceps (Figure 3). Using tissue between the suture marks, three samples of the wound 5 mm in length were dissected perpendicular to the incision with a calibrated double bladed knife. The breaking strength of each sample was measured with an Instron Tester Model TM using a C-cell with pneumatic clamps.

Collagen Content

The posterior wound was frozen after it was excised from the animal until analysis for total collagen and the soluble fraction was carried out as described by Jackson, et al. (1958). The Hyp determinations of the collagen fractions were analyzed using the method of Stegemann (1958). Spectrometry was accomplished with an Acta III Spectrophotometer (Beckman) using a wavelength setting of 550 nm. A tungsten source was used with a dynode setting of 500 volts. Samples were done in duplicate.

Histology

A fourth tissue sample was taken from the anterior wound for a semiquantitative histological examination. The tissue was fixed in 10%
TABLE I

Daily Calendar of Vitamin E Supplements, Irradiation Treatment, Day of Wounding, and the Healing Period

<table>
<thead>
<tr>
<th>Groups</th>
<th>Supplements</th>
<th>Irradiation and/or Wounded</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>*</td>
<td>*</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>*</td>
<td>600 Rad</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>SO**</td>
<td>600 Rad</td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>10IU†</td>
<td>600 Rad</td>
<td>7</td>
</tr>
<tr>
<td>E</td>
<td>20IU</td>
<td>600 Rad</td>
<td>8</td>
</tr>
<tr>
<td>F</td>
<td>40IU</td>
<td>600 Rad</td>
<td></td>
</tr>
</tbody>
</table>

Duration of Wound Healing: 21

*no treatment given

**0.2 ml sesame oil administered

†International Units of Vitamin E administered with sesame oil as a vehicle
Figure 3. Excised Tissue Showing Subcutaneous Scar

The subcutaneous tissue of the wounded skin is exposed. The samples for breaking strength were taken between the suture marks.
formalin. Skin sections were cut at 5-6 μm from paraffin blocks with a microtome blade. Routine slide preparations were made with hemotoxylin-eosin and trichrome staining. The microscopic assessment was based on three criteria: 1) cellular infiltration, 2) epithelialization and, 3) collagen formation. The final score for collagen formation was the addition of the mean scores for density, width, and thickness of a new collagen present. Each criteria was given a score from 0 (none present) to 4 (high amount present). The slides were graded in conjunction with a person who was blind to the group that the slides were from.

**Experiment 2**

In this experiment, there were 30 male Sprague-Dawley rats (mean body weight ± 1 S.D. = 257 ± 14 grams). The animals were randomly divided into three groups. Group I was the non-irradiated control; Group II received radiation and sesame oil; Group III received radiation and 120 IU of Vitamin E. The procedure for the preoperative treatment was the same as Experiment 1. The surgical procedure and healing period were equivalent to the first experiment with the exception that mercilene suture (Ethicon) was used instead of prolene.

**Breaking Strength**

The anterior wound was used to determine the breaking strength as previously described in Experiment 1.

**Lipid Peroxidation**

The posterior wound was used to determine the relative amount of fluorescent products due to lipid peroxidation. The procedure used for
this determination was that of Fletcher, et al. (1973) with the omission of the water wash. An Amico-Bowman spectrophotofluorometer (American Instrument Co.) was used with an excitation wavelength of 360 nm and emission wavelength of 450 nm. The photomultiplier setting was 0.2. The excitation and emission slits were 0.3 mm, allowing for the passage of 16.5 nm of rays. The relative fluorescence of each sample was recorded.

**Experiment 3**

Ethane Production

The intent of this experiment was to induce lipid peroxidation in rats and measure the ethane expired. The closed system used to collect ethane was the one described by Hafeman and Hoekstra (1977, 1977a). The total volume of the system used was 3.28 liters. Oxygen was introduced at a rate of 60 ml/min with an air flow through the system at a rate of 5.7 L/hr. A Hewlett-Packard gas chromatograph 402 was used to measure air samples from the system for ethane. The oven temperature was 190°C with the Helium flow set at approximately 50 ml/min. The injection port and flame detector exits were 233°C and 260°C, respectively. The retention times for methane, ethane, and propane were 0.26, 2.91, and 16.18 minutes, respectively.

**Statistical Methods**

The Duncan's Multiple Range Test was used to analyze the results of the breaking strength, total collagen, percent soluble collagen, and relative fluorescence between the experimental groups.
The results of the three experiments are given below. Vitamin E had the most significant effect on breaking strength.

**Experiment 1**

**Breaking Strength**

Increasing doses of Vitamin E significantly correlated with an increase in the breaking strength (r = 0.92). However, none of the groups that were irradiated had a greater breaking strength than the non-irradiated control. Within the irradiated groups, the sesame oil carrier did not have a significant affect on the breaking strengths.

Data from groups B and D include only five rats. The reason is that one rat per group died while under the ether anesthesia during irradiation therapy. The mean weight and weight gain were not significantly different between groups (Table II). There were no signs of wound infection throughout the postoperative period.

A total of 87 wound samples were used to compare the breaking strengths between the groups. The mean breaking strengths between the groups supplemented with 60 IU and 120 IU of Vitamin E and the non-irradiated group (E, F, and A, respectively) were significantly greater than the irradiated groups that received no placebo or sesame oil; groups B and C (p < .05). The mean breaking strength for group D which received a total of 30 IU of Vitamin E could not be statistically separated from
### TABLE II

**Results of Experiment 1: Weight Gain, Breaking Strength, and Histologic Scores**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>NO RAD (A)</th>
<th>RAD (B)</th>
<th>SESAME OIL</th>
<th>30 IU</th>
<th>60 IU</th>
<th>120 IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight Gain (grams)</td>
<td>103±16</td>
<td>110±20</td>
<td>102±16</td>
<td>102±25</td>
<td>100±16</td>
<td>110±22</td>
</tr>
<tr>
<td>% Breaking Strength of Control (A)</td>
<td>100%</td>
<td>74.6%</td>
<td>76.8%</td>
<td>86.7%</td>
<td>89.6%</td>
<td>94.9%</td>
</tr>
<tr>
<td>% Breaking Strength of Irradiated Group (B)</td>
<td>134.1%</td>
<td>100%</td>
<td>103.0%</td>
<td>116.3%</td>
<td>120.2%</td>
<td>127.3%</td>
</tr>
<tr>
<td>Epithelialization (histological score)</td>
<td>2.6</td>
<td>1.8</td>
<td>2.3</td>
<td>2.2</td>
<td>2.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Cellular Infiltration (histological score)</td>
<td>1.2</td>
<td>1.4</td>
<td>1.3</td>
<td>0.8</td>
<td>1.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Collagen Formation (histological score)</td>
<td>7.2</td>
<td>3.2</td>
<td>5.5</td>
<td>5.0</td>
<td>6.1</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Six rats per group except for the Rad and the Rad with 60 IU group which had five rats each. The weight gain is in grams ± 1 standard deviation. The semiquantitative mean scores for histology are from 0 (none present) to 4 (high amount present). Collagen formation is the addition of the mean scores for density, width, and thickness of new collagen present. There was a great amount of variability within groups.
the high or low breaking strength groups (Figure 4).

The breaking strength of the non-irradiated control group is considered to be 100%. Therefore, the non-supplemented irradiated rats in group B had a mean breaking strength that was 74% of the control group receiving no radiation. On the other hand, the 120 IU group had a breaking strength that was 95% of the group receiving no radiation and 127% of the group receiving 600 rads and no supplemental treatment (Table II).

Collagen Content

The results of the total amount of Hyp per gram of wound and percent soluble collagen fraction were not significantly different between groups. However, there was less total collagen in the untreated irradiated group compared to the non-irradiated control. The amount of total Hyp and the percent of soluble collagen that was measured did not statistically correlate with the treatments that the experimental groups received (Figures 5 and 6).

Histology

There was a great amount of variation within the groups. The general findings are given below. Microscopic evaluation demonstrated that the irradiated groups had less new collagen in the area of the wound compared to the non-irradiated control. There was a consistent increase in the amount of new collagen in the irradiated group treated with sesame oil or Vitamin E. However, it was not possible to correlate an increase in new collagen with the amount of Vitamin E used for treatment. The score for epithelialization was lower for the irradiated group compared to the non-irradiated group or the groups receiving sesame oil or Vitamin E prior to irradiation. The difference in epithelialization was not as
The radiation groups with no treatment and sesame oil are significantly different than the radiation groups with 60 IU and 120 IU Vitamin E and significantly different from the no radiation group (p < 0.01). The radiation group with 30 IU Vitamin E is not significantly different from any of the groups. Vertical lines represent 1 standard deviation.

Figure 4. Breaking Strength of Wounds; Experiment 1
Figure 5. Amount of Hydroxyproline Per Gram of Wound

Vertical lines represent 1 standard deviation from the mean.
Figure 6. Percent Soluble Collagen

Vertical lines represent 1 standard deviation from the mean.
great as with the difference in collagen formation. The amount of cellular infiltration between the groups was not different 14 days postoperatively (Table II).

Experiment 2

Breaking Strength

In this experiment, none of the animals demonstrated signs of infected wounds. One animal in groups II and III respectively, died because of ether intoxication. A similar pattern compared to Experiment 1 persisted in regards to weight gain and breaking strength (Table III). The non-irradiated group had the highest breaking strength 14 days postoperatively. The irradiated groups had a decrease in their breaking strengths. However, in this experiment, all the wounds had lower breaking strengths than in Experiment 1. The non-irradiated group (I) had a significantly higher breaking strength than the irradiated group that received sesame oil (II) \((p < .05)\). The non-irradiated control was not significantly greater than the group that received radiation and 120 IU of Vitamin E (III). Group III was not able to be statistically different from Group II (Table III).

Lipid Peroxidation

In Figure 7, the emission and excitation spectrum for the fluorescent products is given. This is the same as the one reported by Fletcher, et al. (1973). The relative fluorescence of the wounds was not statistically different between any of the three groups (Table III).
TABLE III

Results of Experiment 2:
Relative Fluorescence, Breaking Strength, and Weight Gain

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>CONTROL</th>
<th>+ VITAMIN E</th>
<th>+ SESAME OIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Fluorescence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± 1 standard deviation</td>
<td>6.6±1.9</td>
<td>5.8±1.3</td>
<td>5.5±1.4</td>
</tr>
<tr>
<td>Breaking Strength</td>
<td>(A)</td>
<td>(A,B)</td>
<td>(B)</td>
</tr>
<tr>
<td>(grams/5 mm wound)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± 1 standard deviation</td>
<td>174±5</td>
<td>166±8</td>
<td>149±6</td>
</tr>
<tr>
<td>Weight Gain (grams)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± 1 standard deviation</td>
<td>101±13</td>
<td>105±16</td>
<td>106±10</td>
</tr>
</tbody>
</table>

A,B - results with different letters are significantly different; p<.05

Nine to ten rats per group for the breaking strength analysis; 7-8 groups for fluorescent product analysis. Statistical analysis was with the Duncan's Multiple Range Test.
Emission and Excitation spectra of fluorescent product after isolation.

Figure 7. Fluorescent Products
Experiment 3

Ethane Production

There were numerous problems encountered with the apparatus used for this experiment. It took several trials before enough leaks were found and fixed so the system retained 95% of a known amount of ethane for five hours. However, I was not able to measure any ethane production from rats either injected with carbon tetrachloride or irradiated with 1,000 rads. A large percent of the difficulty revolved around not having the appropriate materials to reproduce the closed system that was set up by Hafeman and Hoekstra (1977a; 1977b). Additionally, out of seven rats, three did not remain alive for more than five hours in the closed system. I am not sure if the deaths were caused by carbon dioxide poisoning or due to toxicity of fumes from the excreta. For the preceding reasons, no data was obtained that could be used to analyze the relation between radiation, lipid peroxidation, and Vitamin E.
CHAPTER 4

DISCUSSION

The fact that Vitamin E consistently improved the breaking strength of the wounds receiving radiation provides the strongest evidence in these studies that radiation induces lipid peroxidation as the only positively proven effect of Vitamin E as its free radical scavenging capacity. The positive effect of sesame oil in Experiments 1 and 2 is not unwarranted. Shinoda, et al. (1968) also reported that sesame oil had some radioprotective properties. This may be due to the Vitamin E content of the oil. The Vitamin E content has been reported to be 2.2 milligram percent (Slover, 1971).

In retrospect, the inconsistency in the collagen measurements was due to the sampling error. When the eschar was excised for analysis, some of the normal tissue surrounding the wound accompanied the sample. A better method to measure the collagen metabolism would have been to determine the rate of Pro hydroxylation. Unfortunately, this problem was not realized until the methods and results were fully scrutinized. The histology results, although only semiquantitative, did tend to show that Vitamin E has some positive effect on collagen formation.

The unfavorable effect of preoperative irradiation manifests itself within the first 3-5 days postoperatively, as explained in the Introduction in the section, "Radiation and Wound Healing". Even though after five days the rate of collagen synthesis, gain in breaking strength, and the rate of contraction return to normal, these parameters of wound
healing are lower than non-irradiated wounds 10-14 days postoperative because of the initial delay. This is probably due to the high amount of peroxides that are formed at the time of radiation due to the formation of free radicals that initiate lipid peroxidation. After the free radicals and other oxidants have returned to the normal physiological level and lipid peroxidation is decreased, then the normal healing process returns. Therefore, it is surprising that in this study there was no measurable difference in the amount of fluorescent products formed. These products were expected to be present and remain in the area of radiation. I am not able to explain this discrepancy. Although it was disappointing not to accumulate data on lipid peroxidation by measuring the ethane production, I am able to do some speculation. When Hafeman and Hoekstra (1977a; 1977b) measured expired pentane, they had to use extremely deficient diets in both Selenium and Vitamin E and additionally induce lipid peroxidation by injecting carbon tetrachloride. When they measured the air samples for rats that had only deficient diets and no carbon tetrachloride, there was very little ethane production. Perhaps chow fed rats locally irradiated would not have enough ethane production to measure and therefore not correlate with the breaking strength of wounds.

Using electron microscopy, it has been determined that native collagen fibrils undergo irregular swelling and a loss of contrast when subjected to electrons (Grant, et al., 1970; Cox and Grant, 1969). These changes are primarily due to the rupture of intermolecular and intramolecular hydrogen bonds leading to the disorganization of the specific bonding regions of the tropocollagen molecule. Secondly, these changes occur because of the scission of the polypeptide chains resulting in fragmentation (Grant, et al., 1970). Periodic splitting of collagen
fibrils due to electron irradiation has also been illustrated with electron microscopy (Filisko, et al., 1972). Jeleska and Dancewicz (1972) irradiated in vitro an oxygen free tropocollagen solution. A decrease in the ε amino group's ability to bind with fluorodinitrobenzene was determined. These authors hypothesized that the decrease was due to conformational changes in the polypeptide strand.

With Vitamin E deficient diets, the normal metabolism of collagen is changed. There is a greater amount of the acetate and the total soluble fraction in normal skin and the stability of the insoluble collagen to gel at 4°C is depressed (Brown, et al., 1967). On the other hand, Ehrlich, et al. (1972) administered 190 IU of Vitamin E intramuscularly three times prior to wounding. These animals had a depressed healing response in regards to tensile strength and total Hyp. This report, in using a total dosage of 570 IU for 400 gram rats, probably induced a prolonged prothrombin time (Corrigan and Marcus, 1974).

In a clinical situation, it is important that the initial phase of wound healing proceed at a normal rate. It is within the first few days after surgery that wounds are the most susceptible to infection due to the lack of a sufficient protective barrier. It is for this reason that post-irradiated surgical patients have a higher risk of complications (Habel, 1965; Yarington, et al., 1976). Ancillary to the depression of wound healing, the immune response is also slowed down with irradiation therapy (Meyer, et al., 1966). The unfortunate result may be a full scale necrotizing infection (Daly, 1978). The administration of Vitamin E enhances several immune responses. The primary response of animals to bacteria and toxins are increased with Vitamin E. This is due to both an increase in cell mediated immunity and the humoral immune

Finally, Vitamin E has been reported by Chen (1974) to be required in greater amounts to protect older tissue from peroxidation. Since most radiation therapy involves the elderly, the clinical use of Vitamin E may be very important for the overall health of these patients. To determine if a patient is adequate in Vitamin E, Horwitt, et al. (1972) suggest that the serum \( \alpha \) tocopherol not be less than 0.8 mg to 1 g total lipids.
REFERENCES


