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By

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ABSTRACT

INTESTINAL RADIATION INJURY: ITS RELATIONSHIP TO CYTOKINES, BLOOD FLOW, AND RADIOPROTECTANTS

By
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The purpose of this investigation was to: 1) develop a model of localized pelvic radiation (XRT); 2) investigate the effects of this model on ileal and colonic blood flow and histology; 3) evaluate changes in tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6); and 4) evaluate the potential of radio-protection of sodium meclofenamate, vitamin A, and elemental diet. Animals were anesthetized and treated with 900 rads once a week for 5 weeks for a total of 4500 rads. Control animals received anesthesia only. Histology confirmed the presence of radiation injury. Biocellular assay for TNFα revealed peak values within 1 hour post XRT and a progressive increase during the course of radiotherapy. IL-6 did not show any significant changes. Ileal blood flow increased at 1 week and decreased at 5 weeks post XRT, while colonic blood flow was unchanged. TNFα significantly decreased with sodium meclofenamate; however, no other agents affected TNFα or IL-6. Elemental diet and sodium meclofenamate prevented the changes in blood flow within the terminal ileum at 1 and 5 weeks, while vitamin A increased blood flow at both time points. Colonic blood flow was unchanged by any agent. All agents showed benefit in preventing the histologic injury post XRT in both the ileum and colon.

DEDICATION

This project is dedicated to my family; Barbara, Mark, Eric and Steven James. Through them I have realized that no accomplishment is greater than the love and understanding of ones family. I shall forever be in debt for these hours stolen from you.

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INTRODUCTION

The use of radiation in the treatment of human disease dates back to just after its discovery by Roentgen in 1895.[1] The first patient cured by radiation therapy was reported in the literature in 1899.[2] Since then the advancement and application to the treatment of malignant disease have been outstanding. The first successful use of therapeutic radiation was with squamous cell carcinoma of the larynx. In 1922, Coutard and Hautant showed that laryngeal carcinoma could be cured with radiation therapy, thereby sparing the morbidity and mortality of radical surgery.[3] Today, radiation therapy remains a therapeutic option in almost 50% of all cancer patients.[4] When one considers that in 1990 there were approximately 1.1 million cases of invasive carcinoma to the viscera, the magnitude of the problem and its application becomes obvious.[5] But radiation therapy is not without its side effects. Damage to tissue adjacent to the malignancy can occur and this injury is permanent and progressive. While acute radiation injury mimics that of non-specific inflammation, the chronic side effects (defined as those occurring greater than six months) can appear as

late as 20 years post injury - even after the patient has been cured of his or her primary disease. The chronic side effects of progressive vasculitis and fibrosis are the most feared and difficult to overcome. These side effects occur in only 15-20%[6] of patients, but when one considers the numbers quoted above, the potential for suffering becomes enormous. It is with these thoughts in mind that this project was undertaken.

Aims and Rationale of the Study

The basic aims of the study are to investigate the mechanisms of intestinal radiation injury and explore the possibility of lessening or preventing this injury. In this study, we have attempted to create an animal model of pelvic radiation injury that is analogous to that which occurs in patients undergoing pelvic radiation therapy. We feel that our fractional multi-dose approach avoids the extrapolation and errors that can occur when comparing pre-existing single large dose models that have been currently utilized. With this model in place, we attempted to delineate the effect that pelvic radiation had upon the production of Tumor necrosis factor alpha, Interleukin-6, intestinal blood flow, and histopathology. In the first part of the study, we compared these factors between radiated and unradiated controls. With the first part of the study completed, we then investigated the effects of various agents with

potential radioprotectant properties. Sodium meclofenamate was chosen because of its role as an immune modulator. It is known to decrease the immune response due to its actions on the cyclo-oxygenase and lipo-oxygenase pathways. Vitamin A was chosen because of its opposite effect. It is a known immune stimulator which increases white blood cell number and function. Elemental diet was chosen because it worked in a fashion dissimilar to the above agents. Its mechanism of action is believed to be physically protective to the intestinal brush border. In this part of the study, these agents were given to rats receiving the same radiation protocol as in Part I. The same parameters of blood flow, tumor necrosis factor, Interleukin-6, and histopathology were compared between these groups and to the radiated and unradiated controls.

The ultimate purpose and rationale of the study is the clinical applicability of these agents. All of these agents have been or could be used in the patient care setting. When one considers the large number of patients undergoing radiation each year the potential benefits could be immense.

Definition of Radiation Injury

Radiation injury can be simply stated as any cellular tissue or organ injury resulting from the use of ionizing radiation. However, a more precise definition and use of that definition as causative in radiation injury can be problematic. Radiation injury varies with the type of radiation source utilized. External sources of ionizing radiation can be delivered as either alpha, beta, gamma or x-rays. The dose can be delivered externally through direct application, or through intravenous, intracavitary or interstitial routes. Also, the dose delivered to the tissue depends on the particular ability of that form of radiation to penetrate tissues. This is described as the linear energy transfer or LET. It is defined as the amount of energy delivered to a tissue over a fixed distance. An energy source that penetrates a tissue more deeply will have a lower linear energy transfer as it dissipates its energy over a longer distance.

The absorbed dose of radiation is expressed in rads which is equal to 1000 ergs or 10^{12} primary ionizations per gram of tissue from any type of ionizing radiation. One rad equals 0.01 gray which also can be identified as a centigray (cg), in which one centigray equals one rad.

The most important factor in all these calculations is the dose to the target area. Only then can one estimate the

potential injury to the tissues receiving the radiation dose. Other factors to consider are: 1) the elapsed time of the dose; 2) the size of the dose fractionation; and 3) the biological nature of the tissue. The sensitivity of the tissue is proportional to its proliferative activity. Bone marrow, GI mucosa, skin and its dermal appendages represent highly proliferative and therefore sensitive organs. Injury to these tissues represent some of the major side effects and dose limiting restrictions of radiation therapy. Dermatitis, alopecia, and bone marrow suppression are representative of these changes.

Despite different tissue sensitivities, the primary cellular events occurring with radiation are similar. The energy from ionized radiation causes free radical production from intracellular water. These short lived molecules have localized effects on cellular components causing macromolecular damage. The half life of these free radicals can be increased by increasing the concentration of oxygen. Also, there can be direct injury by high energy electrons. However, most injury appears to be due to hydroxyl radical production from ionized water.[2] DNA injury appears to be the most critical, with resultant DNA breakage and point mutation of chromosomes. This can result in prompt death from mitotic arrest or a period of temporary non-mitotic growth followed by cellular death. Also, this injury can be

sublethal, and be repaired with a continuation of normal cellular function.[2]

On a macroscopic scale, acute radiation injury presents as intestinal mucosal slough and hemorrhage. This is seen in more severe cases and in the case of total body radiation (greater than 400 rads total body dose). This is invariably accompanied by bone marrow suppression with severe consequences for the individual. The earliest changes seen are within the crypts of the intestinal mucosa. The crypt cells represent the regenerative source of the intestinal mucosa. The production of new enterocytes are blocked and mucosal atrophy and necrosis is seen. Also, active transport into the cell is affected due to an overall decrease in cellular function. These events result in diarrhea, malabsorption and mucosal hemorrhage. Further cellular breakdown leads to disruption of the intestinal barrier function and bacterial translocation. In less severe cases, a decrease in mitotic activity with partial necrosis of crypt cells is seen. This results in decreased mucosal and villous height with a decrease in the absorptive capacity of the bowel. Depending on the total surface area involved, this may or may not cause noticeable symptoms.

Late radiation injury has a much more variable presentation. Its time to presentation can vary from weeks to years. Its pathogenesis is due to a progressive vasculitis which leads to collagen deposition and fibrosis.

This leads to decreased tissue blood supply and hypoxia which is causative in the symptoms of chronic radiation injury. Therefore, chronic injury is not due to an alteration of epithelial proliferation but ischemia secondary to a decreased blood supply. This ischemia leads to ulceration, necrosis, perforation, fibrosis, stricture and intestinal obstruction. Low blood flow states such as decreased cardiac output or atherosclerosis can increase symptoms or increase the progression of this disease. This can make sub-clinical disease present as a fulminant process. Also, patients with previous abdominal surgery, intra-abdominal infection or treatment with radiosensitizing drugs such as 5-Hydroxyfluorouracil or doxorubicin are at increased risk for complications. Associated with this gastrointestinal injury are dermatitis, dermal and epidermal atrophy, telangiectasias, hyperkeratosis and ulceration of the exposed abdominal wall skin.

Grossly, acute radiation injury appears as an acute inflammatory reaction. One sees a red, injected inflamed serosa with friable mucosa and contact bleeding. Chronic radiation injury is manifested as a serosal surface which becomes gray, opaque with multiple fibrinous adhesions. Fibrosis and stricture are also common. The mucosa appears pale with lack of the normal appearing submucosal vascular pattern. The rectum presents with a much more specific syndrome of proctitis. These symptoms include bleeding,

pain, tenesmus and ulceration. These symptoms can progress until the need for fecal diversion in the form of a colostomy may be necessary. Ulceration can lead to the devastating complication of recto-vaginal fistula which may necessitate resection. These symptoms can be part of both the acute and chronic syndrome.[7]

Animal Models

Radiation Therapy

A significant body of literature exists describing the effects of radiation on normal tissue.[2] Also multiple studies have shown the specific effects of radiation on both the large and small bowel. However, most of these studies have relied on a single dose of either total abdominal or total body radiation. Cento Neto[8] investigated the effects of radiation on the rectal mucosal histology of mice. In his study he treated the animals with 400 rads of total body radiation. This actually represents the LD50 dose in humans. Crowley's[9] work with dogs on small bowel and anastomotic healing dealt with total abdominal radiation as a single dose. Studies of localized pelvic radiation have all limited their dose to the anatomic pelvis.[10,11,12,13] However, these studies consisted of large amounts of single dose radiation (1000-3000 rads). The work of Hubman[10,14] indicated that the LD50 dose for rectal obstruction in the rat is 2150 rads single dose. Since this lesion is almost

uniformly fatal, this is, in actuality, the LD50 dose for localized single dose radiation to the pelvis. Another study by Black et al[15], used much higher doses, but divided these doses into multiple fractions. These investigators primarily described the histopathological effects and were able to obtain long term survivors (greater than one year) using 10,000 rads of localized pelvic radiation divided in 10 fractions. To our knowledge this represents the only report in the literature of attempts to study pelvic radiation in a fractionated dose similar to that delivered in humans. This seems peculiar due to the multiple reports documenting the difference in effects of fractionated dosing and the potential decrease in side effects caused by fractionation.[16,17,18]

Most of these studies were set out with the purpose of describing the histopathologic effects of radiation upon either the large or small bowel. Most of the gross histopathologic changes have been mentioned in the above section on the definition of radiation injury. However, some of these studies have added further insight. In the study of small intestinal anastomotic healing by Crowley et al[9], there was shown to be no deleterious effects of intestinal anastomoses performed after 1500 rads of total abdominal radiation in dogs. However, this study did not measure the anastomoses long term (only 22 days post radiation therapy). Similar work by Schaur[13] and Bubrick[12]

showed no decrease in anastomotic healing after 2000 and 4000 rads of pelvic radiation in dogs. All these results are despite the presence of acute radiation changes seen histopathologically. Studies in rats, however, have shown different results. Work by Ormiston[19] on the healing of surgical wounds, showed a marked decrease in wound healing that was directly dose dependent.

With regards to the rectum, there have been several studies investigating specific effects of radiation therapy to this organ. Studies by Centeno Neto[8] have shown an increase in mucosal goblet cells post radiation. This is followed by a sharp decrease with a return to almost near normal levels over several weeks. Chronic radiation ulcer and colitis cystica profunda have also been described as being dose dependent in their formation.[15,20] Perhaps most of the work on rectal radiation injury has dealt with the development of fibrosis and obstruction. Multiple studies have shown rectal obstruction to be a lethal end point of pelvic radiation in the rat. This obstruction is dose dependant and appears to have an ED50 of approximately 2150 to 2300 rads.[10,14] This is accompanied by multiple histologic changes such as vascular sclerosis, fibrosis, mucosal atrophy, ulceration, atypical cellular regeneration and colitis cystica profunda.

To quantitate these pathological effects in the rat, Black et al[15] created a grading scale based on 5 points of

observation: 1) ulceration; 2) colitis cystica profunda; 3) atypical epithelial regeneration; 4) fibrosis; and 5) vascular sclerosis. This was then applied to a model of increasing doses of radiation and was shown to create a reproducible increase in the severity of this injury score with an increase in the dose of radiation. Most other studies have described the individual pathology described in Black's studies but have not attempted to quantitate these changes in such a manner. This grading scale formed the basis for the grading scale used in our study.

Although the histologic changes of radiation are well described, there exists no data in the literature describing radiation affects on intestinal blood flow. With this in mind we elected to study the blood flow to the terminal ileum and colon as well as to evaluate their histopathologic changes. We also included the proximal jejunum in the evaluation since this would serve as an internal control. It was out of the field of radiation and therefore should be unaffected by the local effects of pelvic radiation. However, it could be influenced by systemic factors released by this local injury. This model allowed us to investigate those potential changes.

Nominal Standard Dose Equation

Animal Models

Almost all of the animal models of radiation injury have utilized a single dose and directly or indirectly extrapolated this by the nominal standard dose equation. This equation was proposed by Ellis in 1969[21], to allow for comparison of single dose to multiple dose radiation. The formula is as follows:

$$D = (NSD)\ T^{0.11} \times N^{0.24}$$

where D = total dose of radiation, NSD = the nominal standard dose, T = total time in days in which the total dose of radiation is administered and N = the number of irradiated fractions. Limitations of this equation are that it does not allow for interruption in therapy or split dose therapy. Actual treatment with variation of dose fraction has shown variation in the doses calculated by as much as 30%[22,23]. It is our contention that this formula does not allow for factors such as the sequential nature of radiation injury, the cumulative effects of injury, the maturation of the inflammatory response and possible changes in the pathophysiology occurring during a course of radiation therapy which may last 4 to 6 weeks. These factors make comparison between single and multiple dose animal models difficult, and comparison to clinical studies irrelevant.

Animal Models: Radiation and Cytokine Production

Many studies have been performed to evaluate the effects of abdominal radiation on cytokine production, especially that of Tumor Necrosis Factor alpha (TNF) and Interleukin 1 (IL-1). TNF is a soluble peptide released by activated macrophages and many other cell types. The gene for TNF is located on chromosome 6 and codes for a 233 amino acid precursor protein of which 76 amino acids are removed prior to its secretion. It has a broad spectrum of biological actions on both the immune and non-immune cells. The general effects of TNF include cytolysis, increase in macrophage activity with tumor cell killing and modulation of the non-specific inflammatory response. It also decreases lipoprotein lipase activity leading to decreased energy availability and cachexia. TNF has also been shown to increase fever, the production of acute phase reactants, interleukin 6 and interleukin 1 production by macrophages. This increase in interleukin 1 production further increases tumor necrosis factor and serves as a positive feedback. TNF is stimulatory on activated T-cells and increases interleukin 2 receptor expression. It also increases interferon gama production suggesting a role in anti-viral immune function.[24,25]

Many of the roles of TNF are similar to that of interleukin 1. Interleukin 1 is produced primarily by macrophages but also by keratinocytes and endothelial cells

and some T and B cells. It is also made by Langerhan cells, smooth muscle cells, neutrophils and kidney mesangial cells. The macrophage and keratinocytes remain the major producers. There are two genes for interleukin 1 (interleukin 1 alpha and interleukin 1 beta). Both are located on the long arm of chromosome 2. Both are initially translated as 31 kilodaltons proteins that undergo extracellular proteolysis with proteinases released simultaneously from the macrophage. These then make the active form of interleukin 1.

Interleukin 1 functions as a major mediator of inflammation. It has many similar effects of TNF causing an increase in fever, acute phase reactants, decrease in plasma iron and zinc, and an increase in plasma copper. Interleukin 1 increases prostaglandin E2 release, increases the release of neutrophils from bone marrow and increases the production of colony stimulating factors which increase growth and differentiation of hematopoietic stem cells. Interleukin 1 also has neuroendocrine effects on the hypothalamus causing fever, increased release of corticotropin releasing factor, suppression of appetite and induction of slow wave sleep.

Substantial evidence exists that TNF and interleukin 1 are increased by exogenous sources as a part of the inflammatory response. However, the specific roles that these agents play in radiation injury is unclear. Studies by Neta et al[26,27], have shown interleukin 1 to be beneficial

in whole body radiation in mice. This was believed to be because of interleukin 1's stimulatory effect on bone marrow. Work by McBride[28] using total body radiation in mice reveal an increase in radiation induced complications (primary adhesion formation) and death in animals supplemented with interleukin 1 or lipopolysaccharides. This is in contradiction to work by Wu[29] showing an increasing crypt survival of mice treated with total body radiation and human recombinant interleukin 1.

TNF has also been shown to be radio-protective in similar models. Urbascheck[30] measured splenic granulocyte precursors as a measure of myelopoiesis. In this study the authors injected minute amounts of lipopolysaccharides to stimulate both TNF and interleukin 1 release. Mice showed an increase in survival with this method and with direct injection of recombinant TNF. Interleukin 1 showed an increase in the rate of survival but only when TNF was given subcutaneously. In Neta's[26,31] study TNF effect was also evident but interleukin 1 was found to have more of an advantage than TNF alone. Together they appeared to have an additive effect. These studies have dealt only with survival rates or the indirect measures of radiation side effects such as adhesion formation. In none of these studies was the influence of these cytokines measured against gastrointestinal function, blood flow or histopathologic changes. Also, the use of total body

radiation or total abdominal radiation superimposes hematopoietic effects upon that of gastrointestinal function.

The relationship of interleukin 6 to radiation injury has not been documented in the literature to date. Interleukin 6 is produced by T-cells, macrophages, fibroblast and a variety of transformed cells. It is coded on chromosome 7 and translates into a protein of 190 amino acids in its active form. Its effects are similar to interleukin 1 and TNF. Both tumor necrosis factor and interleukin 1 increase interleukin 6 and vice versa. Interleukin 6 plays a role in the increase of acute phase reactions, and production of fever similar to that of TNF and interleukin 1. In fact, the increase in acute phase reaction by tumor necrosis factor and interleukin 1 are believed to be related to their increase in interleukin 6. Interleukin 6's major role appears to be an accessory signal to T and B cells, possibly permitting a growth response to interleukin 1.[24,25]

Radio Protectants and Vitamin A

The search for methods of protecting collateral radiation injury began almost immediately after the discovery of the complication itself. The compounds can be divided into two large classes, those that decrease the subsequent inflammatory response (such as corticosteroids

and non-steroidal anti-inflammatory agents), and those that scavenge or decrease the free radical production by radiation (Vitamin E, Vitamin C, Sulphydryl containing compounds, and or relative hypoxia).[2] All of these agents have been tried with varying degrees of success. Included in the discussion are those agents utilized in our experiment and their related compounds.

Vitamin A is an essential fat soluble vitamin derived from carotenes. These carotenes are pro-vitamin A and the most common form is beta carotene. The most common source in the human diet consists of liver, butter, yellow and green fruits and vegetables. Many modifications of Vitamin A exists with Vitamin A aldehyde having the most biological activity. Vitamin A acetate utilized in our study has similar biological activity. This is also the most commercially available form. Vitamin A is essential for normal growth and development. It also has a critical role in visual development and its deficiency can lead to blindness.

The mechanisms of action of Vitamin A are diverse. It appears to help regulate normal cellular growth and differentiation as a deficiency of this can lead to an increased risk of developing epithelial carcinoma.[32] These studies showed that a deficiency of Vitamin A causes hyperplasia and an increase in DNA synthesis. This occurs primarily in respiratory, mammary, urinary bladder and skin.

This was reversed with replacement of Vitamin A. Also, a decrease in RNA synthesis has been observed with a resultant decrease in the production of glycoproteins and glycolipids. This suggests a role of Vitamin A in transcription and translation. Also, glycoprotein modification may interfere with cell adhesion and regulation. Vitamin A has also been shown to increase the production of mucous and increase in goblet cell concentration in intestinal epithelium.[33] Studies have revealed a destabilization of lysosomal membranes and activation of lysosomal enzymes with an increase in Vitamin A. All of these factors have been used to explain the beneficial effects of Vitamin A in protecting organisms from environmental insults such as infection, wound injury, radiation, and carcinogenesis.

Specifically, Vitamin A has been shown to decrease the mortality in an LD50 model of total body radiation.[34] Its activity shifted the LD50 dose to the right by 100 rads. Tannock et al[35], showed that an increase in Vitamin A decreased the dose of XRT needed to achieve local tumor control in mice. This only occurred in an immunogenic tumor. This data is supported by Winsey, Wu[29] and Levenson[36] which showed an increase in thymic size and an increase in white blood cell count in animals treated with whole body radiation. Work by Barbul[37] further supports this by showing that supplemental Vitamin A reversed the adrenal hypertrophy seen with stress thereby reversing the immunosuppressive

effects of increased cortisol. This reversed the thymic atrophy seen under high stress conditions. Jurin[38] noted that Vitamin A treated animals had an increase in cellularity of the lymph nodes and increase in antibody production when measured by hemagglutination. He also showed that these animals had an accelerated rejection rate for non-homotypic skin grafts.

Vitamin A has been used as a radio-protectant in the model of intestinal injury. Wensey et al[36], used Vitamin A supplementation in a rat model of abdominal radiation and colonic anastomosis. They showed that the anastomotic site in Vitamin A treated animals had increased bursting strength and increased hydroxyproline content measured 7 days post radiation. Work by Levenson[34] supported that the oral route of intake is important in this intestinal model. This is due to direct stimulation of the gut mucosal associated lymphoid tissue resulting in more site specific activation of the immune system. Other studies have shown Vitamin A to increase wound healing and increase wound strength in soft tissue injury following radiation therapy.

Very little data exists concerning the relationship of Vitamin A to blood flow. No known studies have investigated whether Vitamin A increases or decreases blood flow in a model of radiation injury. Some studies have speculated the increase in blood flow may be due to increased capillary

ingrowth, secondary to acceleration of the inflammatory and healing process.[37] This data is less than substantial.

Sodium Meclofenamate

Sodium Meclofenamate is a derivative of the fenamate class of compounds. They are aspirin-like drugs which are a derivative of n-phenylanthramide acid. These agents have been shown to have anti-inflammatory, analgesic and anti-pyretic activity.[33] The exact mechanism of action of sodium meclofenamate is unknown. It has been shown to decrease cyclooxygenase activity and thereby decrease prostaglandin synthesis. It has also been suggested that it competes with prostaglandin binding sites to further decrease their effectiveness.[33] The significance of this action lies in the fact that an elevation of prostaglandins (specifically PGE2) have been shown to increase radiation injury and have been shown to be increased in neoplastic tissue.[39] Also, a decrease in 15 hydroxyl prostaglandin dehydrogenase has been demonstrated in tissue treated with radiation.[40] This leads to a further decrease in the degradation of prostaglandin and contributes to the overall increase.[41] Studies using cyclooxygenase inhibitors like indomethacin and sodium meclofenanate have shown a decrease in radiation induced esophagitis and cystitis.[39,42] Sodium meclofenamate has also been reported to decrease radiation therapy induced brain damage in man and primates, and to decrease ultraviolet

radiation induced carcinogenesis. In addition, sodium meclofenamate is an inhibitor of lipooxygenase. This inhibits leukotriene production and has further effects on modulation of the immune response.

Studies in both humans and rats have shown sodium meclofenamate agent to be very safe. Human studies reveal only moderate side effects of nausea and diarrhea at 400 mgs per day (5.7ml per kilogram per day). No bleeding disorders or increase in peptic or gastric ulceration were noted.[33] The LD50 of this drug in rats is 126ml per kilogram.[43] In rats, long term studies have shown no adverse effects on rats maintained on 5mg per kilograms per day. Rats maintained on 7ml per kilogram per day showed slight weight loss and decrease in food intake after 28 days.[43] Based on this data a dose of 5mg per kilogram per day was chosen for our study. This represents the highest dose tested to be safe over a long term period (greater than 3 months). The rats showed no side effects of nausea, vomiting, diarrhea or weight loss during this study period. This was important as we did not want to confound the side effects of drug therapy with that of radiation injury.

Sodium meclofenamate has been shown to have radio-protective effects in humans and primates. Mahafzah[42] showed an increase in the acute side effects of radiation but a decrease in the chronic side effects of radiation in patients treated with sodium meclofenamate. A small cohort

of patients were followed for three years with sodium meclofenamate suggested an increased benefit. This did not reach statistical significance. Small sample sized could not eliminate the possibility of Type 2 error. Ambrus et al[44,45], showed a marked decrease in radiation induced esophagitis and cystitis in monkeys treated with 2,000 rads of single dose radiation. Radiation esophagitis and cystitis were determined by endoscopy and biopsy. Despite these studies, no data exists concerning the use of this agent in rats or animals treated with multi-dose radiation therapy. Our anticipation is that the results would be similar however. Also, no data exists concerning the effects of sodium meclofenamate on intestinal blood flow following radiation therapy.

The animals in our study received 5 mg per kilogram per day of sodium meclofenamate. This was suspended in methylcellulose and mixed with baby food. The methylcellulose was inert and the baby food vehicle is a common practice in delivering oral medication in veterinary medicine (personal communication). This mixture was administered directly po to avoid the potential morbidity and mortality of gavage. Confirmation of dose was by visual determination of the dose delivered and watching the animals take the mixture readily. The amount of baby food delivered was 0.5 cc per day and its seems unlikely that this amount would have an effect on overall food intake.

Elemental Diet

Dietary manipulation and its ability to prevent radiation injury represent a considerable body of literature. The use of an elemental diet has been shown to be of potential benefit in both animal and human studies. Pageau and Bonous[46] showed that the use of an elemental diet limits the side effects of both GI and hematopoietic injury after single dose radiation. They showed an increase in tritiated thymidine uptake in both hematopoietic tissue and in the GI tract after treatment with an elemental diet. Also, they noted an increase in mitotic activity within the jejunum of these animals. This benefit was most significant in the lower doses of radiation but did persist at the higher dosages. McArdle[47] showed similar results with pelvic radiation in dogs as did Beitler in rats. Beitler's[48] results showed a benefit from an elemental diet to the ileum but not to the jejunum when evaluating tritiated thymidine uptake. Clinical studies have also substantiated the benefits of an elemental diet in protection against radiation injury.

Studies by Douglas[49] and McArdle[47] showed a decrease in both subjective and objective symptoms in patients with pelvic radiation therapy. Douglas's study was not randomized and utilized patients with end stage carcinoma. The symptoms measured in McArdle's study were supported by objective evidence, i.e. the use of histology and electron

microscopy. Both of these studies substantiated a benefit from the use of an elemental diet.

The mechanism of the beneficial effect of an elemental diet is best described in the work by Bounous.[46,50] In studies using both radiation and hemorrhagic shock, he has been able to show a significant protection with the use of an elemental diet. Elemental diet helped preserve the glycocalyx and intestinal brush border and thereby preserve the absorptive surface area of the small bowel. This results in better absorption of nutrients specifically that of amino acids. This may relate to the benefit noted by Souba[51] in animals fed glutamine supplemented diets. Elemental diets also decrease the output of pancreatico biliary secretions. These secretions present a second potential injury to the already damaged intestinal mucosa. Bile salts and pancreatic enzymes can gain access to the enterocytes once the protective mucus layer and gylcocalyx have been damaged by inflammation. Other studies have confirmed this by showing that protein hydrosolates in an elemental diet have decreased trypsin activity within the lumen of the small bowel. This has been confirmed by the prevention of intestinal injury in dogs who have underwent pancreatico-biliary ligation. Other potential injurious agents are hypertonic solutions and the physical trauma of undigested macromolecules. Theoretically an elemental diet should prevent this trauma and this has been proposed as a

potential mechanism for the radio-protective effects of an elemental diet.

The potential benefits of glutamine demonstrated by Souba and Klineberg[52] may represent a mechanism by which an elemental diet is beneficial. By eliminating the need for an enzymatic digestion of protein, glutamine may be more easily absorbed. Glutamine has been shown to be a principle fuel for the enterocyte.[51,53] Most studies in the literature do not reveal the glutamine concentration within their elemental diet. This lends support to the conclusion of Klineberg[52] suggesting glutamines' primary role in intestinal reabsorption and reparation after injury.

The composition of an elemental diet in the literature varies. Most studies use casein hydrosylate as the protein source. This is enzymatically digested and contains all the essential amino acids. The elemental diet we utilized was commercially prepared by Purina Mills, Inc. The contents of this diet are contained within the table below. The protein source was that of an acid supplemented with d + l methionine. Carbohydrate source was that of sucrose and fatty acids were supplied via corn oil. Supplemental minerals and vitamins were also added. This yielded a mixture of 24.46% protein, 7.4% fat and 62.36% carbohydrate. These values are similar to those used by Beitler[48], Bounous[46] and Hagen[49]. In all of these above mentioned studies, animals tolerated this diet without difficulty. Previous

studies have confirmed that this diet is safe and able to sustain normal growth and maturation in the rat for an indefinite period of time.

Literature describing the relationship of an elemental diet to blood flow remains scarce. In a paper by Ottaway[54], he noticed a decrease in the percentage of cardiac output to the terminal ileum and colon in mice fed an elemental diet. These tissues also had a decrease in mass which may explain the difference in blood flow noted. He also stated that the overall intestinal blood flow remained the same. No other data exists to confirm or refute this. However, the above mentioned studies seem to be contradictory that they describe an increase in proliferation and a decrease in injury. Ottaway's animals were not radiated and this may represent a lack of trophic affects of a more proximally absorbed diet. Correlation between the two models remains difficult.

Table 1

Contents of Elemental Diet

Hydrolyzed Casein (Enzymatic)	24.46%
Sucrose	20.79
Dextrin	41.57
AIN-76 Vitamin Mix	1.00
AIN-76 Mineral Mix	3.50
DL-Methionine	0.05
Choline Bitartrate	0.20
Calcium Carbonate	0.77
Calcium Phos. DiBasic	0.17
Corn Oil	7.49

Microsphere Technique

Blood flow determinations were performed using the reference sample method with microspheres of Strontium 85. The reference sample technique is based upon injecting a known quantity of radioactivity into the left ventricle of an animal and measuring the amount of radioactivity distributed to the organs and a reference sample of arterial blood. The sample is withdrawn from the femoral artery during injection of the radioactive compound. After measuring the initial counts per minute of the injectate and the counts per minute of the organ in reference blood and the reference withdrawal rate you may use the equation:

$$\text{Blood Flow} = \frac{\text{Organ CPM} \times \text{Reference Blood Withdrawal Rate}}{\text{Reference Blood CPM}}$$

Dividing this value by the weight of the tissue and blood flow can be expressed in ml per minute per gram of tissue. This technique has been validated in many animal species (cats, rats, dogs, and turkeys).[55,56,57] It has also been shown to be a reproducible technique for determining cardiac output. For this calculation, one must determine the counts per minute left remaining in the injectate. The formula is then used as:

$$\frac{(\text{Initial CPM} - \text{Remaining CPM of Injectate}) \times \text{RBWR}}{\text{Reference Blood CPM}}$$

With RBWR being defined as reference blood withdrawal rate. Dividing this value by weight in 100 grams in cardiac output is expressed in ml per minute per 100 grams body weight.

The basic technique used in all the references is basically the same. A PE50 catheter is placed into the right femoral artery and secured. This is attached to a standard withdrawal pump and set at the chosen withdrawal rate. A catheter is placed in the right carotid and threaded into the left ventricle. Confirmation of the placement into the left ventricle is by characteristic wave form and a decrease in the diastolic pressure. At this point, the microspheres are injected. In our experiment we used the method described by Premer and Granger[55,58] in which the withdrawal rate utilized was 0.69 ml per minute for a total of 75 seconds. Microsphere injection occurred after 10 seconds of withdrawal and continued for 20 seconds. Withdrawal then continued for another 45 seconds for a total of 75 seconds. Carotid catheters were flushed with minimal amounts of saline to allow for continuous monitoring of blood pressure and heart rate.

The dose of microspheres in the rat can be calculated to avoid hemodynamic disturbances. Studies by Stanik[59] revealed that up to 1×10^6 microspheres could be given with only minor changes in the heart rate. No change in any parameter was noted when 3.6×10^5 microspheres were used for injection. Neither of these injections altered the

cardiac output. In our experiment 0.2 ml of solution per animal was utilized which contained 1.8×10^5 microspheres per injection. The microspheres were suspended in a solution of 10% dextran and 0.05% Tween 80. Of concern was the potential hemodynamic effects of the suspending solution. Previous work in our laboratory (Ping Wang, M.D.- unpublished data) has shown the solution does not have any hemodynamic effect in the rat model.

Microsphere size chosen was that of 15±3 microns. This preparation is commercially available. This micron size allows for adequate trapping within the tissue capillaries. Studies by Malik[60] reveal that 90% of the 15 micron microspheres are removed in the first pass of the circulation. Also, 15 micron microspheres allow for a sufficient number of microspheres within the injectate. It is necessary to have enough counts per minute (and therefore microspheres) distributed to the tissues to allow for accurate calculation. Placement of the catheters within the left ventricle allows for adequate mixing of the microspheres and accurate determination of cardiac output.

Confirmation of blood flow technique was performed by measuring the counts per minute in both the kidneys and lungs. This technique is used by all the authors quoted. This confirmation is based on the premise that equal mixing of the microspheres will result in even distribution of counts per minute within the right and left kidney.

Difference between kidneys should be less than 5%.[60] Also, blood flow in the lungs should be low indicating an intact ventricular septum. The numbers utilized most commonly in the literature is less than 5% of the cardiac output. These calculations were performed for each animal in our study.

To date, no data exists in the literature discussing the use of microspheres in the irradiated rat intestine. Multiple studies have evaluated cardiac output and regional blood flow in normal rats.[57] We utilized these values from the literature for comparison. Normal cardiac output for a 200-250 gram rat ranged from 22.8 to 27.8 ml per minute per 100 grams of tissue.[56,60] Normal organ blood flow for the intestine was 0.64±13 for stomach 0.54±0.06. These are equivalent to 16.1%±1.1 and 2.05%±.25 of the cardiac output respectively.[56] In our study, the stomach was not measured but "intestine" was broken down into jejunum, colon and terminal ileum. No specific data was available for this type of comparison.

Appropriateness of Animal Model

Based on the above presented information, we feel that our animal model has several distinct advantages over those previously reported in the literature. Total body radiation superimposes myeloid suppression on that of severe gastrointestinal injury. Measurement of specific and local effects of GI function must be considered in this light.

These have been eliminated or dramatically reduced in our model. The beneficial affects of cytokines in previous studies (TNF and interleukin 1) may represent the potential myeloproliferative benefit and not any specific radio-protectant effects within the GI tract. However, no literature exists to document or refute this.

The dosage schedule used in our study directly mimics that used clinically in patients with pelvic malignancies. There was no need for the extrapolation of the dose by the nominal standard dose equation. One limitation is once weekly dosages (patient received daily dosing). Daily doses of anesthesia would have been necessary to deliver this dose of radiation would not have been appropriate as this would increase the risk to our animals. Our animal model does contain the pattern of sequential injury and recovery present in the human model. With this in mind, we attempted to define the repeated insult in terms of histologic alteration and the production of interleukin 6, and TNF blood flow. We feel that our model is more appropriate and accurate, and allows for direct correlation with human studies.

Also, we can follow the possible progression of this response during the course of therapy, i.e. does the animals ability to generate TNF and/or IL-6 change with time. This kind of information is not available from the present models of radiation injury in the literature. By creating moderate

injury without evidence of significant mortality, clinical relevance is maintained. The nature of the radioprotective agents and their prior usage also allows for easy determination of the clinical efficacy and applicability of these agents used in this study.

In our model, we have afforded the opportunity to study the effect of pelvic radiation on non-radiated GI function. The proximal jejunum was out of the field of radiation therapy. It was subjected to the same analysis as that of the terminal ileum and colon. If any systemic effects of this localized radiation model occurred, it was hypothesized that the jejunum may serve as a potential indicator of these effects.

Materials and Method

Animals

Animals used for this project consisted of female Sprague-Dawley rats 200 to 250 grams. Animals were obtained from Charles River Corporation and certified as germ free. They were housed in the University Laboratory Animal Research facility on campus during the entire course of this study. Routine care was provided by the ULAR veterinary staff.

Female rats were chosen because of lack of interference of the external genitalia with the absorbed radiated dose.

The ventral location of the scrotum and penis within the radiated field would call for an adjustment of the radiation dose to achieve the desired dose in the more dorsal colon. This increased dose could lead to an injury to the urethra and scrotal skin. This could lead to potential side effects and complications necessitating the removal of these animals from the study. Also, the female Sprague-Dawley rats are commonly used in models of abdominal radiation therapy.

Animals were allowed approximately one week of adjustment time prior to the start of radiation therapy. All animals had free access to food and water during the entire study period. Those animals on the Vitamin A and elemental diets had free access to these diets in unlimited quantities.

Anesthesia

Anesthesia consisted of a mixture of ketamine hydrochloride 100mg per ml, xylazine 20mg per ml, acepromazine maleate 10mg per ml. This was given subcutaneously in a ratio of 1.5mg ketamine to 1.5mg xylazine to 0.5mg of acepromazine. The intraperitoneal route was not utilized because of the necrotizing effects of ketamine.[61] Studies have shown that this agent can cause significant muscle necrosis. To avoid these potential side effects, the mixture was given subcutaneously in the nape of the neck. Initial test dosing with this agent did result in mild dermal necrosis, however diluting the mixture 3 to 1

with normal saline prevented such further problems. Dose consisted of .08mls of non-diluted mixture or 1.5 to 1.8 mls of the diluted mixture. Supplemental dosages of anesthesia were given as necessary.

This mixture was chosen because it affords analgesia/anesthesia and muscle relaxation. The animals had to be completely still for approximately 20 minutes during radiation therapy and muscle rigidity would have interfered with placement beneath the radio-protective lead shielding. Since its duration of action lasted from 1/2 to 1 hour, it also provided anesthesia necessary for blood drawing prior to and after completion of the radiation therapy. It served as the only source of anesthesia during the entire experiment.

Radiation Therapy

Dose of radiation consisted of 900 rads per animal once a week for 5 weeks for a total of 4500 rads. This dose was chosen to mimic that of pelvic radiation used in humans. No data in the literature exists using multiple dose radiation therapy similar to our model. Therefore, there was no literature to compare the severity of the injury created by this dose. Personal communication[67] revealed that 4500 rads to the rat pelvis should create a reproducible, moderately severe radiation injury. To confirm this, a preliminary

portion of the study was undertaken, and once established, the continuation with Part II proceeded.

Radiation therapy was performed in the radiation suite of the Small Animal Clinic on the Michigan State University campus. The procedure occurred under the supervision of Dr. Ulreh Mostofsky, DVM. The radiation was delivered by a Siemen's Stabilipan II. Target to skin distance (TSD) was 76 centimeters. Settings were 300 kv at 12 MA which had a half value layer (HVL) of 1.6mm of copper. Dose consisted of 45 rads per minute to a field of 3.5 x 3.5 cm^2 per animal. Each animal was treated for 20 minutes which yielded 900 rads per animal. This dose was confirmed by using a Vitaveen Radocon III dosometer with a 550-5 probe. Accuracy of this unit is ± 2 %. Animals were shielded beneath 1/4 inch lead shielding effectively limiting all radiation to the exposed areas. Animals were radiated 8 at a time. Two groups of 4 animals were placed on specially designed platforms that were rotated to account for dispersion or uneven distribution. Upon completion of this, XRT animals were placed into bedding lined containers for transport back to the ULAR Building. Animals were wrapped with towels to prevent hypothermia when appropriate.

Experimental Groups - Part I and Part II

Experimental groups in Part I consisted of control animals and animals receiving the above described radiation therapy. Control animals underwent similar doses of anesthesia and had blood drawn at identical times as that of the treatment animals. Animals were housed in the same room within the ULAR Building and had free access to food and water. Weight was recorded on a weekly basis at the time of radiation therapy. Part II consisted of 5 experimental groups, a control and treatment group as described above and then a group of animals receiving radiation and then receiving either 1) sodium meclofenamate; 2) an elemental diet; or 3) a Vitamin A enhanced diet. The dose of sodium meclofenamate consisted of 5mgs per kilogram as described previously. This was prepared by opening the capsule form and suspending the product with methyl- cellulose. This was then sequentially mixed with Gerber Dutch Apple Dessert (Gerber Products, Fremont, MI) to assure uniform mixing. Mixture consisted of 250mg of sodium meclofenamate per 125ml of baby food. This was then delivered by direct oral placement of the mixture by syringe and soft plastic catheter. Measurement of the delivery of dose was done by the direct observation of the amount delivered through the syringe. All animals took this dose readily and it consisted of .5 to .6 mls of the 2mg per cc sodium

meclofenamate baby food mixture. This was given to all animals on a daily a.m. basis. The mixture was refrigerated between usage and remixed before each delivery. The elemental diet group received ad libum amounts of a specifically prepared hydrolyzed casein diet. This was prepared by Purina Mills Inc. This diet was placed on cage racks in similar fashion to the standard diet. All animals took this diet freely without any noticeable complication.

The Vitamin A supplemented diet consisted of a standard rat chow with additional Vitamin A. This was mixed in an amount of 662 international units per kilogram. Of note, this diet was air dried to prevent damage to the heat labile Vitamin A. This resulted in somewhat of a texture difference in the diet. However, the components were the same. All animals took this diet freely without difficulty. All experimental groups were housed together within the same room under constant temperature and humidity and controlled lighting and ventilatory conditions.

Blood draw

During Part I of this study, animals had blood drawn at multiple time points before and after radiation therapy. Both control and treatment animals had blood drawn prior to radiation but after onset of anesthesia, blood was drawn at 1/2 hour 1, 2 and 4 hours after radiation. This was an attempt to delineate the effect of time on the levels of

both TNF and IL-6. Once it was determined that the peak of TNF occurred between 30 minutes and 1 hour (see results), animals in Part II had blood drawn in approximately 30 minutes to 45 minutes post radiation therapy.

Blood was drawn via a tail artery in all animals. Supplemental anesthesia was given as necessary during the more prolonged portion of this experiment. In Part II, the initial dose of anesthesia was frequently sufficient for the single blood draw. Blood was withdrawn using a 25 gauge needle and 1ml syringe and placed immediately into Autostep 10 cc serum separator tubes. These were chilled on ice prior to centrifugation. Approximately 1/2ml of blood was drawn at each time point. During Part I of the study, supplemental subcutaneous saline equal to the amount of blood drawn (2.5mls) was given at the completion of the first blood draw. This was given subcutaneous in the nape of the neck. During Part II, this procedure was not necessary.

Blood was then centrifuged at 3600 rpm on a Sorvall rt600b refrigerated centrifuge (DuPont, Willmington, Delaware), for 15 minutes. Serum was immediately removed using sterile technique and placed in Sarstedt disposable storage vials and stored at -80° until performance of the bioassay.

ANALYTICAL TECHNIQUE

MICROSPHERES

The microsphere technique used in Part I and Part II of this study is essentially the same as described in the introductory section of this experiment. The referenced sample method was used in all animals under identical circumstances. Animals were fasted overnight and anesthetized with the non-dilute mixture of Ketamine, Xylazine and Acepromazine subcutaneously. This was delivered by subcutaneous injection. When an appropriate level of anesthesia was obtained, the animals were shaved on the neck, groin bilaterally and midline abdomen. The animal was then secured and the right femoral artery was dissected free under direct observation. This was secured with several 5-0 silk free ties and then cannulated with a section of PE-50 polyethylene tubing. The tubing tip was modified to allow for easy access into the artery. This was advanced several millimeters to allow for a secure placement using several 5-0 silk ties placed around both the artery and catheter. The tubing was connected to a 3ml syringe via 20 gauge stub adapter. The syringe was flushed with minimal amounts of normal saline to insure patency. The syringe was then placed into a Harvard 22 infusion withdrawal pump (Harvard Apparatus, South Natick, MA) and manually checked for patency and flow into the artery. Once secured, attention was then turned towards the right carotid artery.

Again, under direct visualization the right common carotid artery was dissected free and secured with silk sutures. Cannulation was performed in a similar fashion as that of the right femoral artery using P-50 tubing. The tubing was connected to a 20 gauge stub adapter and then to a pressure transducer (Abbott Labs Chicago, Illinois) and to a Hewlett Packard neonatal pressure monitoring system (Hewlett Packard Corp., W. Germany). Confirmation of the operation of the system was by audio signal of the heart rate and visual inspection of the blood pressure trace. Catheter was flushed with small amounts of saline to insure patency. The catheter was advanced into the left ventricle by monitoring the blood pressure trace on the monitor screen. Confirmation of placement into the left ventricle was made by observing the typical left ventricular pressure tracing and the marked decrease in diastolic pressure. Once in the left ventricle the animal was allowed to stabilize for approximately one minute and to observe for any alteration in blood pressure.

If no alteration in blood pressure had occurred, the withdrawal pump was then started. Confirmation of withdrawal was made by visually observing the blood within the right femoral artery P-50 tubing. After ten seconds of withdrawal, the microspheres were injected over a 20 second period. Upon completion of this, the withdrawal pump continued for exactly 45 seconds for a total of 75 seconds.

Animals were observed for several minutes after completion of the withdrawal. The left ventricular catheter was flushed with approximately 0.5 ml of saline to allow for patency and to continue blood pressure monitoring. After this period of observation, 1 cc of blood was withdrawn from the left ventricular catheter for determination of TNF and IL-6 values. Immediately after this, the animals were euthanized with an intercardiac injection of saturated potassium chloride.

Microsphere injection consisted of 0.2 ml of commercially prepared solution of 15 micron strontium 85 microspheres (10mCI/g). Each 0.2 ml contained an average of 1.8×10^5 microspheres at approximately 5×10^5 counts per minute. This was equal to 4 MCI per injection. The solution contained the microspheres as described, 10% dextran and 0.05% of Tween 80 solution. The microsphere solution was vortexed for 3 to 5 minutes prior to withdrawal for injection. Gama counter determination of counts per minute was made just prior to injection for each animal. This was to insure adequate mixing and prevent settling of the microspheres in solution.

Organs were harvested after euthanasia. Midline laparotomy was made and right and left kidney, 2 centimeters of distal colon, 2 centimeters of terminal ileum, and 2 centimeters of proximal jejunum were then harvested. Also, right and left lung samples were taken from the chest.

Kidneys had the renal capsule removed while the bowel specimens have their luminal contents extruded. All tissues were pressed gently between gauze pads to removed excess blood. Intestines had remaining portions of their mesentery stripped away. One half centimeter segments were taken from the bowel to be used for histopathologic analysis. All specimens were weighed and placed into Biovials (Beckman Corp. Philadelphia, PA) for determination of counts per minute. Calculations were performed by the previously described formula of counts per minutes tissue x reference withdrawal rate divided by reference blood value. This result was then divided by weight and grams to obtain the blood flow data as millimeters per minute per gram of tissue. Cardiac output was also calculated for all animals by the previously described formula of counts per minute in injectate minus counts per minute remaining in the syringe times reference blood withdrawal rate divided by reference blood counts per minute. This data was recorded in pre-prepared data sheets.

ANALYTIC TECHNIQUE

HISTOLOGIC GRADING SCALE

Specimens collected for histopathologic evaluation were placed in 10% formalin. Specimens were pinned under minimal stress to reproduce physiologic conditions. They were fixed in formalin for at least 48 hours in all cases. Slides were then prepared in a standard fashion using hematoxylin and eosin stains and examined using standard light microscopy.

A grading scale was devised using the standard parameters of inflammation based on previous studies in the literature.[15] A separate grading scale was established for both the colon and small bowel. (see table) This was done to include histologic differences between the organs. Epithelial height was measured using an eye piece micrometer. Also, specimens were coded and the viewer was blinded to the nature of the treatment groups.

The scale was devised to have a normal tissue produce a value close to or equal to zero. One possible flaw is that increased upper epithelial height could register the same score as a decrease epithelial height. The individual grading points were also examined and this did not prove to be the case. For example, if a treatment groups grading score was increased, it was due to a uniform increase in mucosal height and not an increase and decrease. This case held true for all groups and for all time points.

Figure 1

HISTOGRADE - SMALL BOWEL

1) _____ Increased Epithelial Height (0=Normal, 1, 2)

2) _____ Decreased Epithelial Height (0=Normal, 1, 2)

3) _____ Increased Mucosal Height (0=Normal, 1, 2)

4) _____ Decreased Mucosal Height (0=Normal, 1, 2)

5) _____ Increased Villous Length (0=Normal, 1, 2)

6) _____ Decreased Villous Length (0=Normal, 1, 2)

7) _____ Mitotic Index Increased (0=Normal, 1, 2)

8) _____ Mitotic Index Decreased (0=Normal, 1, 2)

9) _____ Shelving of villi (0=Normal, 1, 2, 3)

10) _____ Submucosal edema & fibrosis (0=Normal, 1, 2, 3)

11) _____ Muscular edema & fibrosis (0=Normal, 1, 2, 3)

12) _____ Vascular Abnormalities (0=Normal, 1, 2, 3)

HISTOLOGIC GRADING SCALE - SMALL BOWEL

Figure 2

HISTOGRADE - COLON

1) _____ Increased Mucosa (0=NL, 1, 2)

2) _____ Decreased mucosa (0=NL, 1, 2)

3) _____ Epithelial Increased (0=NL, 1, 2)

4) _____ Epithelial Decreased (0=NL, 1, 2)

5) _____ Goblet Cell Increased (0=NL, 1, 2)

6) _____ Goblet Cell Decreased (0=NL, 1, 2)

7) _____ Mitotic Index Increased (0=NL, 1, 2)

8) _____ Mitotic Index Decreased (0=NL, 1, 2)

9) _____ Crypt Concentration (0=NL, 1, 2)

10) _____ Colitis Cystica Profunda (0=NL, 1, 2, 3)

11) _____ Submucosal edema & fibrosis (0=NL, 1, 2, 3)

12) _____ Muscular edema & fibrosis (0=NL, 1, 2, 3)

13) _____ Vascular Abnormalities (0=NL, 1, 2, 3)

HISTOLOGIC GRADING SCALE - COLON

Statistical Analysis

Statistical analysis consisted of a students t-test and a one way ANOVA with post-hoc comparison by Fischer's least significant difference. This was used for all parametric data including values of TNF, IL-6 and blood flow. Kurskal Wallis analysis was used for the non-parametric data of the histologic grade. Post-hoc comparisons were made by the multiple comparison test. These statistical functions were run using the Number Cruncher Statistical System statistical package (Kaysville, Utah) and significance was set at $p<0.05$ for all calculations.

Tumor Necrosis Factor and Interleukin 6

Cytokine Assay

TNF activity was determined by the method of Espevich and Nissen-Meyer.[62] This is a cytotoxicity assay utilizing WEHI 164 clone 13 with varying dilutions of serum. The assay has been previously described and is well utilized in our laboratory at MSU.[63] In short, the assay consists of utilizing duplicate samples and increasing dilutions of RPMI-1640 medium with 10% fetal calf serum. Dilutions utilized in this study were 1:1 to 1:128. These were then mixed with 100 microliters of the WEHI 164 cells at a concentration of 5×10^5 cells per ml. These were incubated overnight and after 24 hours were mixed with 20 microliters of 3(4,5 dimethylethiazol 2yl)2,5 diphenyltetrazolium

bromide (5 micrograms per ml of RPMI-164) (Sigma Chemical Company). This is incorporated into the remaining viable WEHI cells during incubation. After 4 hours of incubation the reaction is stopped using 100 microliters of isopropyl alcohol. This dissolves the dark blue crystals formed during the previous reaction. After overnight incubation at room temperature, absorbance is read at 620 nanometers on a Biotech EL311 automated plate reader (Biotech, Inc. Winoski, Vermont). The activity of TNF was determined in units per ml by extrapolation off the standard curve done with simultaneous known concentrations of TNF standard. (20 units per ml Angen Biologicals, Thousand Oaks, California)

Interleukin 6 was performed in an identical fashion except for the cell line utilized was of the 7 TDI.[64] Cells were mixed to serum samples with identical dilutions as that of TNF. Samples were incubated for 72 hours and then treated with 3(4,5 dimethylthiazol 2yl)2,5 diphenyltetrazolium bromide and isopropyl alcohol as previously described. Values were determined from a standard curve in a similar fashion to TNF.

Results

Phase I TNF

Results from this portion of the study are contained within the chart below. The values of TNF are represented as units per ml. Time points are prior to the start of XRT,

1/2 hour, 1, 2, and 4 hours post radiation. Control animals received anesthesia only and had blood drawn at identical time points. Each animal had blood drawn at each week of the experiment. There is an N of 5 in each group and results are expressed as the mean ± the standard error of the mean. Peak values are contained within the highlighted boxes.

CHART 1

TNF - Part I Week 1-5

	Group	Pre	1/2 hr	1 hour	2 hours	4 hours
Week 1	XRT	0	4.5±1.41	1.44±6.02	0.91±0.547	.212±.23
	Control	.12±.119	6.41±0.99	4.87±3	0	0.047±0.047
Week 2	XRT	0	668±209	408±211	0	37±37
	Control	0	524±236	388±336	67±75	0
Week 3	XRT	6.75±5.5	16.5±6.17*	11.0±5.22	0	3.75±3.75
	Control	0	3.2±3.31	1.50±1.75	0	0
Week 4	XRT	0	5.2±3.31	0	0	0
	Control	0.20±.22	0.40±4.47	.175±.17	0	0
Week 5	XRT	0	7.35±4.88*	4.41±2.50	0	0
	Control	0	0.299±.317	.787±.662	0	0

Values of TNF in units/ml. Highlighted boxes are peak values.

The data reveals that the peak TNF occurred between 1/2 hour and 1 hour for both control and treatment groups. The asterisk denotes where the comparison between peaks was statistically significant ($p<.05$ students t-test). This occurred at week 3 and 5 only. Week 4's difference did not reach statistical significance despite being 13 time the

control value. This was due to large error variance. This data is represented graphically in graphs 1 through 5 (page 89-93).

To compare the data week to week we must take into the account the variability of the cellular assay. To accomplish this, we have listed the data as percent of control in the graph below. Here the peak in the treatment group (XRT only) is compared to the peak in the control group for that week. Results are represented as percent of control and are unitless.

CHART 2

TNF - Part I - Percent of Non-Radiated Controls

	Week 1	Week 2	Week 3	Week 4	Week 5
XRT % of non-radiated control	178	127	515*	1300	971*

*asterisk denotes significant value

There is a statistically significant difference between the means with the peak of TNF occurring at week 4 at 13 times the control value. This is represented graphically in graph 7 on page 95. There is also a statistically significant difference between the control and the treatment group peaks occurring at week 3 and 5 respectively.

Part I: Interleukin 6

The results of the investigation of Interleukin 6 in this portion of the study are contained within the chart below. The values of Interleukin 6 are represented as units per ml. Time points are the same TNF at prior to radiation 1/2, 1, 2, and 4 hours post radiation therapy. Control animals received anesthesia only and had blood drawn at identical times. There is an N of 5 in each group and results are expressed as the mean value ± the standard error of the mean. Peak values are contained within the highlighted box.

CHART 3

IL-6 - Part I Week 1-5

	Group	Pre	1/2 hr	1 hour	2 hours	4 hours
Week 1	XRT	751±650	586±387	6562±201	17580±5146	27912±12908
	Control	8525±158	24467±186	4843±38	0	0
Week 2	XRT	37±27	26.3±6.75	47.0±17	24.2±2.2	36.1±6.85
	Control	12.1±13.5	588±301	102±52	193.7±87	984±326
Week 3	XRT	1008±507	8690±7338	2174±116	0	1700±616
	Control	619±534	14±2.12	36210±1520	3532±1100	1514±1693
Week 4	XRT	1171±788	1293±361	279±155	16569±7920	3116±2698
	Control	829±413	9543±978	16690±737	4676±2455	16243±11272
Week 5	XRT	190±83	264±13	328±12	318±25	346±6.5
	Control	284±28	286±37	258±20.3	259±20	227±42

Values are units/ml. Highlighted boxes are peak values.

The chart reveals that the peak for Interleukin 6 was inconsistent for each group. It appears that the peaks for Interleukin 6 in the radiated group occurred more frequently after two hours post XRT when compared to controls. However, this did not reach statistical significance. These values are expressed graphically in graphs 8 through 12 on page 96-100.

When expressed as percent control in the chart below there was no statistically significance between the means and the radiated group and no difference between control and radiated values. The results contained in this chart as represented as percent of control. (see graph 13 on page 101.)

CHART 4

IL-6 - Part I Percent of Non-Radiated Controls

	Week 1	Week 2	Week 3	Week 4	Week 5
XRT % non-radiated control	114	11.8	238.7	99	121

Comparison of the results between TNF and Interleukin 6 are summarized in graph 13 on page 101.

Results Part I - Blood Flow

In this section, the results for the blood flow portion of Phase I are reported. The chart contains data for the blood flow comparison between radiated and control groups. This is presented for colon, ileum and jejunum. All values are in ml per minute per gram of tissue and reported as mean ± the standard error of the mean.

CHART 5

BLOOD FLOW - PART I

	Ileum	Jejunum	Colon
Control	0.717±0.81	1.465±0.195	0.517±0.079
XRT	1.25±0.203*	1.54±0.257	0.447±0.042

Units are ml/min/gram tissue
Asterisk denotes statistically significant difference

The asterisk denotes a statistically significant difference between control values in the radiated group for ileal blood flow only. There was a statistically significant difference in blood flow at 1 week post XRT in the ileum. The radiated group had a higher blood flow at 1 week post XRT and a decreased blood flow at 5 weeks post XRT. There is no difference between blood flow in the jejunum and colon when compared between treatment and control groups at either time point.

Part II

The results from this portion of the investigation are contained with the chart below. All values are expressed as units per ml and reported as means ± standard error of the means. Group A represents animals that received radiation only, Group B represents animals that received radiation and treatment with sodium meclofenamate, Group C consists of

animals with radiation and an elemental diet, Group D is the control and Group E is radiation therapy and Vitamin A. There is an N of 8 in each group.

CHART 6

TNF - Part II Week 1-5 and 1 + 5 Weeks Post XRT

	Group A	Group B	Group C	Group D	Group E
Week 1	7.54±3.09	3.62±0.97	3.03±1.61	2.9±0.97	1.39±0.657
Week 2	0.09±0.03	0.12±0.055	0.148±0.050	0.09±0.03	0.19±0.038
Week 3	1.04±0.91	0.404±0.37	8.13±5.99	0.525± 0.428	3.98±2.96
Week 4*	1162±199.6	1246±406.7	4.84±3.21	932±349	8.9±6.12
Week 5*	2.89±1.70	0.042± 0.0399	2.63±1.57	0.219± 0.166	7.99±4.58
1 Week Post	272±184.6	161±133	8.56±2.83	340±790	8.03±1.91
5 Weeks Post	257±188	644±283	100±38.5	82.±36.2	692±213

Legend

Group A - radiation only
Group B - radiation + sodium meclofenamate
Group C - radiation + an elemental diet
Group D - no radiation
Group E - radiation and Vitamin A

There is a statistically significant difference between the means of these groups at week 4 and 5 (asterisk).

When analyzed as percent control the data has the identical statistical results with a difference between the means at week 4 and 5. These results are contained within the chart below.

CHART 7

TNF - Part II Week 1-5 + 1 + 5 Weeks Post XRT
Percent of Control

	Group A	Group B	Group C	Group D	Group E
Week 1	254	103	104	100	41.8
Week 2	94	127	156	100	201
Week 3*	198	75	1476*	100	268
Week 4*	124*	133	931*	100	1651*
Week 5*	1318*	19	1202*	100	3655*
1 Week Post	95	56	51.2	100	47.75
5 Weeks Post	313	785	121	100	1012

Values are expressed as % control

Legend
Group A - radiation only
Group B - radiation + sodium meclofenamate
Group C - radiation + an elemental diet
Group D - no radiation
Group E - radiation and Vitamin A

Within week 4 and 5, Group A, C & E are greater than the control while Group B is not statistically different than that of the control value. This is represented graphically in graph 14 page 102.

When expressed individually as group versus control as percent control the data is easier to interpret. This is contained within the chart below and represented in graphs 16 through 19 on pages 104 through 107.

CHART 8

TNF - Part II Week 1-5 + 1 + 5 Weeks Post XRT
Percent Control

% Control	Week 1	Week 2	Week 3	Week 4	Week 5	1 Week Post	5 Weeks Post
Group A*	254	94	198	124	1318	95	313
Group B*	103.7	113.2	75	133	19.5	56	785
Group C	104	156	1476	931	1202	51	121
Group E*	41.8	201	268.4	1651.7	3655.8	47.7	1012

Values are expressed as % control

Legend
Group A - radiation only
Group B - radiation + sodium meclofenamate
Group C - radiation + an elemental diet
Group D - no radiation
Group E - radiation and Vitamin A

Group A had its peak at week 5 as did Group E. Group B's peak occurred at 5 weeks after the completion of radiation therapy. Groups A, B, and E had a statistically significant difference between the means. The asterisk within the graphs show where values are statistically greater than that of control. Group C, despite large values, did not reach statistical significance due to large error variances. See graphs 15-18 on pages 103-106.

Part II - Interleukin 6

The results from this portion of the study are contained within the chart below. All values are means ± the standard error of the mean error expressed as units per ml. Group A represents radiated animals only, Group B radiation and sodium meclofenamate, Group C radiation and

elemental diet, Group D controls and Group E radiation and Vitamin A.

CHART 9

IL-6 - Part II Week 1-5 + 1 + 5 Weeks Post XRT

	Group A	Group B	Group C	Group D	Group E
Week 1	5.46±1.79	3.72±1.44	5.73±1.11	4.00± 0.636	2.19±0.97
Week 2	10.4±2.62	9.5±2.2	11.8±2.39	12.3±3.02	12.1±1.63
Week 3*	2.28±1.47*	0.255±0.19	3.62±2.37*	3.657± 0.310	1.10±0.465 *
Week 4	3.8±1.34	1.71±1.46	38.7±1.71	4.6±0.91	10.5±8.23
Week 5*	1.97±0.93*	1.50±0.53	38.3±20.9*	0.20±0.13	9.2±4.65*
1 Week Post	2.87±1.90	1.97±0.66	7.64±3.74	16.12± 14.6	0.715± 0.355
5 Weeks Post	2.69±0.78	3.31±1.12	1.6±0.567	6.9±3.74	6.28±3.7

Values are units/ml

Legend

Group A - radiation only
Group B - radiation + sodium meclofenamate
Group C - radiation + an elemental diet
Group D - no radiation
Group E - radiation and Vitamin A

Asterisk denotes that there is a statistically significant difference between the means at weeks 3 and 5. When analyzed as percent control, the statistical difference remains the same. At week 3 the elemental diet group shows a higher value than the control group, with all other groups being equal. At week 5, Groups A, C and E were also greater than control. See graph 19 on page 107.

CHART 10

IL-6 Part II - Week 1-5 + 1 + 5 Weeks Post XRT
Percent Control

	Group A	Group B	Group C	Group D	Group E
Week 1	136	93	143	100	54.7
Week 2	82	77	95	100	99
Week 3*	347	43	485	100	167
Week 4	82	37	841	100	229
Week 5*	753	584	14154	100	2939
1 Week Post	58	38	47	100	4.12
5 Weeks Post	37	48	23	100	214

Values are expressed as % control

Legend
Group A - radiation only
Group B - radiation + sodium meclofenamate
Group C - radiation + an elemental diet
Group D - no radiation
Group E - radiation and Vitamin A

When expressed individually as groups versus control as percent control, the data is easier to interpret. This is contained within the chart below and graphs 20 through 24 on page 108-112.

CHART 11

IL-6 Part II - Week 1-5 + 1 + 5 Weeks Post XRT
Percent Control

	Week 1	Week 2	Week 3	Week 4	Week 5	1 Week Post	5 Weeks Post
Group A	149	82.6	347	82.5	753	58	37
Group B	93	77.5	49.5	37	584	38.6	48.26
Group C	143	95.7	485	841	14154	47	23
Group E	54	99	167	229	2939	4.12	214

Values are expressed as % control

Legend
Group A - radiation only
Group B - radiation + sodium meclofenamate
Group C - radiation + an elemental diet
Group D - no radiation
Group E - radiation and Vitamin A

The asterisk denotes that there is a statistically significant difference between the means within Groups A, B, C and E. All of these groups have their peak occurring at week 5 with a statistically significant difference between the peaks and the control values.

Part II - Blood Flow

The results of the blood flow portion are contained within the charts below. All results were expressed as the mean ± the standard error of the mean and are in units of ml per minute per gram of tissue. There is an N of 8 in each group.

CHART 12

BLOOD FLOW 1 WEEK POST XRT

Blood Flow Ileum *	Group A	Group B	Group C	Group D	Group E
	1.25± 0.14*	1.06± 0.117	0.772± 0.104	0.767± 0.183	1.21± 0.118*

Blood Flow Jejunum	Group A	Group B	Group C	Group D	Group E
	1.54± 0.06	1.60± 0.23	0.99± 0.116	1.39± 0.224	1.80± 0.278

Blood Flow Colon *	Group A	Group B	Group C	Group D	Group E
	0.448± 0.029	0.482± 0.058	427± 0.056	0.614± 0.107	0.770± 0.085*

All values are in ml/min/gram tissue
Asterisk denotes a significant difference compared to unradiated control (Group D)

Legend

Group A - radiation only
Group B - radiation + sodium meclofenamate
Group C - radiation + an elemental diet
Group D - no radiation
Group E - radiation and Vitamin A

There was a significant difference between the means for the colon and ileal blood flow at one week post radiation. Post-hoc comparative analysis revealed for ileal blood flow that Groups A and E had a higher blood flow than that of control (denoted by asterisk). Also, Groups B and C

did not differ from the control values. For the colon, the significant difference was that Group E (Vitamin A) was greater than the other groups. All other groups within that treatment group were equal to control. Jejunum blood flow had no significant differences between the different groups with respect to blood flow. These are depicted graphically in graphs 24 through 27 on pages 112-115.

At 5 weeks post radiation therapy the data on blood flow is contained within the chart below. The units are the same as represented for one week post radiation therapy.

CHART 13

BLOOD FLOW 5 WEEKS POST XRT

	Group A	Group B	Group C	Group D	Group E
Blood Flow Ileum *	.423± 0.033*	.7135± 0.131	1.02± 0.305	.7175± 0.06	1.46± 0.292*

	Group A	Group B	Group C	Group D	Group E
Blood Flow Jejunum	1.30± .17	1.40± .227	1.19± 0.244	1.18± 0.07	1.92± 0.247

	Group A	Group B	Group C	Group D	Group E
Blood Flow Colon	.552± 0.155	.519± 0.101	.753± 0.154	.567± 0.127	0.771± 0.217*

All values are ml/min/gram tissue
Asterisk denotes a significant difference compared to unradiated control (Group D)

Legend

Group A - radiation only
Group B - radiation + sodium meclofenamate
Group C - radiation + an elemental diet
Group D - no radiation
Group E - radiation and Vitamin A

There was a statistically significant difference between the means. Post-hoc analysis of ileal blood flow showed that Group A was less than control and that Group E (Vitamin A) was greater than that of control. Post-hoc analysis of jejunum blood flow revealed that the Vitamin A Group had a statistically significant increase in blood flow as compared to control (denoted by asterisk). There was no difference in colonic blood flow for any of the groups at week 5. See graphs 28 and 29 on pages 116-117.

Part II

Histopathology

Results of the histopathologic grading scale at 1 and 5 weeks post radiation are contained below. All values are unitless and represent the median value of the individual grading scale. There is an N of 8 in each group. Group A represent the animals that received radiation only, Group B radiation and sodium meclofenamate, Group C radiation and an elemental diet, Group D are unradiated controls and Group E represents animals that received Vitamin A and radiation.

The asterisk denotes that there is a statistically significant difference between the median values within the ileum at 1 week post radiation. Post-hoc analysis reveals the Groups A and E are elevated when compared to Groups B, C and D. There is no difference between Groups A and E, and Groups B, C, and D respectively. See graph 30, page 118.

At 5 weeks post radiation there is no statistically significant difference between the means within this group. However, post-hoc analysis reveals that Group A is significantly less than Groups C, D, and E. The difference between Groups A and B was of borderline statistical significance with a p value = .07. There was no difference between the values between Groups B, C, D, and E. See graph 31, page 119.

At 5 weeks post radiation within the ileum there is a statistically significant difference between the means. Within this group, post-hoc analysis revealed that Group D was less than Groups A, B, C and E. There was no statistically significant difference between Groups A, B, C, and E. With respect to the colon there again is a statistically significant difference within the means. Within this chart Group A shows a statistically significant increase over that of Groups B, C, D and E by post-hoc analysis. There is no difference between the median values with respect to groups B, C, D, and E. See graph 32 and 33, page 120 and 121.

Discussion

General Discussion of Radiation Injury

Radiation therapy remains a mainstay in all forms of malignancy. Up to 50% of patients with carcinoma will require radiation therapy as either an adjunct or primary therapy during the course of their disease.[4] As previously stated, the incidence of visceral carcinoma in the United States was approximately 1.1 million in 1990.[5] Patients with pelvic malignancies constitute a significant percentage of this population. Colorectal and gynecologic malignancies make up at least 230,000 individuals.[5] While radiation therapy does have proven benefit in these disease states, up to 10 to 15% of patients will develop side effects.[6] These includes bleeding, proctitis, cystitis, fistula, obstruction and malabsorption. When one considers the number of people involved, potential ways to block radiation side effects would have great potential to decrease human suffering.

A significant body of literature exists describing the effects of radiation injury on the intestine in a variety of animal species. Much work has been done in both the mouse and the rat as well as the human. Work by Hubman[14] and Black[15] have defined the LD50 for rectal obstruction in the rat and delineated the histopathologic changes that occur with this injury. Also, attempts to block these affects have been met with varying degrees of success. Vitamin A has been shown to increase the radiation necessary to affect

an LD50 in mice the LD50 for total body radiation in mice[35] and increase the anastomotic bursting strength in rats[36]. This is believed to be due to Vitamin A's immuno-stimulatory effect.

Sodium meclofenamate has also been shown to decrease the radiation side effects in both humans and animals. Ambrus et al[44,45], showed a decrease in radiation esophagitis in primates while other authors have shown a decrease in chronic cystitis in patients undergoing pelvic XRT. This mechanism is believed to be due to sodium meclofenamate's activity as an immune modulator and its inhibitory effect on prostaglandins and leukotriennes.

Elemental diet benefit is believed to be due to better local nutrition to the injured intestinal mucosa. Also, by decreasing pancreatico-biliary secretions, further injury to an already damaged mucosa is prevented. Benefit of an elemental diet has been shown in both animal and clinical studies[46,47,49]. Whether the benefits of an elemental diet are from sum of its constituents or an individual factor remains a question. Studies by Souba et al[51], have shown that glutamine alone can mimic the effects produced with an elemental diet.

The role of cytokines in abdominal and total body radiation is not as clearly defined. Contradictory results exist showing Interleukin I and TNF to be both radioprotective and deleterious in a model of total

abdominal radiation[28,29,30]. Superimposed on this model is a significant hematopoietic effects seen with total abdominal and total body radiation. To our knowledge, no work exists on documenting the relationship of radiation therapy to Interleukin 6 or the relationship of any of the cytokines to sequential radiation injury.

Very little literature exists to document the effects of radiation therapy on blood flow. Some evidence does exist to suggests that Vitamin A may increase blood flow by increasing angiogenesis in acute inflammation. No data exists for the other radio protectants utilized in this study.

Part I - Cytokines

The results in graphs 1 through 5 show that TNF values appear to peak between 1/2 hour and 1 hour after the completion of radiation therapy. This occurs in both the treatment and control groups. The values for the treatment group peaks are larger than that of control at each week, but did not reach statistical significance until week 3 and 5. Studies have shown that large single doses of abdominal radiation cause bacterial translocation with positive lymph node cultures at 8 hours post radiation.[63] This rise in TNF may represent the early stages of this event. Attempts to measure portal vein endotoxin or quantitative culture of portal vein blood may further delineate this etiology.

Also, the increase in TNF may be in response to the more non-specific inflammatory stimulus caused by the radiation itself. Lastly, the stimulus of the injection of anesthesia did cause an increase in TNF. This is evidenced by the increase in TNF seen with the control group.

Most significant from this data is the relative rise in TNF when portrayed as percent of control values (see graph 1). Here we see the TNF values increased significantly on a week to week basis. This suggests that there is a progressive or maturation of the response to pelvic radiation therapy. This may be explained by a combination of the first two events previously described. A non-specific increase in TNF may occur in the early stages of pelvic radiation (week one and two) followed by a more significant increase in TNF as the severity of injury increases. The progressive injury to the intestinal mucosa may lead to a break in the integrity of the mucosal barrier. This would result in bacterial translocation which would result in an additive increase in TNF. This could be the explanation for the large values occurring at week 4 and 5. (graph 6 on page 94.) The decrease in week 5 is somewhat confusing however. This may represent a statistical or biological variation. This may also represent a further change in the pathophysiology of this sequential injury. TNF may be inhibited or exhausted as the injury progresses.

No data exists to substantiate this theory and at present, this remains speculative.

Interleukin 6

The results of this portion of the study are harder to interpret. The peak value between control and treatment groups showed no consistent pattern. The peaks within the treatment group appeared to occur at later time points but this did not hold up to statistical analysis. Furthermore, the peak values of the control animals were higher than that of treatment animals in several instances. (See graphs 8-13) The results are somewhat confusing considering the relationship of TNF to Interleukin 6. TNF is known to increase Interleukin 6 and indeed Interleukin 6 is directly responsible for inducing the acute phase reaction seen with an inflammatory response. One would suppose that the increase in TNF seen in the previous sections would result in a mirror image of Interleukin 6. This is not the case however. A possibility is the anesthesia itself. Ketamine is a fairly caustic agent known to induce muscle necrosis when injected.[61] We avoided this complication by injecting the agent subcutaneously. However, some element of dermal necrosis may have occurred. This may have induced an inflammatory response that we were unable to distinguish from that induced by the radiation therapy. Other possibilities remain that of the direct effect of one of the

other anesthetic agents. However, these seem unlikely and undocumented.

Blood Flow

Results of the blood flow portion are summarized in charts 24 through 29. Again, these results are largely as anticipated. The histopathologic documentation of acute inflammation in the ileum at 1 week post XRT is the substantiation for the increase in blood flow seen in this organ. This is consistent with the increased vascular permeability, increased cellular infiltrate and increased angiogenesis which are all part of the acute inflammatory response. The blood flow to the colon did increase but was not statistically significant. This is somewhat surprising as the colon was subjected to the same insult as the terminal ileum. This may represent different responses by different organs. This may be due to different amounts of lymphoid tissue contained within these organs. The ileum contains more lymphoid tissue than the colon which may affect the degree of acute inflammation and subsequent alteration in blood flow. It also may represent different distances from the x-ray source. The colon is more dorsal of the terminal ileum. However, this seems unlikely because this distance is only several millimeters in the supine anesthetized rat. The exact mechanism for this difference remains unclear.

Jejunal blood flow did not change with radiation therapy, and this is also as expected. The jejunum was out of the field of radiation and remained shielded at all times during therapy. Therefore, it was not subjected to direct radiation therapy.

Part II - Group A

Perhaps the best way to evaluate the data is by comparing the individual treatment groups versus that of control. This is depicted in graphs 16 through 19 on page 104 through 107. As you can see, Group A follows a similar curve as that of the treatment group in Phase I. In fact, the peak values as percent control are nearly identical - 1300 for Phase I and 1318 for Phase II. As indicated, there was a statistically significant difference between these points at week 5. For this portion of the study, the difference between the means within Group A were border line ($p=.055$). This reflects the difficulty with the large error variance inherent in this bioassay.

The significance of this graph is that it so closely replicates that seen in Part I. This further substantiates the data and increases the credibility of the possible mechanism for the increase in TNF. Also, the peak in this portion of the experiment occurred in week 5 as opposed to week 4 in Part I. This may help explain the decrease seen in week 5 of Part I as biological variation.

The graph also has two other time points not measured in Part I. These include 1 week and 5 weeks after the completion of radiation therapy. The values of TNF at one week post radiation decreased to equal that of control but then increase again at week 5. This is a pattern that is evident in all the treatment groups (graph 15). The mechanism for this remains unclear. Two possibilities exist. First, is that TNF production is being affected at 1 week post radiation similar to that proposed for week 5 of the treatment group in Phase I. More likely, it is that TNF has returned to base line values at 1 week post radiation. Further maturation of the radiation injury may be occurring at 5 weeks post XRT. This may represent a switch from an acute to a chronic form of injury. TNF is known to be a modulator of chronic disease and the data may reflect this. Also, no elevation of IL-6 was seen at this time. This explanation is only speculative and there is no data to support this. Investigation of later time points may help to substantiate this.

The results of Interleukin 6 in Group A are contained in graph 20. These are different than that of Part I. Here, Interleukin 6 shows a significant increase with radiation and peaks at week 5. While these results are more consistent with the literature and our anticipated findings, the contradiction remains unsettling. Further confirmation

of these results needs to be obtained before direct correlation can be made.

Blood flow for Group A follows that of Part I also. There is a significant difference between blood flow in the terminal ileum at 1 week and at 5 weeks post radiation. At 1 week blood flow was increased greater than unradiated controls. This is presumably due to the mechanisms previously described. At 5 weeks post radiation blood flow is decreased compared to unradiated controls. This undoubtedly represents the beginning of more chronic side effects of radiation therapy. These are typified by progressive vasculitis and collagen deposition. This is further substantiated by the histologic grading for Group A (see graphs 28 and 29 on page 116 and 117). The histologic changes from XRT persist in both weeks 1 and 5 after radiation with a significant difference.

Colonic blood flow is again similar to Part I showing no change at weeks 1 and 5. The results are somewhat unexpected given the results obtained from the ileum. Again, this may represent differences between organs, difference of technique, or different response to anesthesia by the colon. However, histologic grade at weeks 1 and 5 did show a significant difference between Group A and control. This certainly confirms that the colon is being affected by radiation but does not explain the difference in the blood flow results.

In summary, pelvic radiation only (Group A) appears to increase a significant effect on TNF. This causes an elevation that is progressive and increases until the end of the treatment period. Pelvic radiation causes significant changes in terminal ileal blood flow but does not affect blood flow to the colon. There is evidence of significant histologic injury and this difference in apparent in both the terminal ileum and colon at 1 and 5 weeks after completion of pelvic radiation.

Group B

Group B represents animals who received radiation and oral sodium meclofenamate. The data is contained within graph 16. The values of TNF do not significantly differ from control values except at 5 weeks post XRT. This most likely does not represent an effect of sodium meclofenamate as the agent was stopped at the completion of radiation five weeks earlier. The elevation TNF at 5 weeks post radiation is similar to that seen in Group A and the other treatment groups at this time point.

The results seen in this graph most likely represent sodium meclofenamates role as an immune modulator. Sodium meclofenamate is known to decrease both prostaglandin and leukotriene production.[33] It also has been shown to decrease the inflammatory response seen with radiation therapy[47]. The results appear to be similar in our model. While

prostaglandin levels were not directly measured in this study, previous works have shown that a decrease in prostaglandins have yielded an increase in the TNF and Interleukin 1.[66] Also, decreasing prostaglandins decreased Interleukin 6. This decrease in prostaglandins was accomplished by the use of ibuprofen. Our results seem to contradict this however. We saw a decrease in TNF without change in IL-6 by an agent that is known to decrease prostaglandins. The difference may be due to sodium meclofenamate itself, its effects on leukotriene production, or the differences between an invitro and an invivo model.

Sodium meclofenamate did not decrease Interleukin 6 in this part of the study when compared to controls. Looking at the chart and graph there is a marked increase in Interleukin 6 at week 5. However, a distressing observation is that Interleukin 6 appears to decrease during weeks 1 through 4 and is decreased at 1 and 5 weeks post radiation therapy when compared to controls. Whether the values of Interleukin 6 at week 4 are accurately representative of the events occurring is unclear. However, the pattern does seem consistent with the histologic and blood flow results described below. Keeping in mind the contradiction between the results of Phase I and Phase II, one must reserve any further conclusion until replication of data occurs.

Sodium meclofenamate's affects on blood flow to the terminal ileum appear to ameliorate the effects of radiation

therapy. This is represented in graphs 24 and 25 on page 112 and 113. As mentioned, a significant increase in blood flow occurred with radiation at 1 week post XRT and a decrease at 5 post XRT. There is no statistically significant difference between sodium meclofenamate and the control groups 1 week and 5 weeks post XRT. No effect of sodium meclofenamate was seen on colonic blood flow at either time point. The affect on the ileum may represent sodium meclofenamate's affect on prostaglandins and their resultant effect on inflammation and blood flow. By blocking the inflammatory response and decreasing prostaglandin production, sodium meclofenamate appears to decrease blood flow at week 1 and prevent further decrease at week 5.

The benefit of sodium meclofenamate is further substantiated by the histologic data (graph 28). This reveals no difference between Group B and controls at 1 week when looking at the ileum. This beneficial effect on the ileum is lost at 5 weeks post radiation (graph 29). The beneficial of sodium meclofenamate appears to persist in the colon for both times points as the histologic grade for the sodium meclofenamate is equal to control during these time points (graphs 30 and 31). However, at one week post XRT the difference between XRT and sodium meclofenamate did not reach statistical significance ($p=0.07$). In light of these

statistical findings it is safe to say the sodium meclofenamate does show some benefit to the colon.

To summarize, sodium meclofenamate appears to block the effects of radiation on TNF production but the Interleukin 6 results are inconsistent. It also eliminates the blood flow changes within the ileum at 1 and 5 weeks and the histologic changes and 1 week post radiation. This benefit is lost at 5 weeks post radiation. Sodium meclofenamate appears to have no affect on colonic blood flow at 1 and 5 weeks post radiation but has a persistent histologic benefit at both 1 and 5 weeks.

Group C

Group C represents animals who underwent radiation and received an elemental diet. The TNF data is contained within graph 17. The slope of this graph and the values of TNF represented as percent control are very similar to that of Group A. The peak in this group occurred at week 3 and remained elevated until week 5. The peak value was 1476 as compared to 1318 in Group A. There is no difference between these values. The results reflect the fact that elemental diet has no direct effect on the inflammatory response. This is further substantiated by the Interleukin 6 results. Here the results show a very similar shape between Group A and Group C.

One can postulate that elemental diet should decrease the secondary inflammation of radiation therapy by preventing injury to the brush border. This is presumed to be due to elemental diets ability to decrease pancreatico-biliary secretions and provide increased localized nutrition. This is supported by the results of blood flow and histology. Ileal blood flow showed no difference between the control group and the elemental diet group but both groups were significantly different than the radiation only animals. This occurred at both 1 and 5 weeks post radiation. No effect of an elemental diet was seen within the colon at either week. Histologic grade in the ileum revealed that an elemental diet reversed the effects of radiation at 1 week but this benefit was lost at 5 weeks.

The effect of elemental diet on blood flow can be due to several mechanisms. One is due to the decreased inflammatory response as previously described. The other is the lack of trophic effects on the terminal ileum. The terminal ileum is significantly down stream from the jejunum where most of the elemental diet is absorbed. Therefore, the lack of lumenal contents may have the effect of decreasing blood flow via lack of a trophic stimulus. Simple diversion of luminal contents has been shown to have similar results. This theory is not supported by the histologic data which did show a benefit to both colon and ileum in 1 week and the colon at 5 weeks. This suggests

that the elemental diet is decreasing the inflammatory response within the ileum at 1 week and with the colon at 1 and 5 weeks. In the ileum this is most likely due to the previously described mechanisms. The mechanisms within the colon remain unclear.

The colonic effects remain an interesting point. Since most of the elemental diets potential benefits are believed to be by modifying small bowel response, how does this benefit the colon? One theory exists that better overall nutrition with an elemental diet benefits the entire GI tract. Also, the harmful effects of pancreatico biliary secretions may also influence the colon. But then why is this effect lost on the ileum at 5 weeks but not lost on the colon at this time point? Is this due to a different response within the organs? These issues remain controversial and speculative at best.

To summarize, elemental diet appears to have no effect on the response to TNF and Interleukin 6 when compared to that of Group A. However, it did eliminate the specific changes in blood flow seen in the ileum at 1 and 5 weeks post radiation. It also reversed the histologic changes seen in the colon and 1 and 5 weeks and in the ileum at 1 week.

Group E

Group E represents animals that received radiation therapy and a Vitamin A supplemented diet. The TNF and IL-6 data are contained within charts 18 and 23. The graph of TNF shows a similar pattern to that of group A but with a marked increase in TNF. While Group A peaked at a value of 1318, the Group E peaked at a value of 3,655 (percent control). This was the highest value of TNF recorded in this study. Also, the pattern for Interleukin 6 is similar to that of Group A but without as large an increase. The results correlate with Vitamin A's function as an immune stimulator. Jurin[38] noted an increase in cellularity in mesenteric lymph nodes in animals treated with Vitamin A. Other studies have shown better survival and better colonic anastomotic healing in rats treated with radiation and Vitamin A.[37] Levenson[36] supported this and stressed the importance of the oral route on intake. While no data exists showing Vitamin A directly increases TNF or Interleukin 6, this is no doubt that there is an overall increase in the inflammatory response.

The effects upon histologic grade by Vitamin A were similar to that of the other treatment groups. Vitamin A decreased the histologic changes in the colon at both 1 and 5 weeks post radiation. The benefit to the ileum was not evident at 1 week and 5 weeks post radiation.

The most interesting effect of Vitamin A is with respect to that of blood flow. Vitamin A had a consistent increase in blood flow in all of the organs treated. Blood flow was increased in the ileum at both 1 and 5 weeks. This increase at week 1 mimicked that of the radiation only group despite a lower histologic score. Blood flow to the colon remained increased at 1 week even while the radiated group did not have a resultant increase. This effect was lost, however, at 5 weeks. Even in the jejunum, treatment with Vitamin A had an increase in blood flow. While blood flow was increased at one week, this did not reach statistical significance. This was significantly increased at 5 weeks. The mechanism behind this remains unclear. Some studies have speculated that an increase in blood flow is due to capillary ingrowth from an accelerated healing process. This has not been reported in the intestine or with any radiated tissues. Mesenteric lymph node hypercellularity has also been documented with Vitamin A treatment.[38] While this may cause a resultant increase in blood flow, it is unlikely in our study. Our specimens had their mesentery removed prior to processing. It may represent hyperplasia of the mucosal associated lymphoid tissue within the bowel wall. This appears to persist for at least 5 weeks post radiation therapy. Unfortunately, evaluation of this lymphoid tissue was not included in our grading scale, as this observation was not anticipated. It may be that this

increase in blood flow is due to an increase in inflammation. This may account for the similarity in the ileum between groups A and E with respect to the grading scale.

In summary, it appears that Vitamin A increases TNF and Interleukin 6 while still providing histologic benefit to the colon. It also markedly increased blood flow in both the colon, ileum and jejunum which overcame the decrease in ileal blood flow seen at 5 weeks.

Summary and Conclusions

In summary, this investigation has created a successful model of pelvic radiation therapy and has delineated significant insight into its pathophysiology. We have shown that TNF increases sequentially with the progression of therapy and Interleukin 6 appears to follow this pattern. Blood flow to the colon and jejunum are not affected by this but blood flow to the ileum is increased at 1 week and decreased at 5 weeks post radiation therapy.

The agents used to prevent this injury all have potential benefit to the target organs. All agents decreased the histologic effects on the colon at 1 and 5 weeks and in the ileum at 1 week (except Vitamin A). This effect was not seen in the ileum at 5 weeks post radiation therapy. All agents (with the exception of Vitamin A) ameliorated the effects of radiation on ileal blood flow. Vitamin A stands alone in uniformly increasing blood flow to all organs studied. These agents appeared to do this independent of TNF and Interleukin 6 values. Sodium meclofenamate decreased TNF while Vitamin A increased TNF. Both agents had similar histologic results. Therefore, it appears that TNF values are not related to the histologic grade of injury. Both radiation therapy alone and Vitamin A increased blood flow when compared to controls. In general, all of these agents have potential benefit in our

experimental protocol. Further investigation is warranted to delineate the mechanisms behind their actions.

Conclusions

1. Forty-five hundred rads delivered as five weekly doses of 900 rads is a satisfactory model of pelvic radiation in the rat.
2. The above-mentioned protocol produces a reproducible intestinal injury with low morbidity and mortality.
3. Tumor necrosis factor alpha values peak at 30 minutes to one-half hour after each dose of radiation.
4. This peak is higher than that of unradiated controls.
5. These values increased significantly during the five week course of radiation therapy.
6. Interleukin-6 values did not show a significant difference between control values in the first part of the study.
7. Blood flow to the radiated terminal ileum increases when compared to controls when measured at one week post radiation injury. Blood flow to the radiated terminal ileum decreased when compared to unradiated controls at five weeks post radiation therapy.
8. Blood flow to the radiated colon did not increase at one and five weeks post radiation when compared to unradiated controls.

9. Blood flow to the unradiated jejunum did not increase at any time point measured when compared to controls.
10. Forty-five hundred rads delivered as a 900 rad dose over a course of five weeks created a quantifiable histologic injury.
11. Sodium meclofenamate prevented the increase in tumor necrosis factor seen with radiation.
12. Sodium meclofenamate prevented the changes in blood flow to the radiated terminal ileum seen at one and five weeks post radiation. It had no effect upon blood flow in the colon.
13. Sodium meclofenamate prevented the histologic changes in the radiated terminal ileum at one week post radiation but not at five week post radiation. It had a similar effect on the colon with the benefit persisting at one and five weeks post radiation.
14. Elemental diet prevented the changes in blood flow to the radiated terminal ileum at one and five weeks post radiation. It had no effects upon the colon.
15. Elemental diet prevented the histopathologic changes in the radiated terminal ileum but not five weeks post radiation therapy.
16. Elemental diet prevented the histologic changes at one and five weeks in the radiated colon.
17. Both an elemental diet and sodium meclofenamate had no effect on blood flow or histology of the jejunum.

18. Vitamin A increased blood flow to the radiated ileum and colon at one week post radiation and to the ileum only at five weeks post radiation. It even increased blood flow to the jejunum at five weeks post radiation.
19. Vitamin A did not prevent the histologic changes in the radiated terminal ileum at one and five weeks post radiation. It did benefit the colon by reducing these changes at one and five weeks post radiation.
20. Vitamin A significantly increased tumor necrosis values when comparing controls to unradiated controls. Tumor necrosis factor values were highest with treatment with Vitamin A.
21. Increasing tumor necrosis factor greater than that of radiated controls (via Vitamin A) did not have a histologic benefit in the ileum but did have benefit at the colon at one and five weeks post radiation.
22. Decreasing tumor necrosis factor significantly below radiated control values had histologic benefit in the terminal ileum at one week and in the colon at one and five weeks post radiation.

FLOW CHART - EXPERIMENTAL PROTOCOL

Part I

Week 1		Blood Draw		Blood Draw - Post			
	Anesthesia	pre	900 rads	30 min	1 hr	2 hr	4 hr
Treatment Group N = 10	→	→	→	→	→	→	→
Control Group N = 10	→	→		→	→	→	→

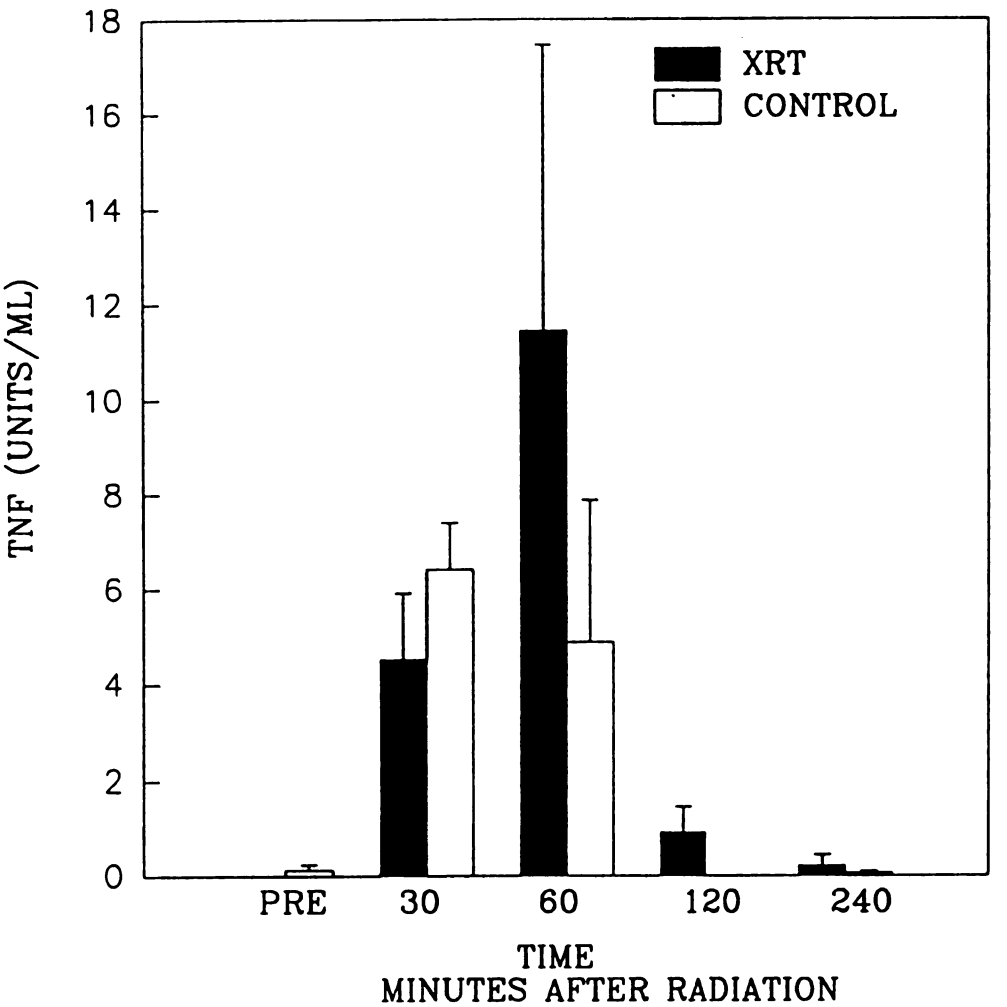
WEEK 2-5 - Same - After completion week 5 blood flow and histopath determinations made

Part II

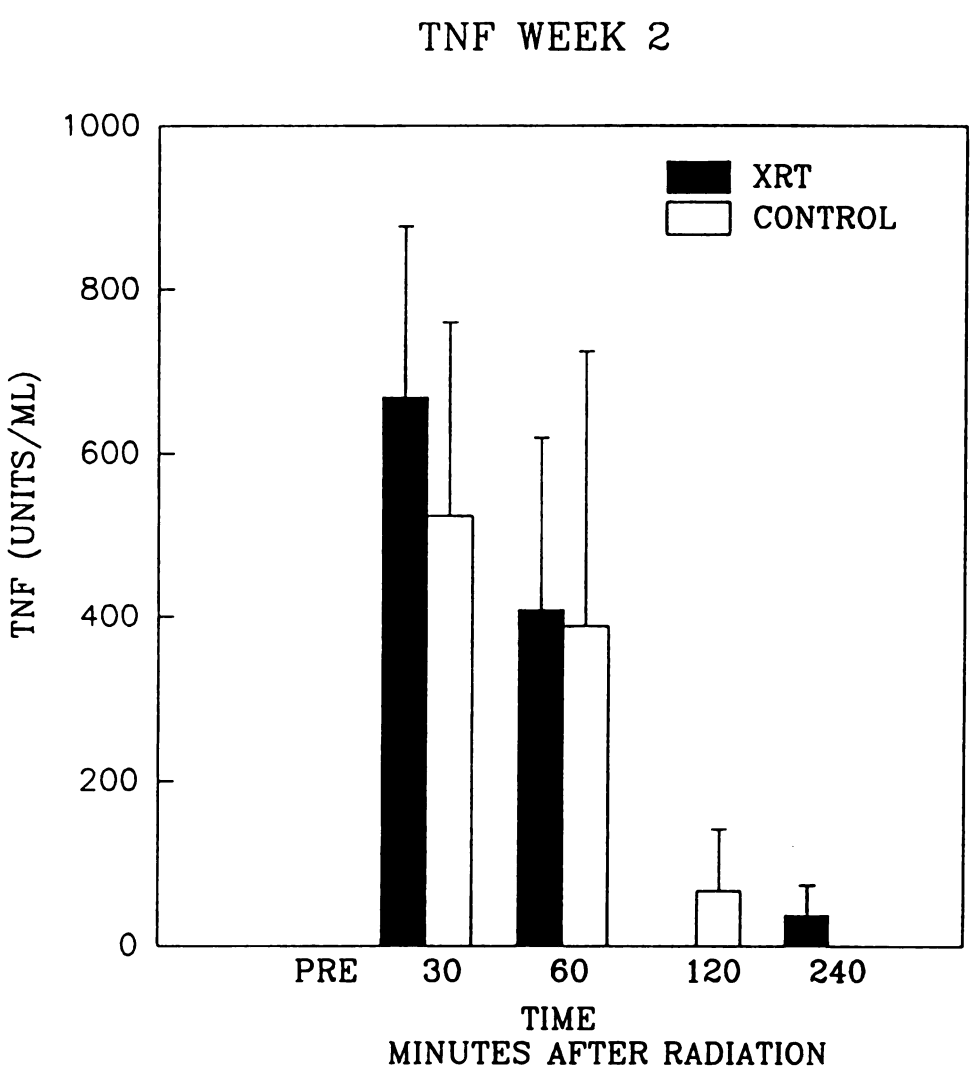
	WEEK 1	WEEK 2-5	COMPLETION WEEK 5	1 WEEK POST COMPLETION RADIATION	5 WEEKS POST COMPLETION OF RADIATION
	START TREATMENT & RADIATION	SAME	STOP TREATMENT STOP RADIATION	BLOOD FLOW, HISTOPATH, TNF, IL-6 (N=10)	BLOOD FLOW, HISTOPATH, TNF, IL-6 (N=10)
Group A (N=20) Radiation Only	→	→	→	→	→
Group B (N=20) Radiation and sodium meclofenamate	→	→	→	→	→
Group C (N=20) Radiation & elemental diet	→	→	→	→	→
Group D (N=20) Unradiated controls				→	→
Group E (N=20) Radiation & Vitamin A	→	→	→	→	→

GRAPH 1

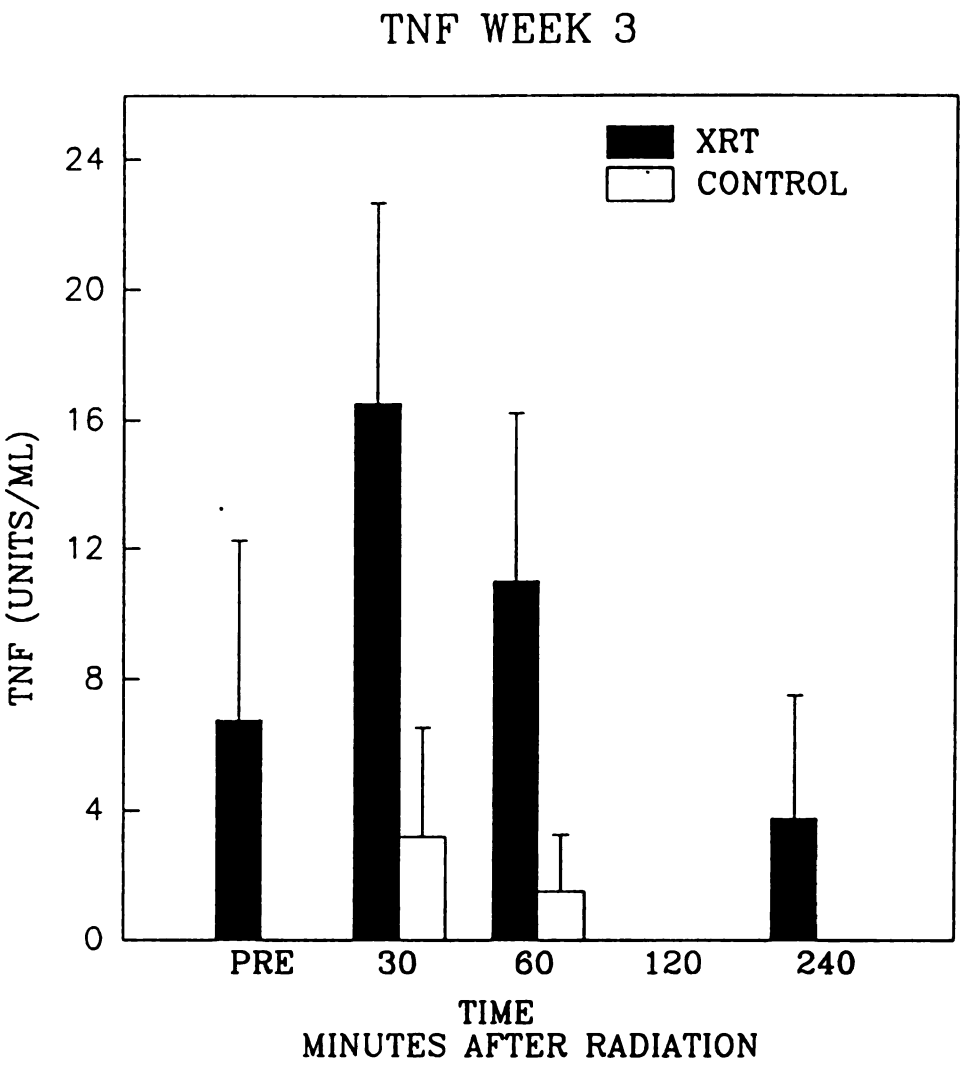
TNF WEEK 1



GRAPH 2

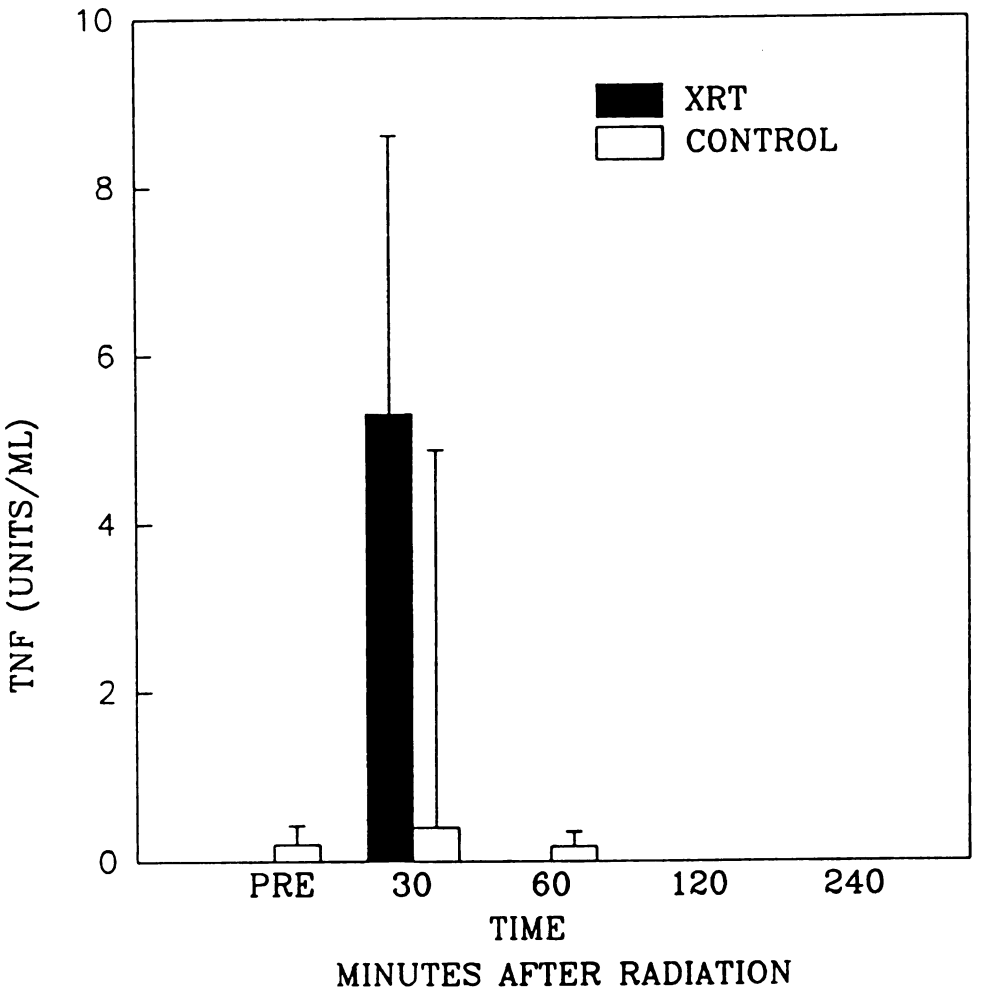

TNF WEEK 2
1000
800
600
400
200
0
TNF (UNITS/ML)
XRT
CONTROL
PRE
30
60
120
240
TIME
MINUTES AFTER RADIATION

GRAPH 3

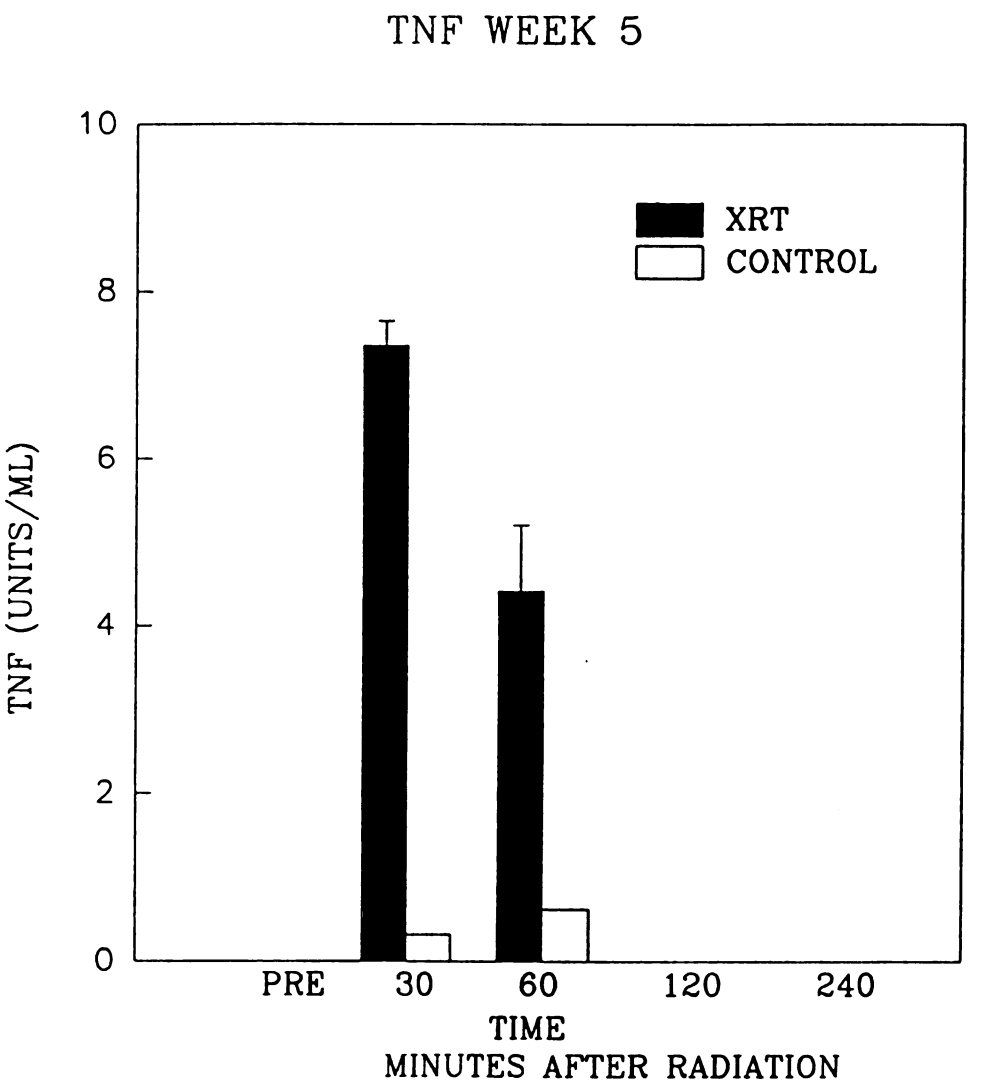

TNF WEEK 3
XRT
CONTROL
TNF (UNITS/ML)
0
4
8
12
16
20
24
PRE
30
60
120
240
TIME
MINUTES AFTER RADIATION

GRAPH 4

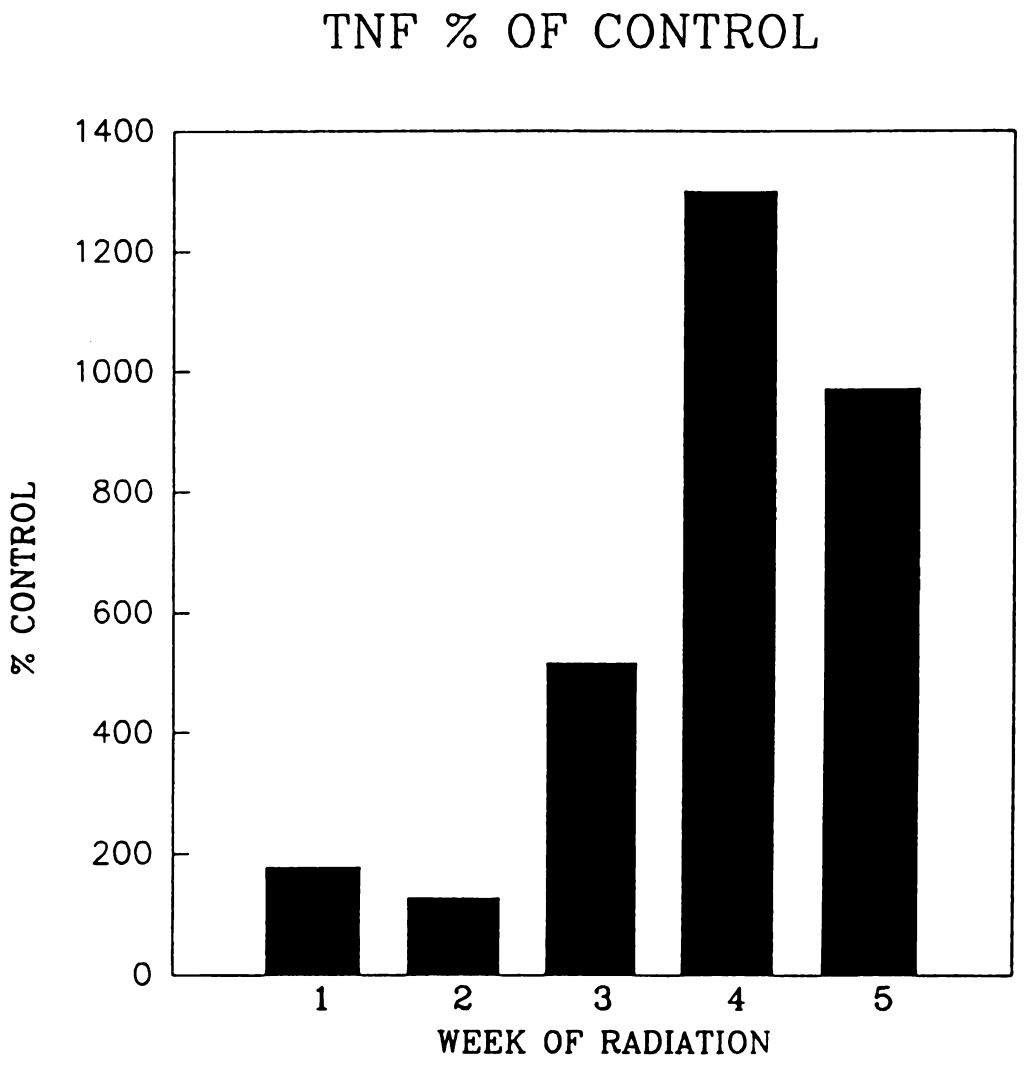
TNF WEEK 4



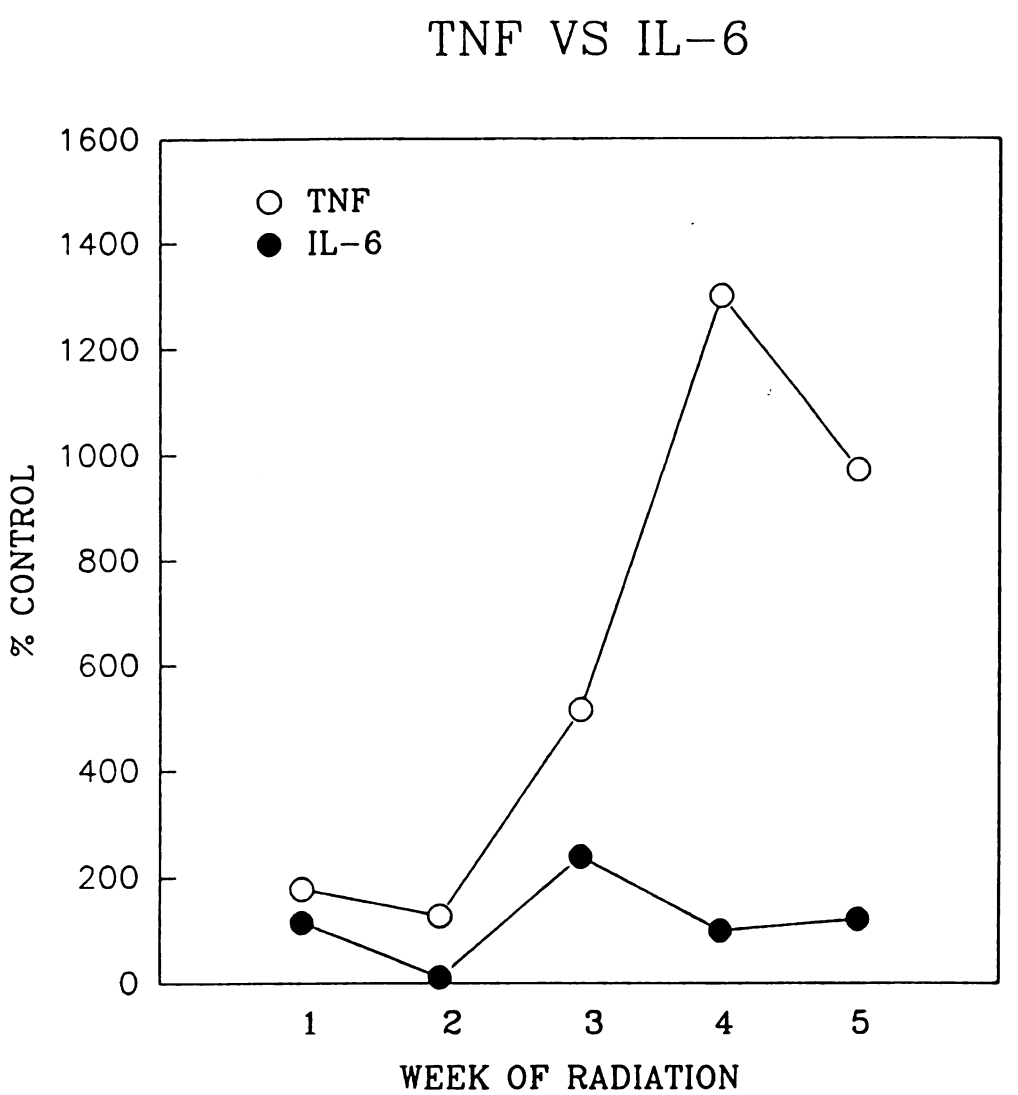
GRAPH 5


TNF WEEK 5
10
8
6
4
2
0
TNF (UNITS/ML)
XRT
CONTROL
PRE
30
60
120
240
TIME
MINUTES AFTER RADIATION

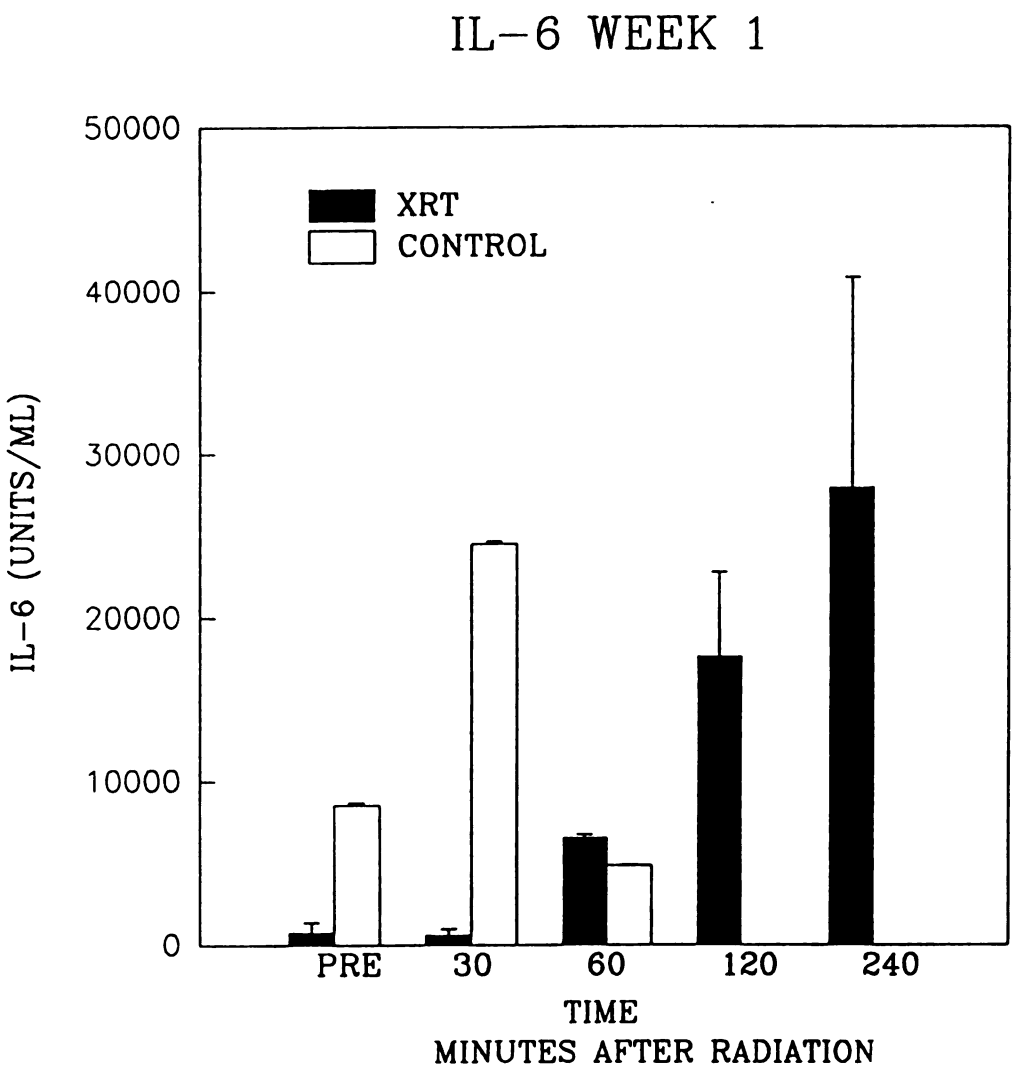
GRAPH 6


TNF % OF CONTROL
1400
1200
1000
800
600
400
200
0
% CONTROL
1
2
3
4
5
WEEK OF RADIATION

GRAPH 7

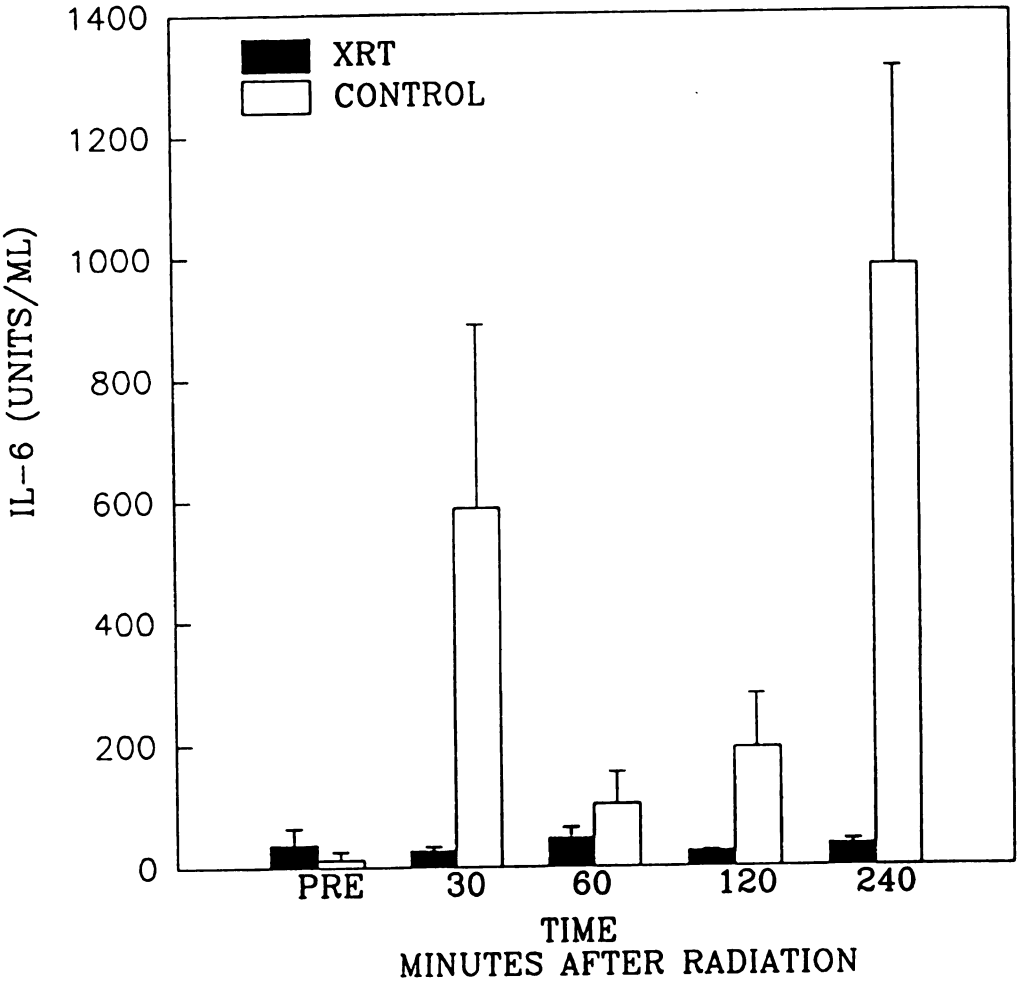

TNF VS IL-6
1600
1400
1200
1000
800
600
400
200
0
% CONTROL
TNF
IL-6
1
2
3
4
5
WEEK OF RADIATION

GRAPH 8


IL-6 WEEK 1
50000
40000
30000
20000
10000
0
IL-6 (UNITS/ML)
XRT
CONTROL
PRE
30
60
120
240
TIME
MINUTES AFTER RADIATION

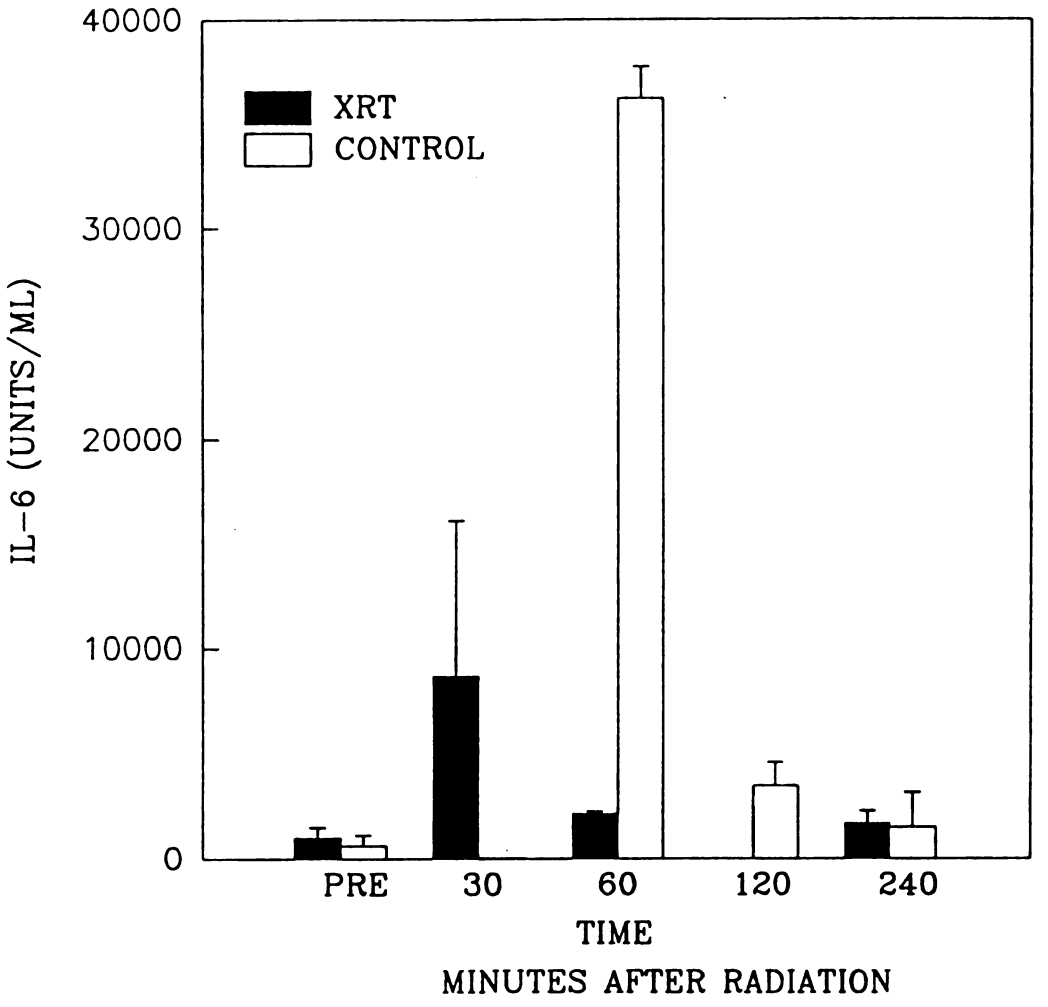
GRAPH 9

IL-6 WEEK 2



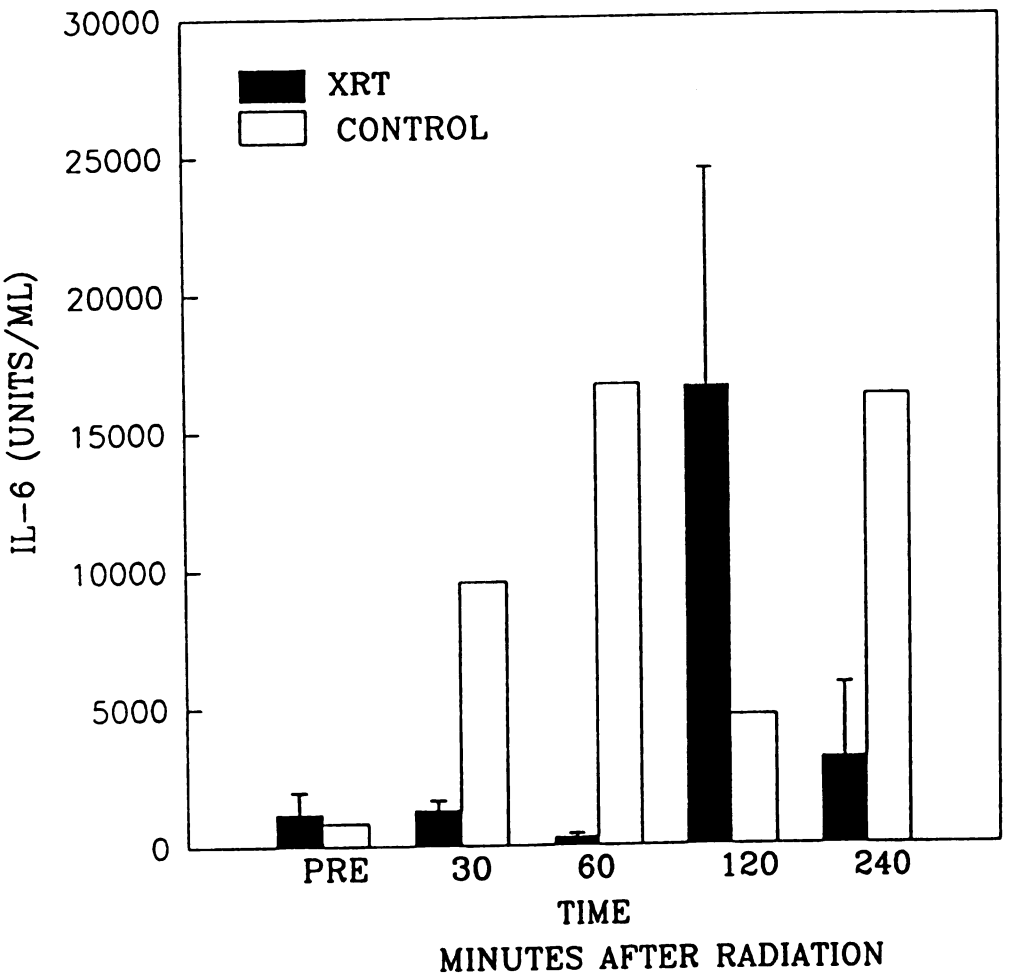
GRAPH 10

IL-6 WEEK 3



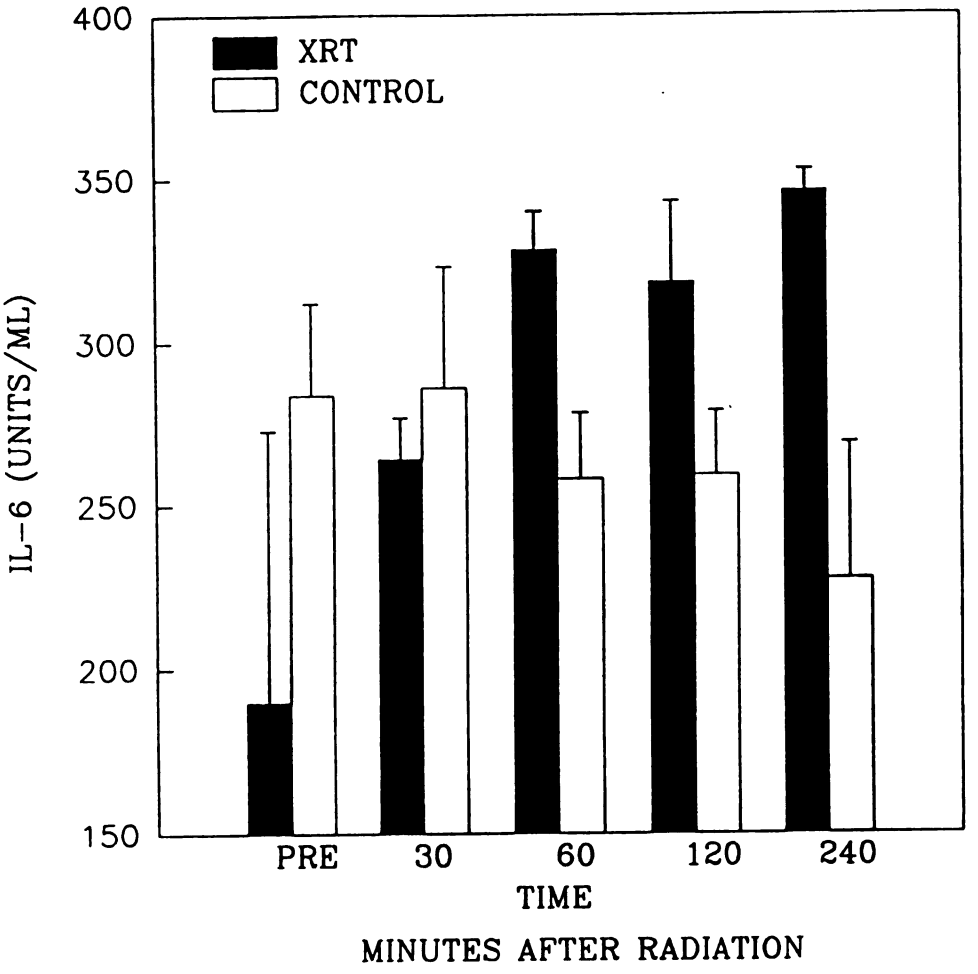
GRAPH 11

IL-6 WEEK 4

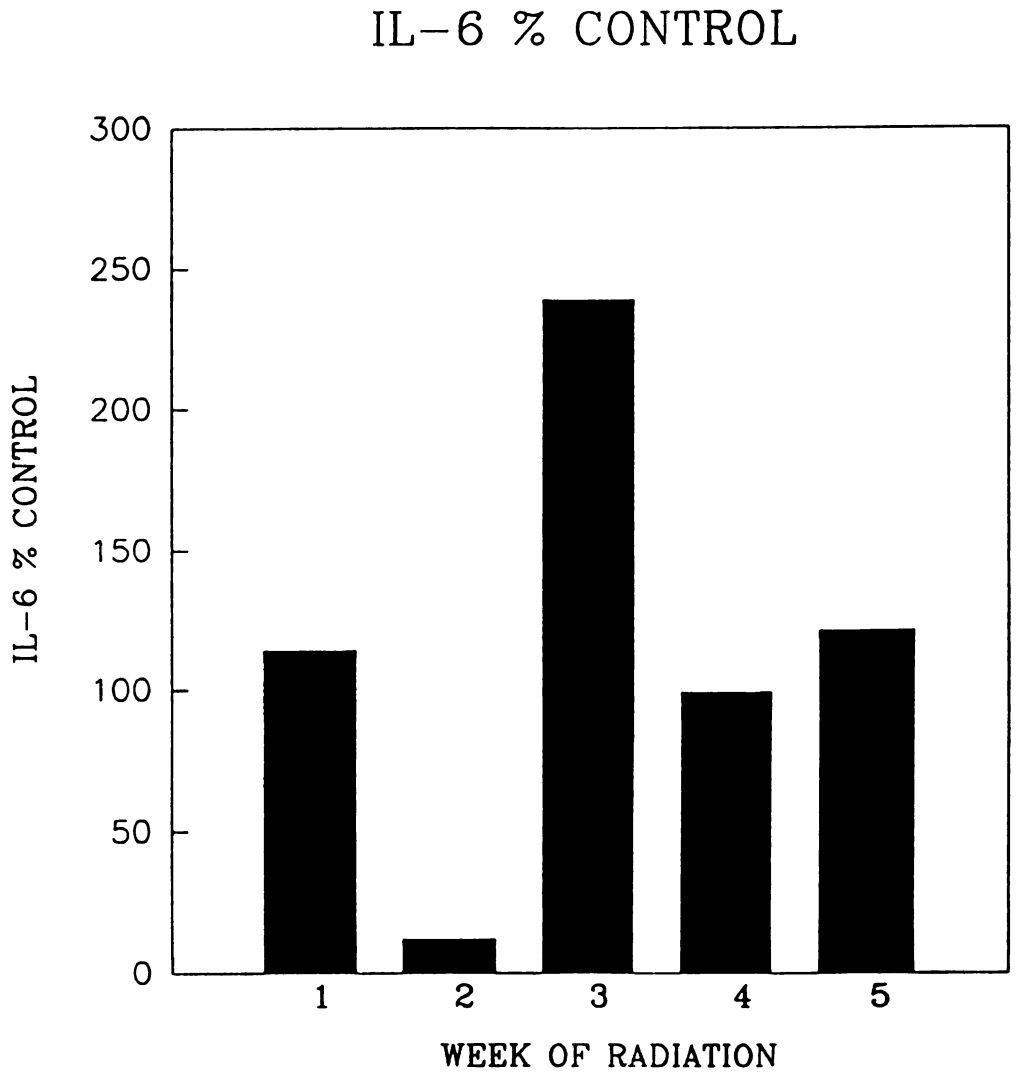


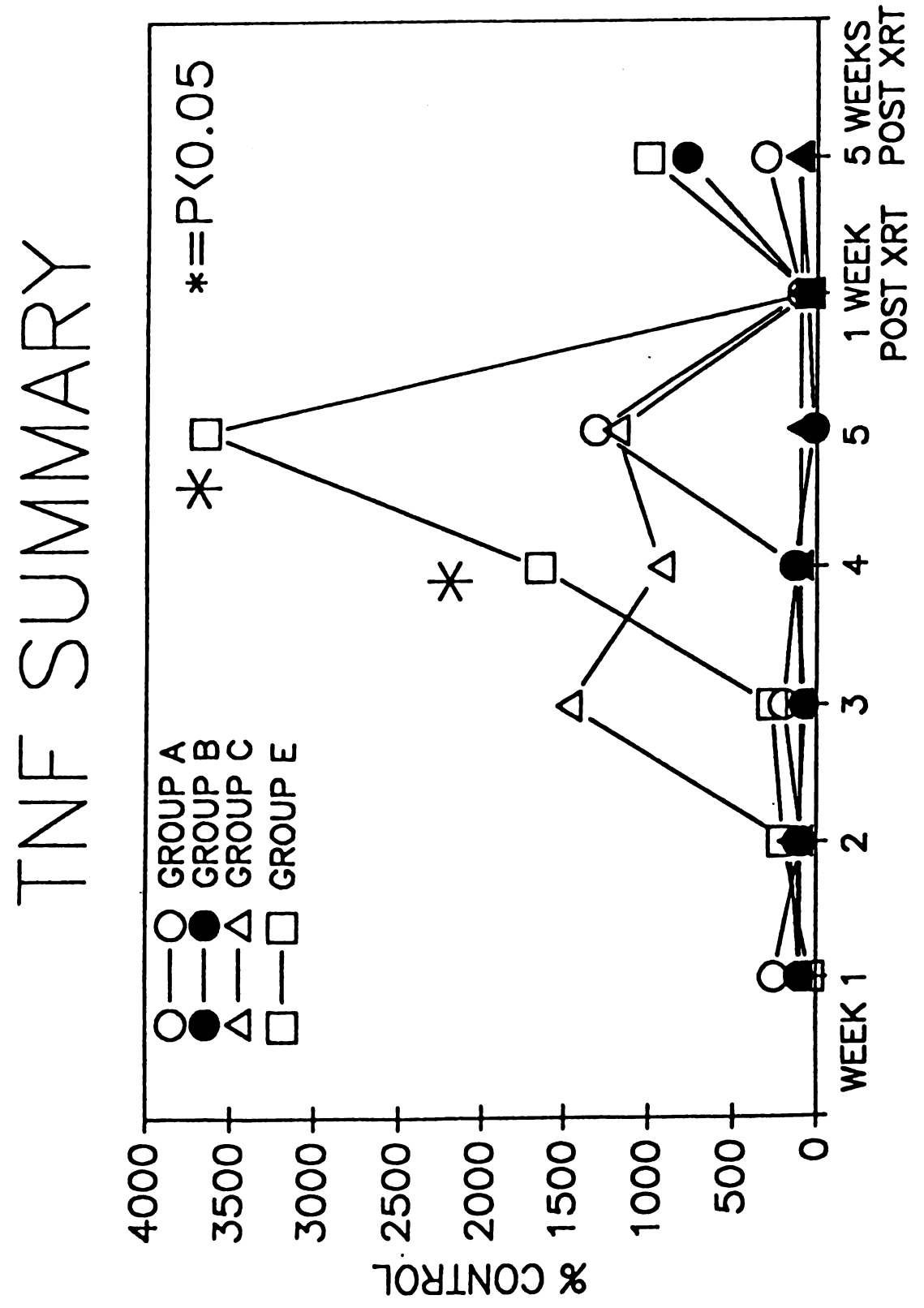
GRAPH 12

IL-6 WEEK 5

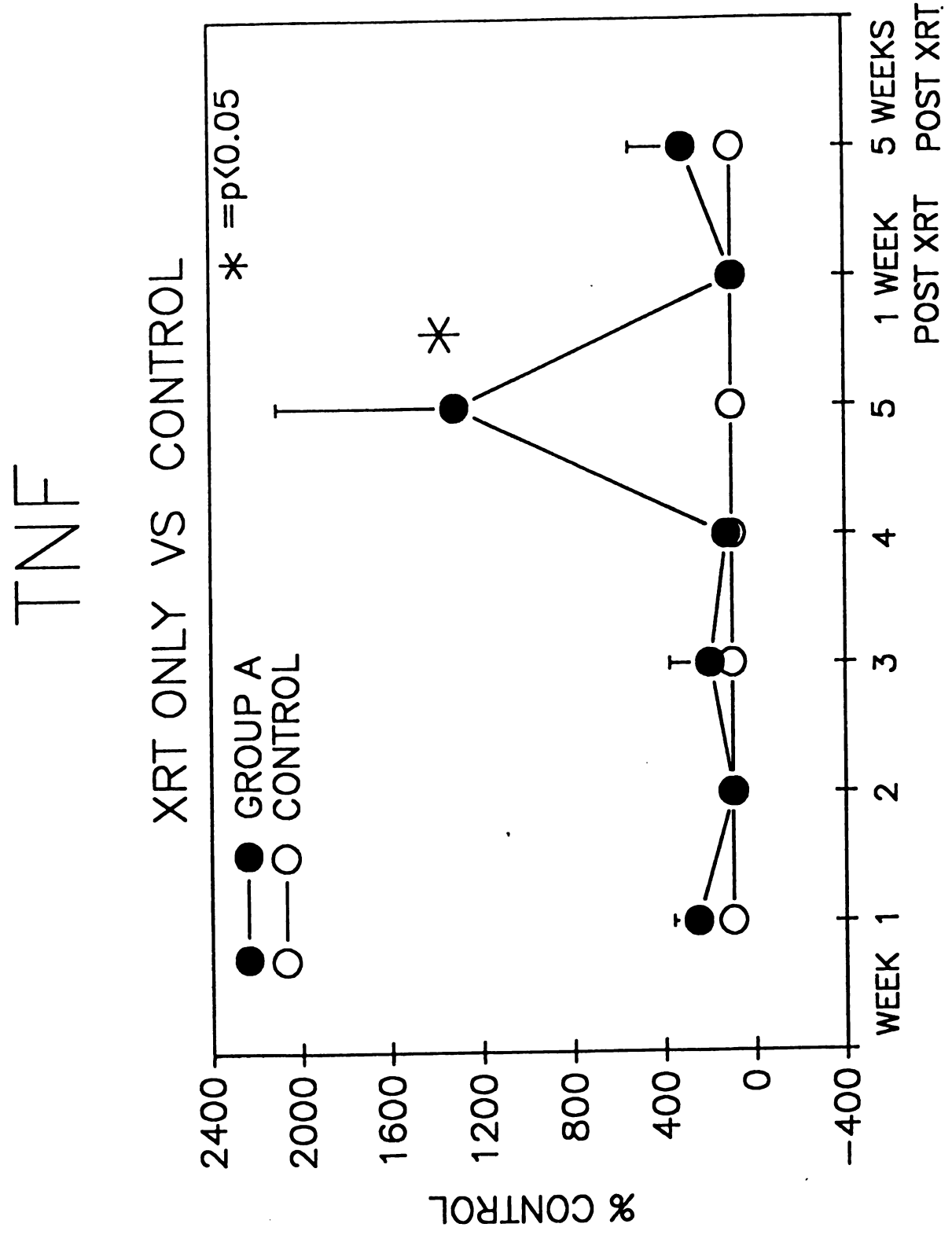


GRAPH 13

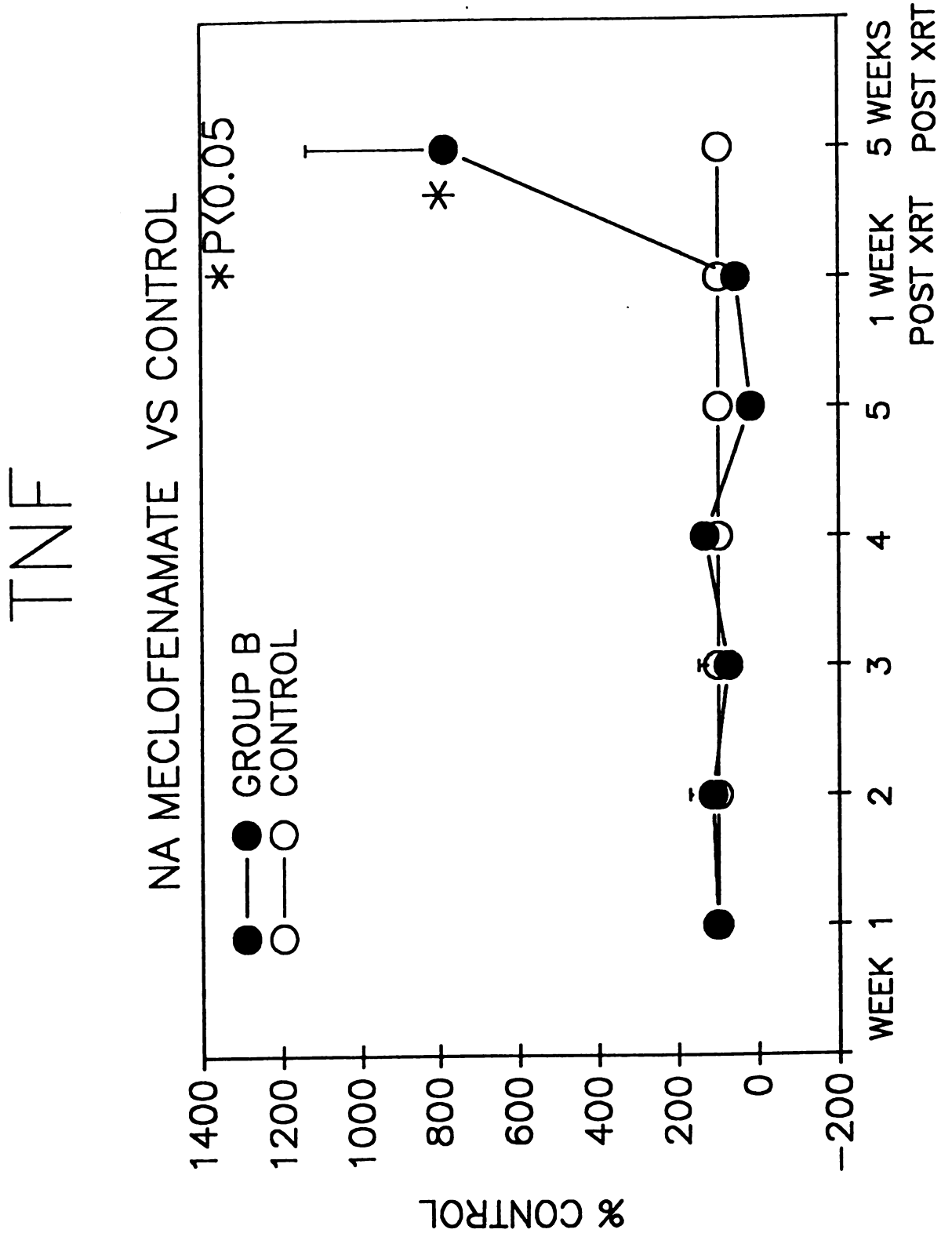

IL-6 % CONTROL
300
250
200
150
100
50
0
IL-6 % CONTROL
1
2
3
4
5
WEEK OF RADIATION


TNF SUMMARY
*=P<0.05
GROUP A
GROUP B
GROUP C
GROUP E
% CONTROL
4000
3500
3000
2500
2000
1500
1000
500
0
WEEK 1
2
3
4
5
1 WEEK POST XRT
5 WEEKS POST XRT

GRAPH 14

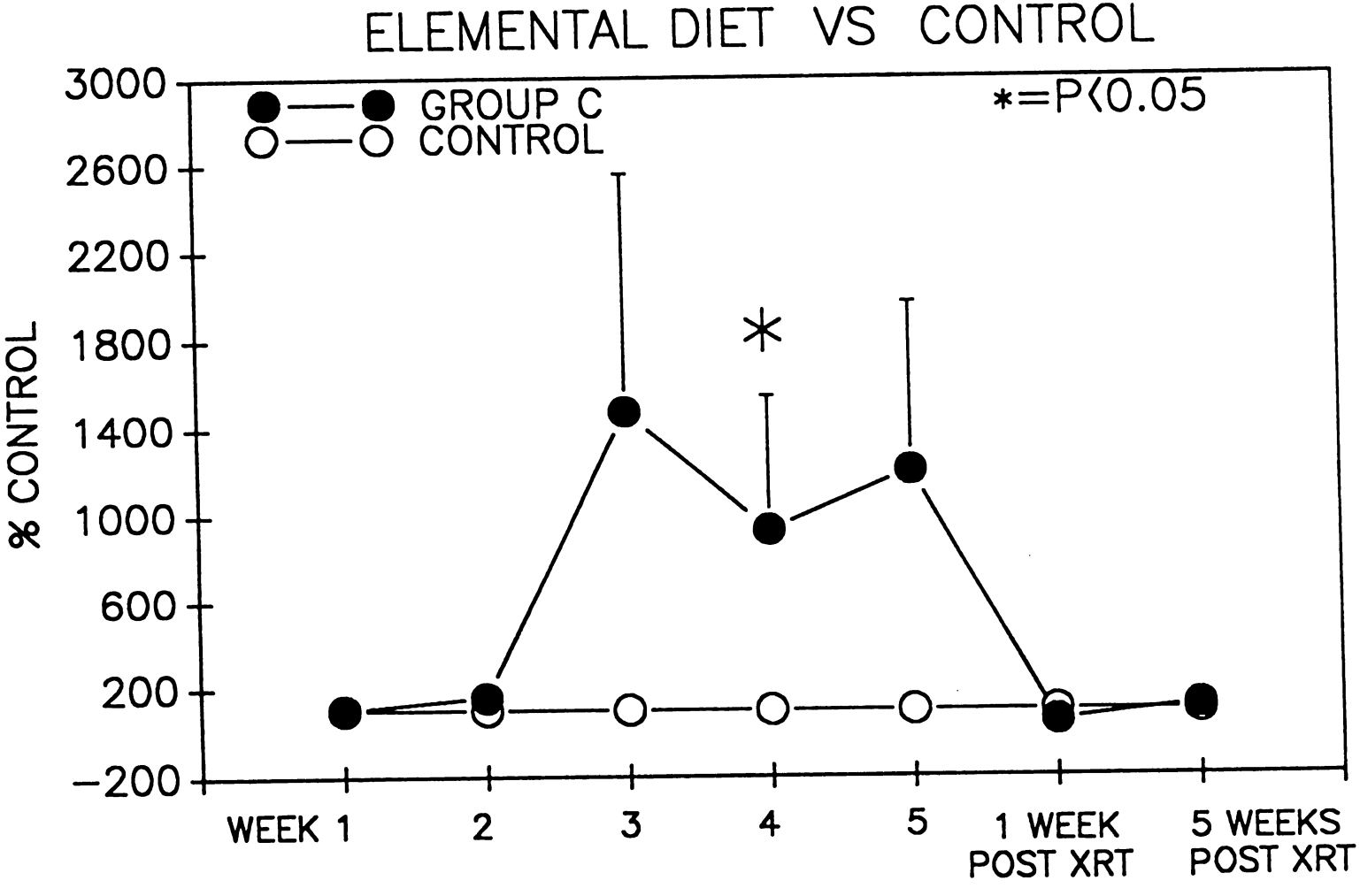

TNF
XRT ONLY VS CONTROL
* =p<0.05
GROUP A
CONTROL
% CONTROL
2400
2000
1600
1200
800
400
0
-400
WEEK 1
2
3
4
5
1 WEEK POST XRT
5 WEEKS POST XRT

GRAPH 15

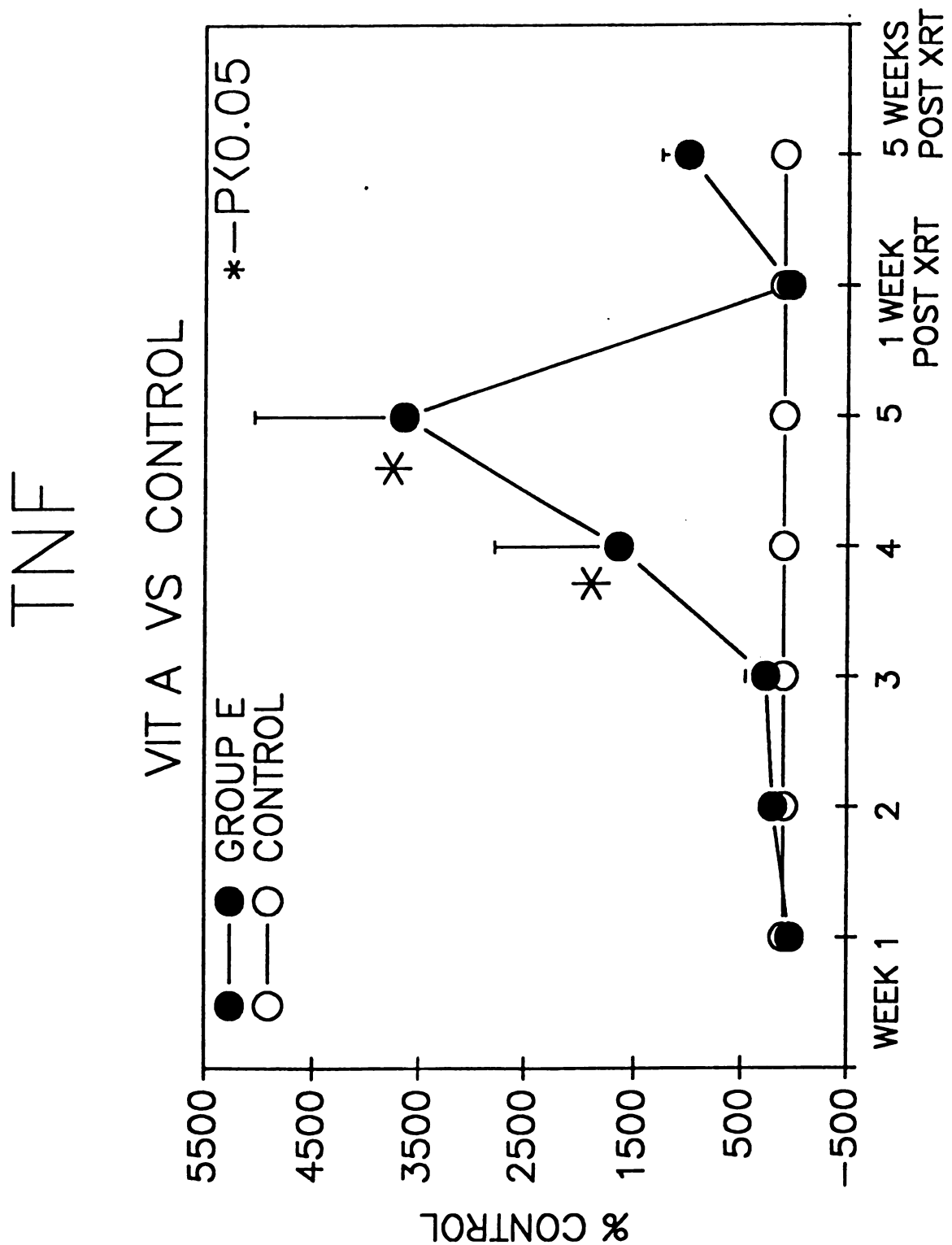

TNF
NA MECLOFENAMATE VS CONTROL
*P<0.05
GROUP B
CONTROL
% CONTROL
1400
1200
1000
800
600
400
200
0
-200
WEEK 1
2
3
4
5
1 WEEK POST XRT
5 WEEKS POST XRT

GRAPH 16

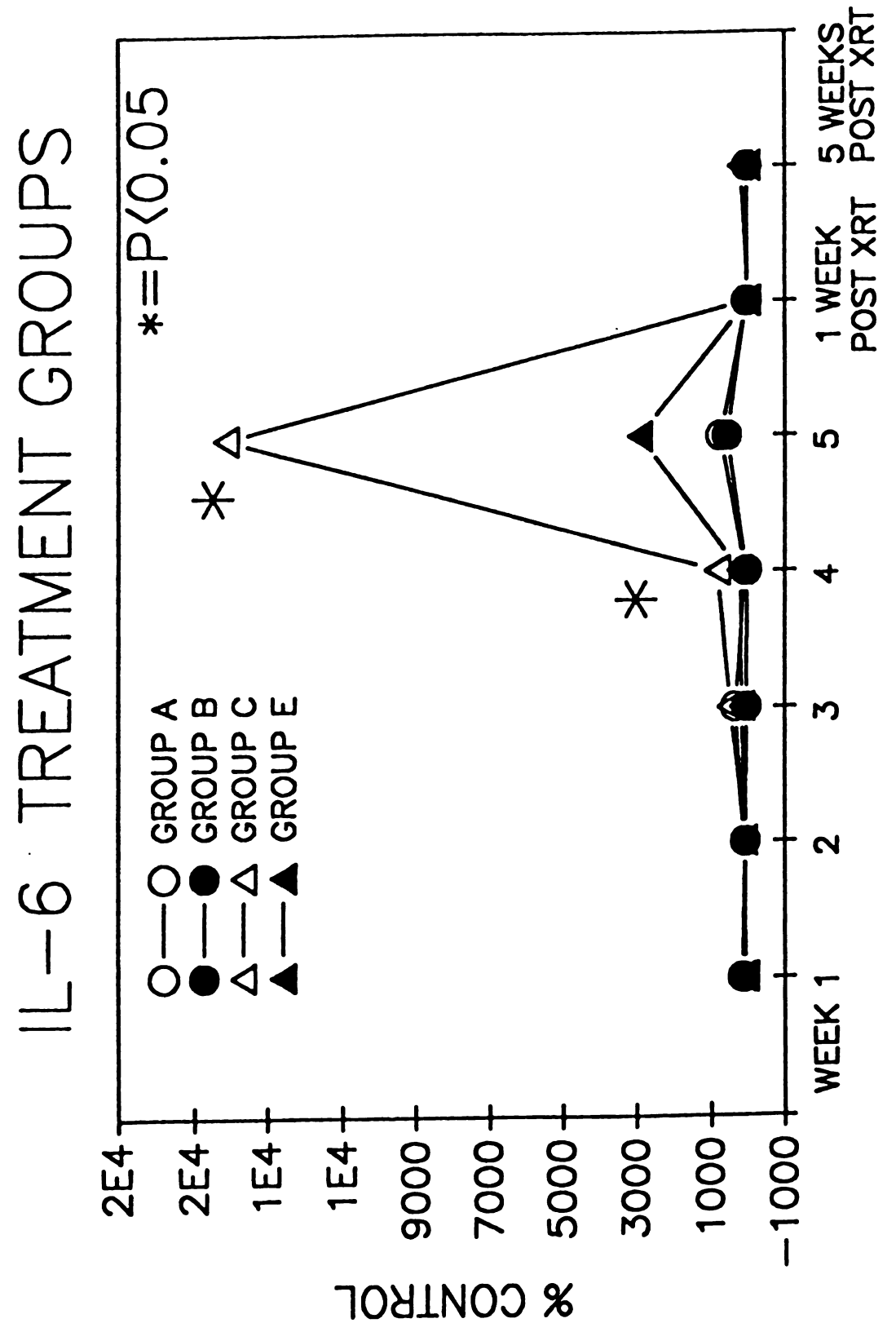
TNF


ELEMENTAL DIET VS CONTROL
GROUP C
CONTROL
*=P<0.05
% CONTROL
3000
2600
2200
1800
1400
1000
600
200
-200
*
WEEK 1
2
3
4
5
1 WEEK POST XRT
5 WEEKS POST XRT

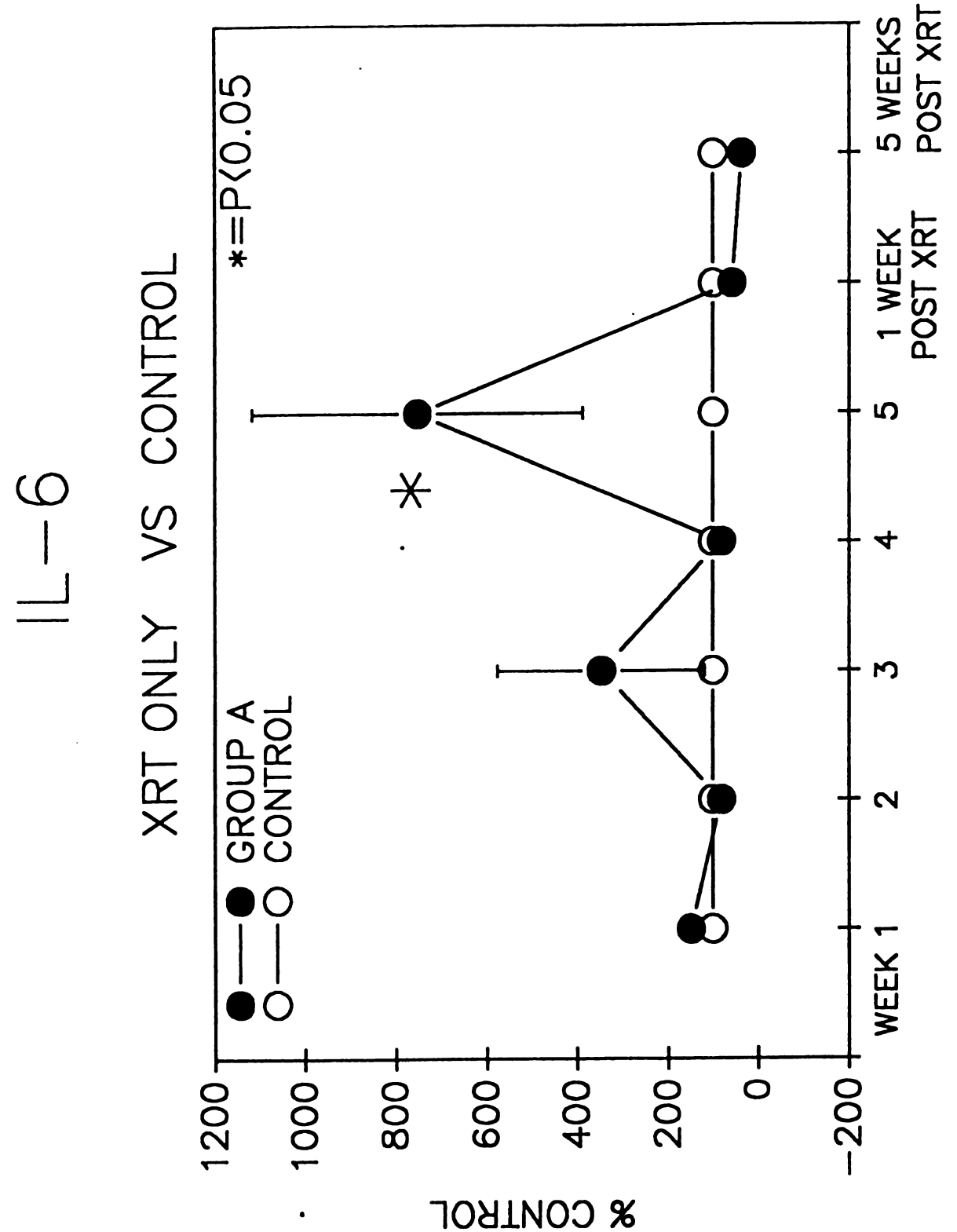
GRAPH 17


TNF
VIT A VS CONTROL
*—P<0.05
GROUP E
CONTROL
% CONTROL
5500
4500
3500
2500
1500
500
−500
WEEK 1
2
3
4
5
1 WEEK POST XRT
5 WEEKS POST XRT

GRAPH 18

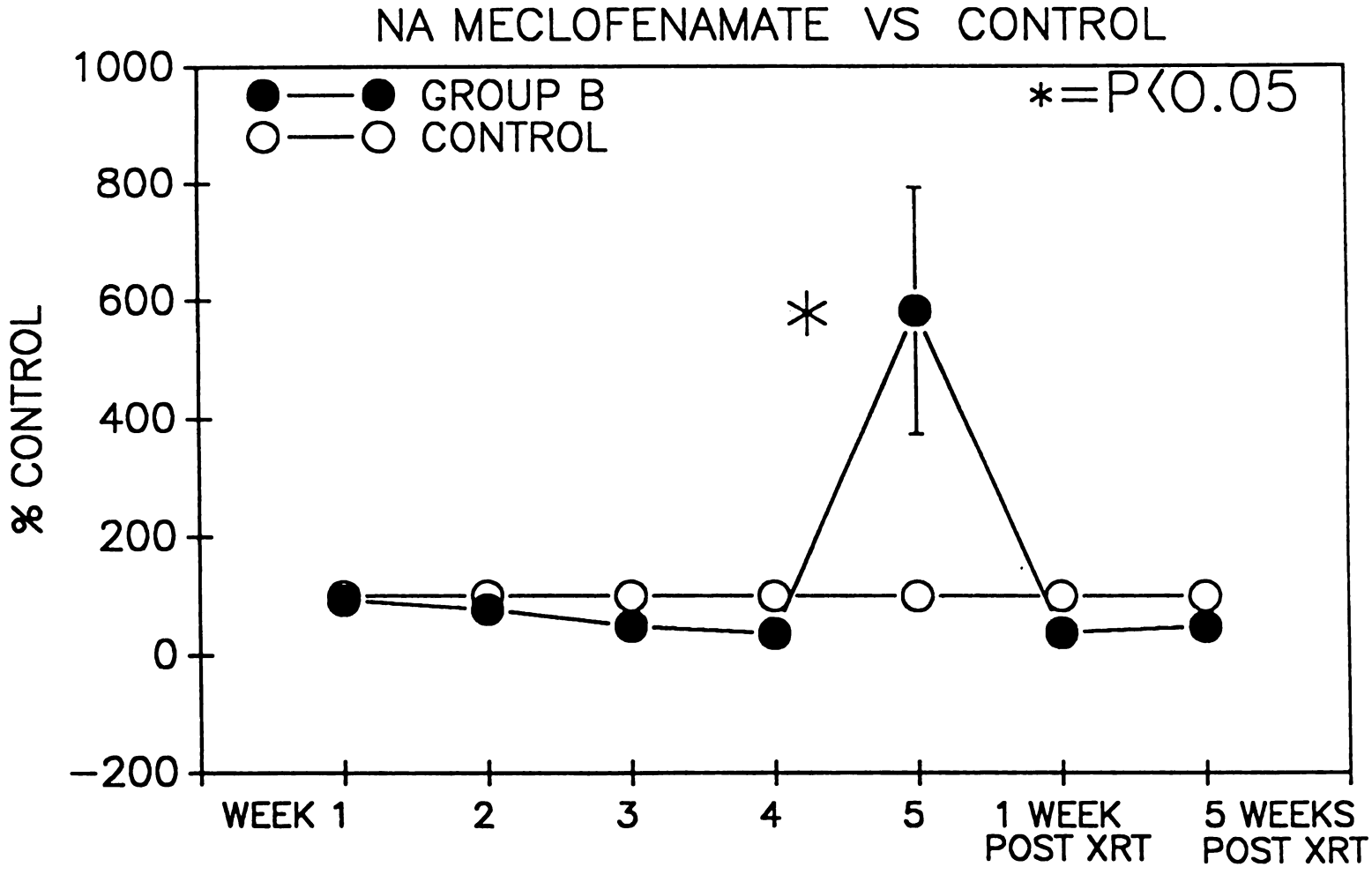

IL-6 TREATMENT GROUPS
*=P<0.05
GROUP A
GROUP B
GROUP C
GROUP E
% CONTROL
2E4
2E4
1E4
1E4
9000
7000
5000
3000
1000
-1000
WEEK 1
2
3
4
5
1 WEEK POST XRT
5 WEEKS POST XRT

GRAPH 19


IL-6
XRT ONLY VS CONTROL
*=P<0.05
GROUP A
CONTROL
% CONTROL
1200
1000
800
600
400
200
0
-200
WEEK 1
2
3
4
5
1 WEEK POST XRT
5 WEEKS POST XRT

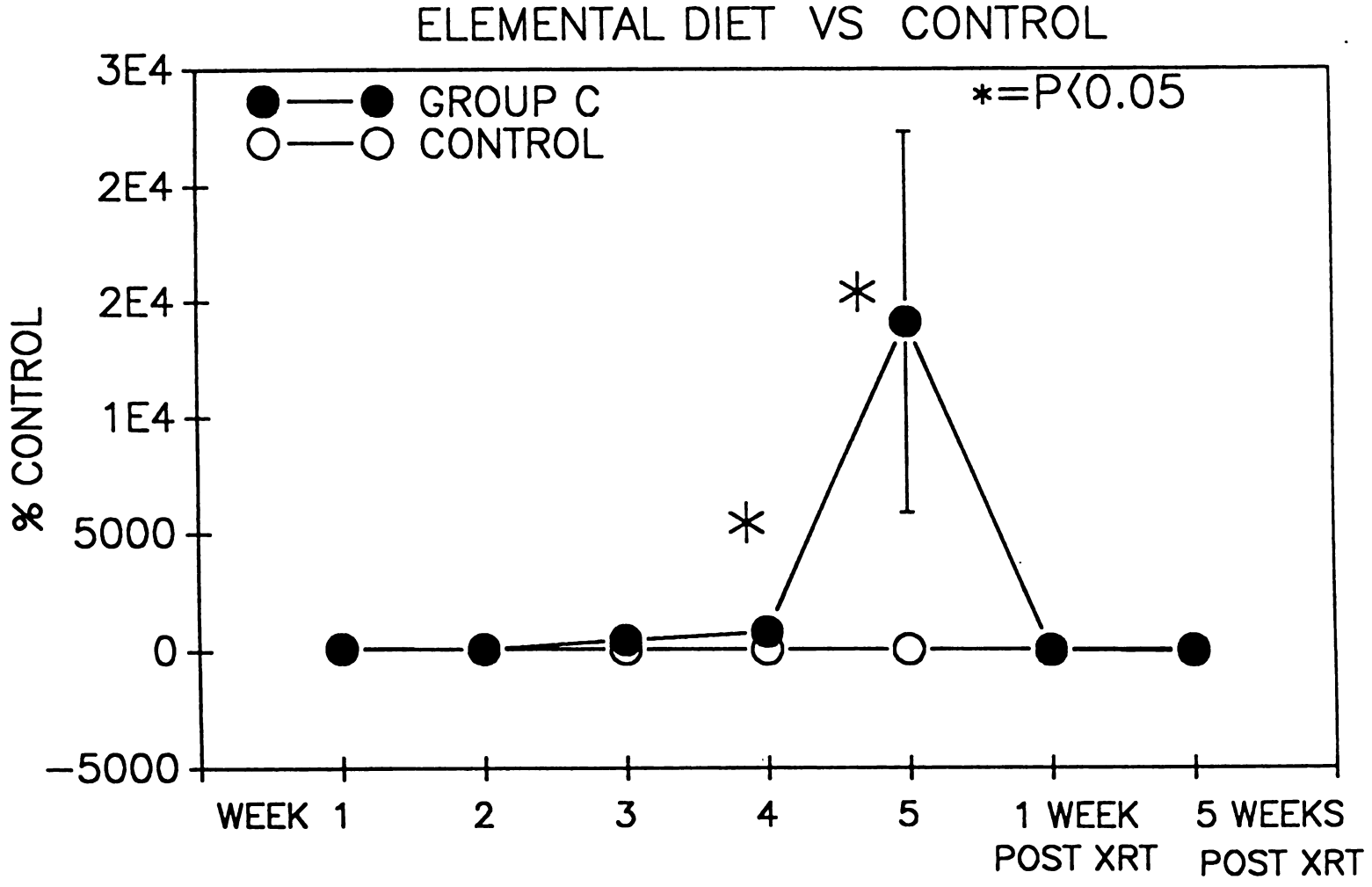
GRAPH 20

IL-6


NA MECLOFENAMATE VS CONTROL
GROUP B
CONTROL
*=P<0.05
% CONTROL
1000
800
600
400
200
0
-200
*
WEEK 1
2
3
4
5
1 WEEK POST XRT
5 WEEKS POST XRT

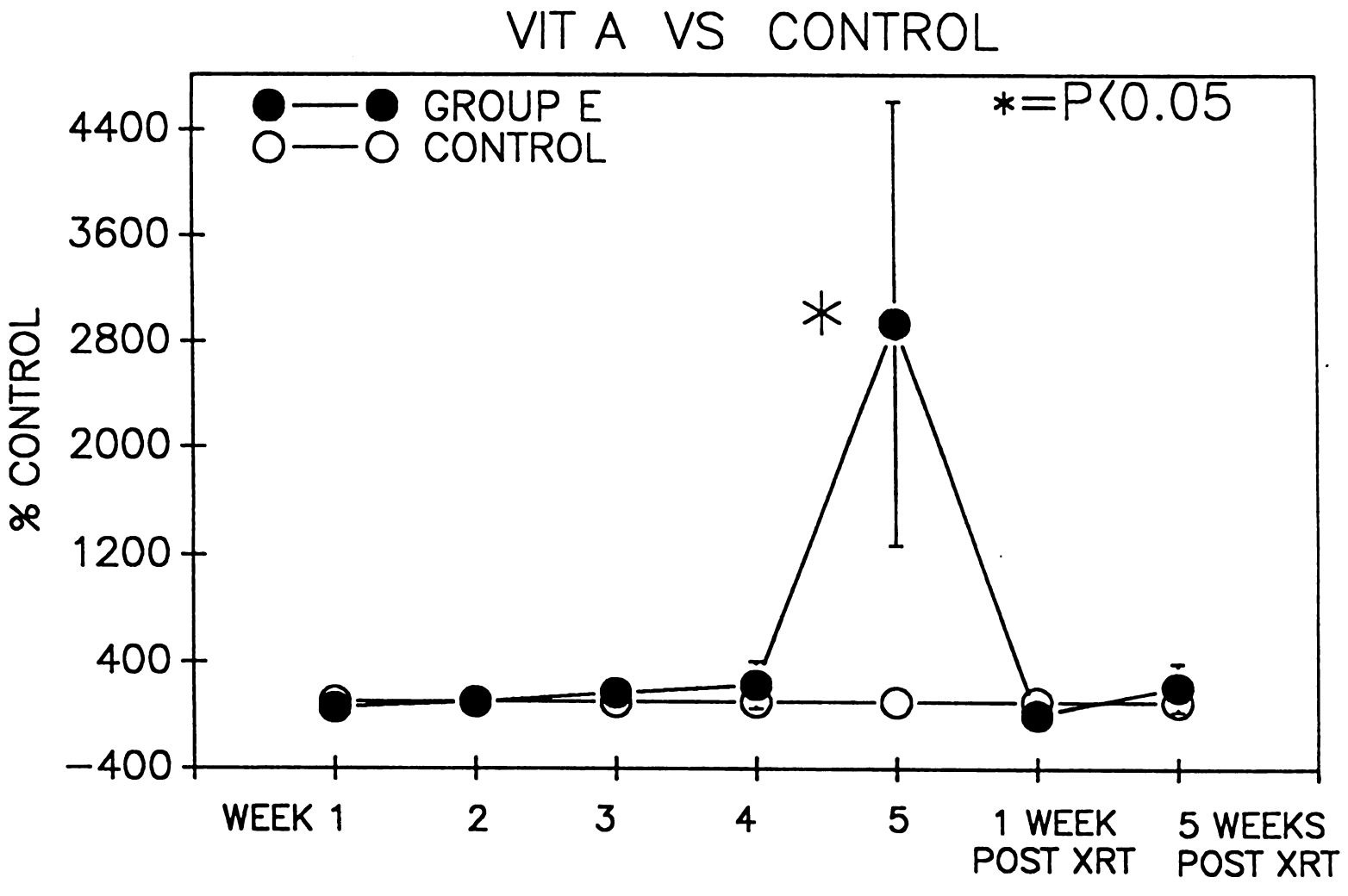
GRAPH 21

IL-6

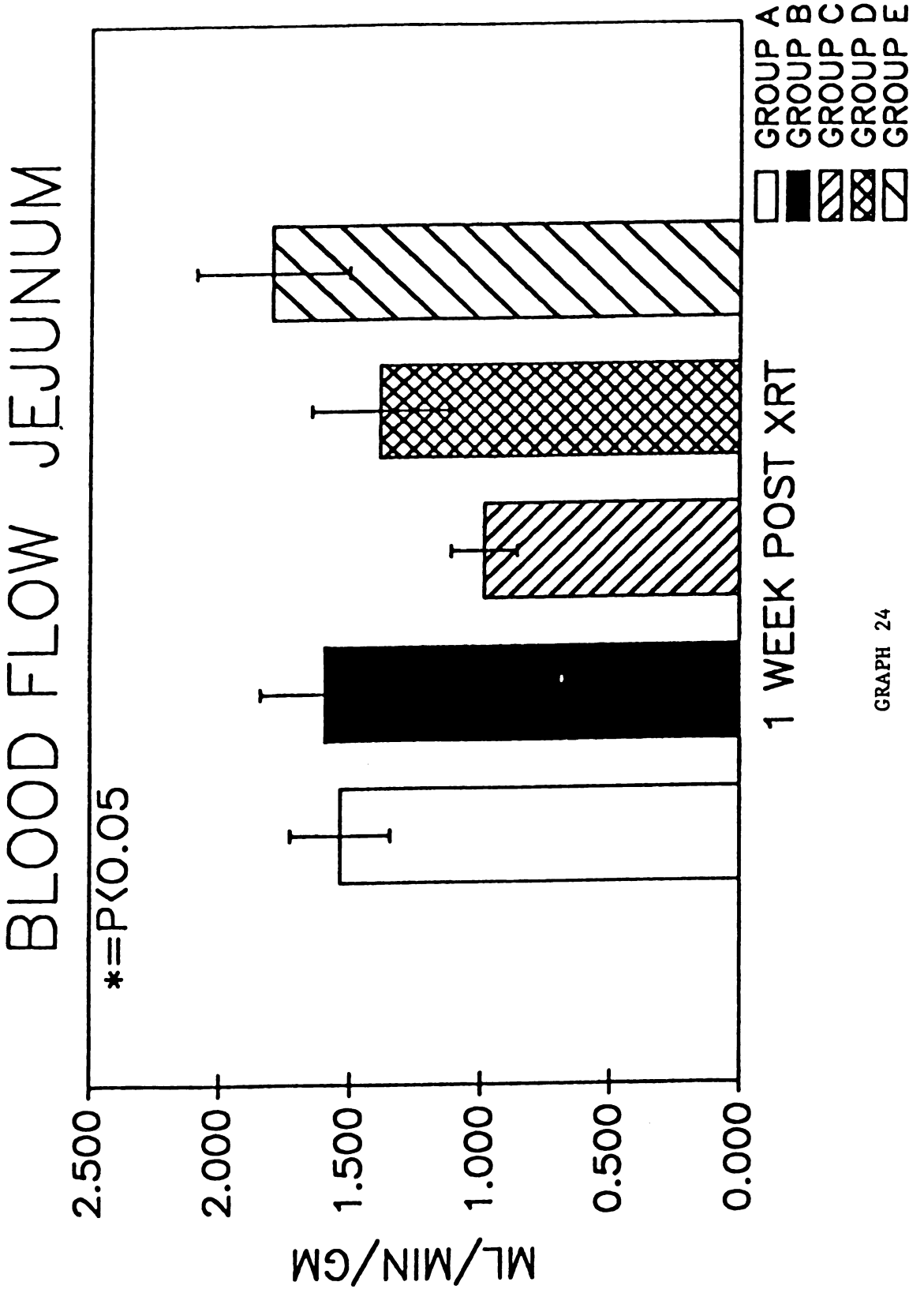

ELEMENTAL DIET VS CONTROL
GROUP C
CONTROL
*=P<0.05
% CONTROL
3E4
2E4
2E4
1E4
5000
0
-5000
WEEK 1
2
3
4
5
1 WEEK POST XRT
5 WEEKS POST XRT

GRAPH 22

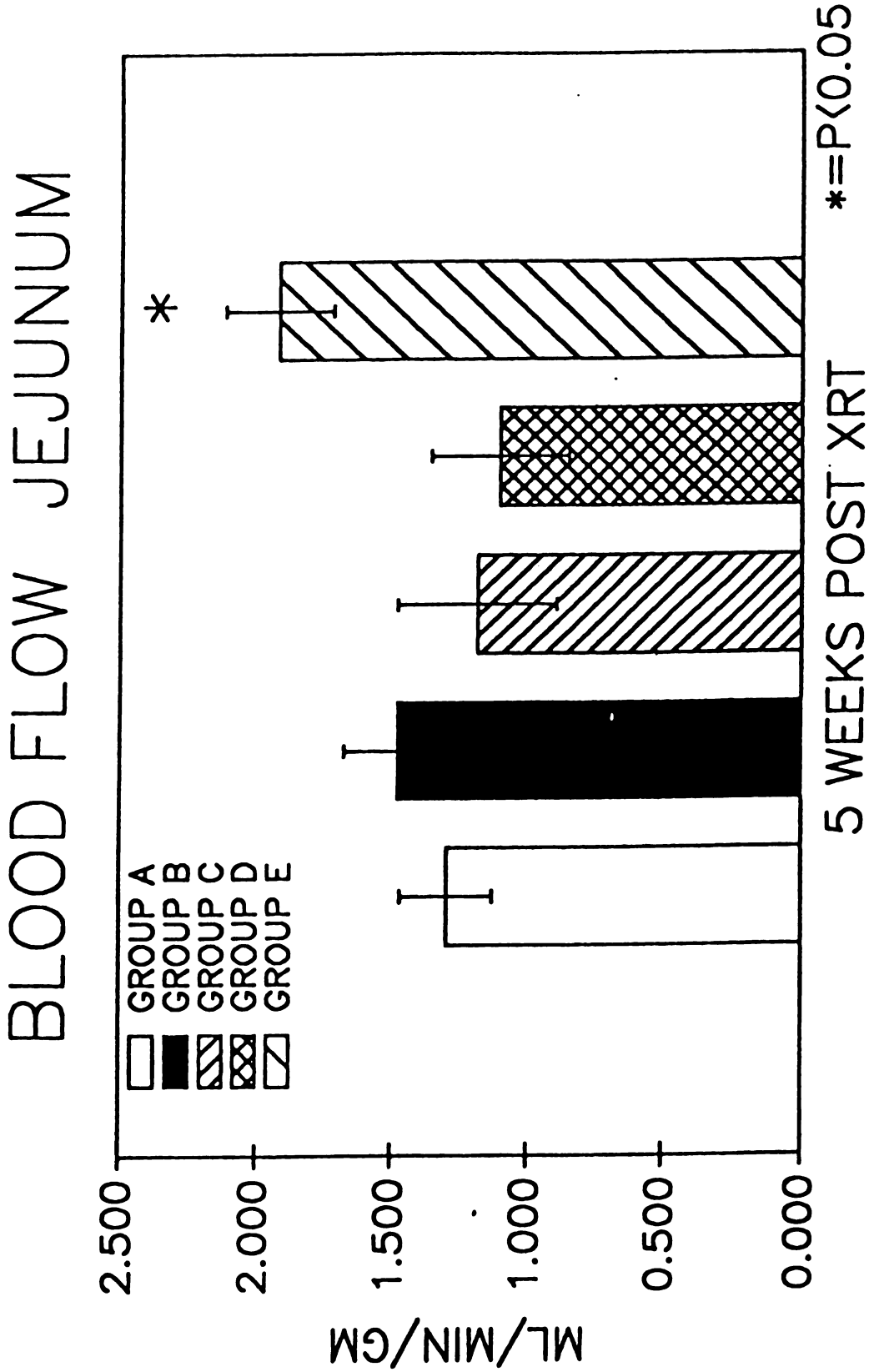
IL-6


VIT A VS CONTROL
GROUP E
CONTROL
*=P<0.05
% CONTROL
4400
3600
2800
2000
1200
400
-400
WEEK 1
2
3
4
5
1 WEEK POST XRT
5 WEEKS POST XRT

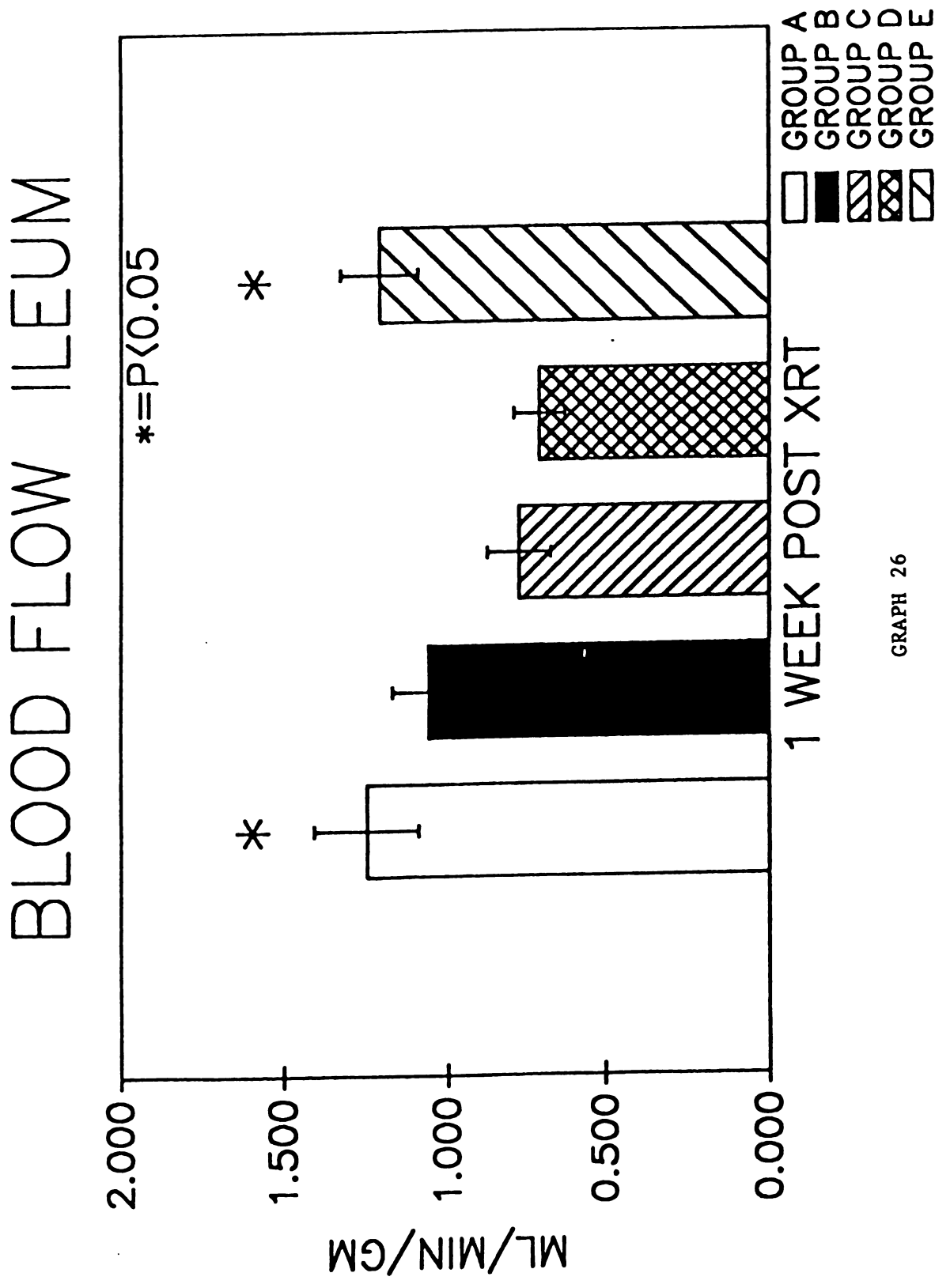
GRAPH 23


BLOOD FLOW JEJUNUM
*=P<0.05
ML/MIN/GM
2.500
2.000
1.500
1.000
0.500
0.000
1 WEEK POST XRT
GROUP A
GROUP B
GROUP C
GROUP D
GROUP E

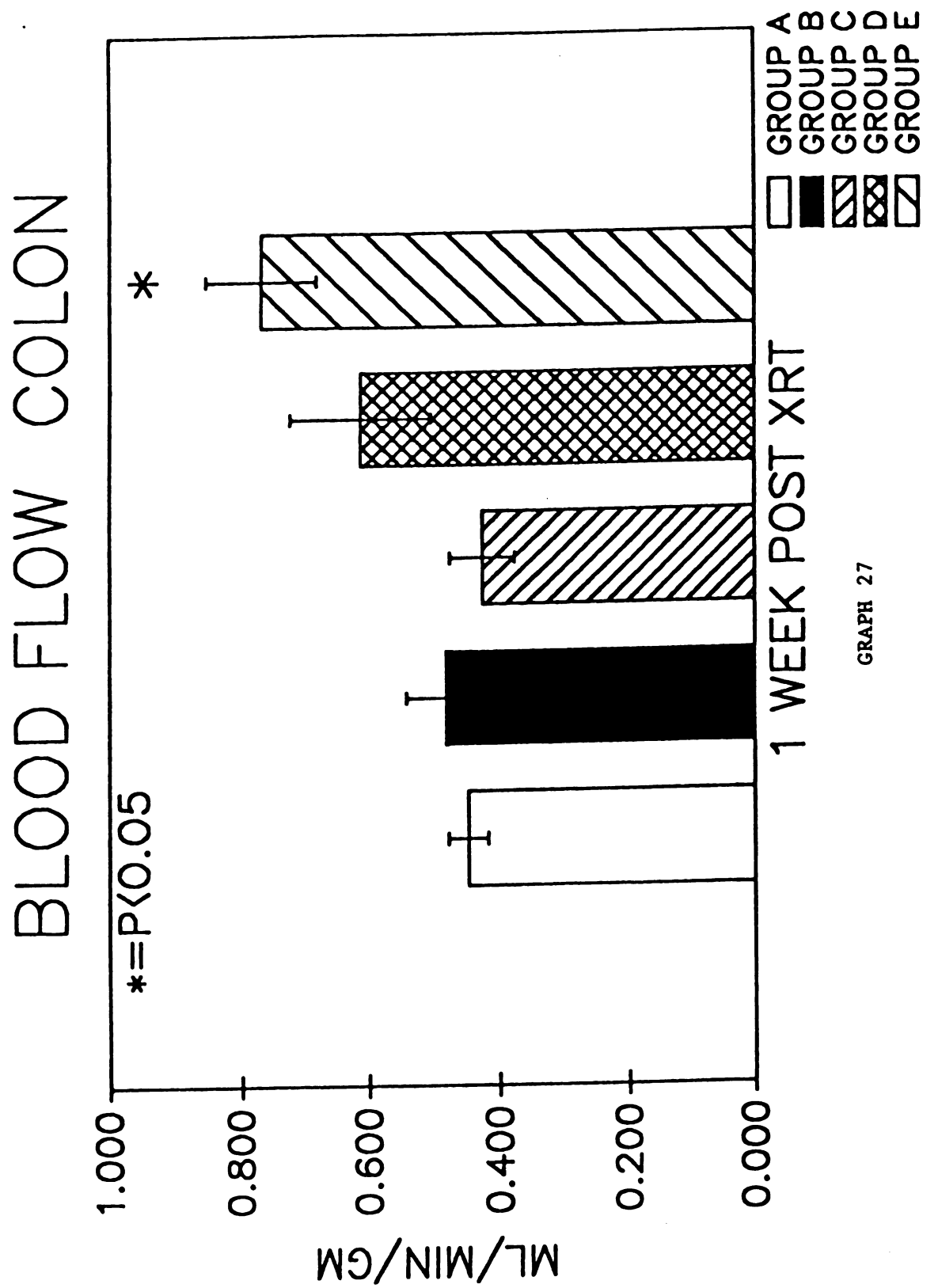
GRAPH 24


BLOOD FLOW JEJUNUM
GROUP A
GROUP B
GROUP C
GROUP D
GROUP E
*
*=P<0.05
5 WEEKS POST XRT
ML/MIN/GM
2.500
2.000
1.500
1.000
0.500
0.000

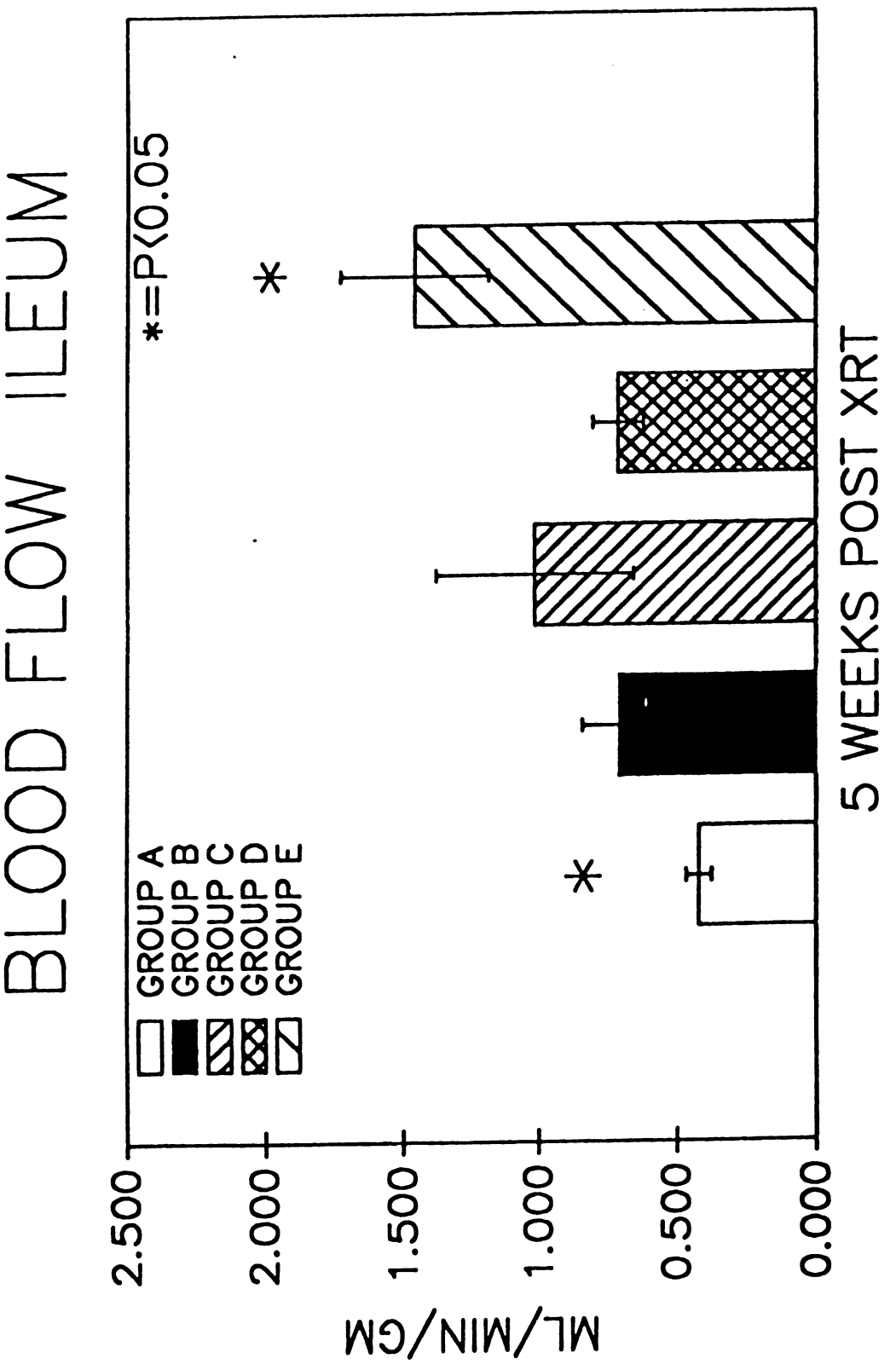
GRAPH 25


BLOOD FLOW ILEUM
*=P<0.05
2.000
1.500
1.000
0.500
0.000
ML/MIN/GM
1 WEEK POST XRT
GROUP A
GROUP B
GROUP C
GROUP D
GROUP E

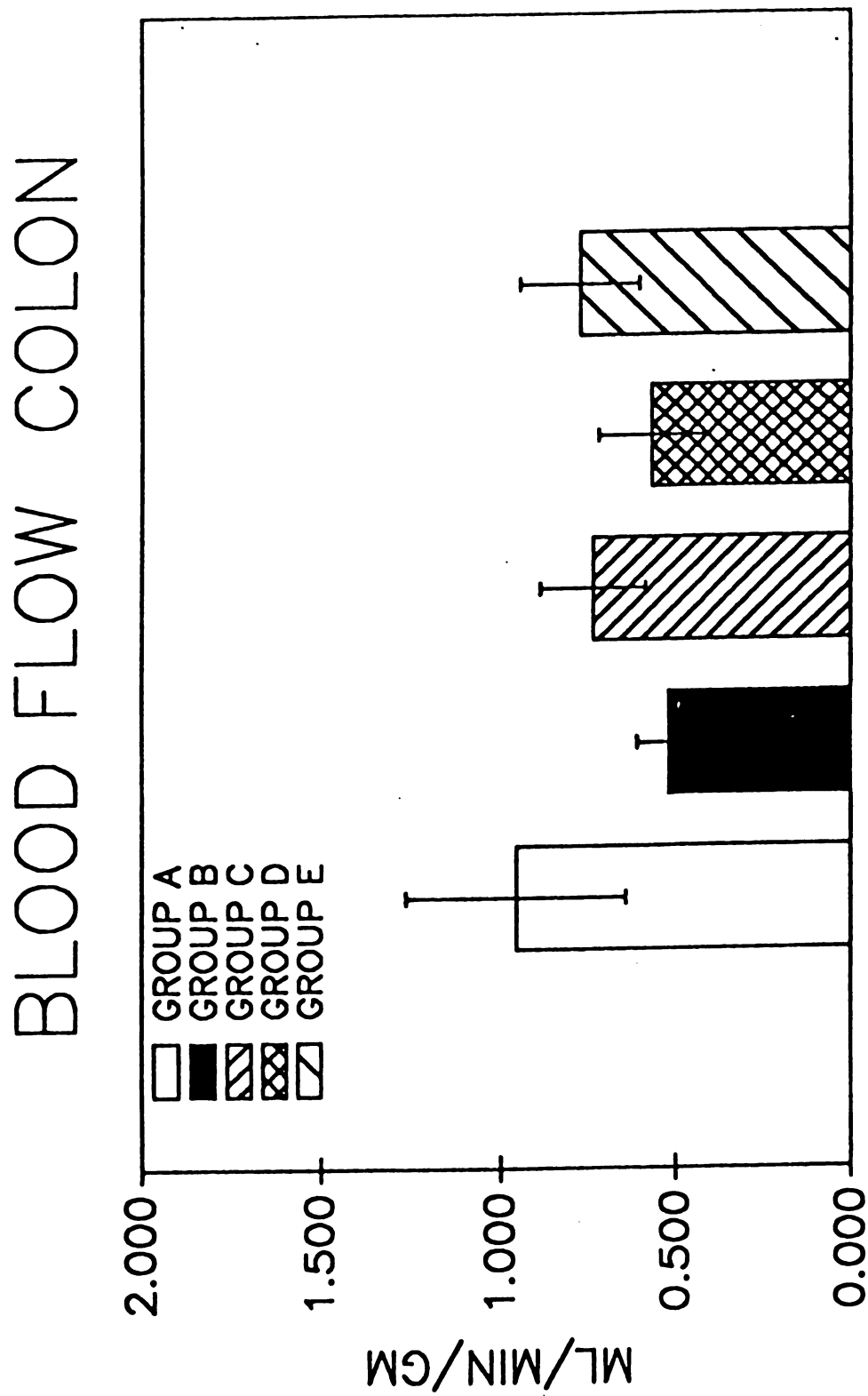
GRAPH 26


BLOOD FLOW COLON
*=P<0.05
*
1 WEEK POST XRT
ML/MIN/GM
1.000
0.800
0.600
0.400
0.200
0.000
GROUP A
GROUP B
GROUP C
GROUP D
GROUP E

GRAPH 27

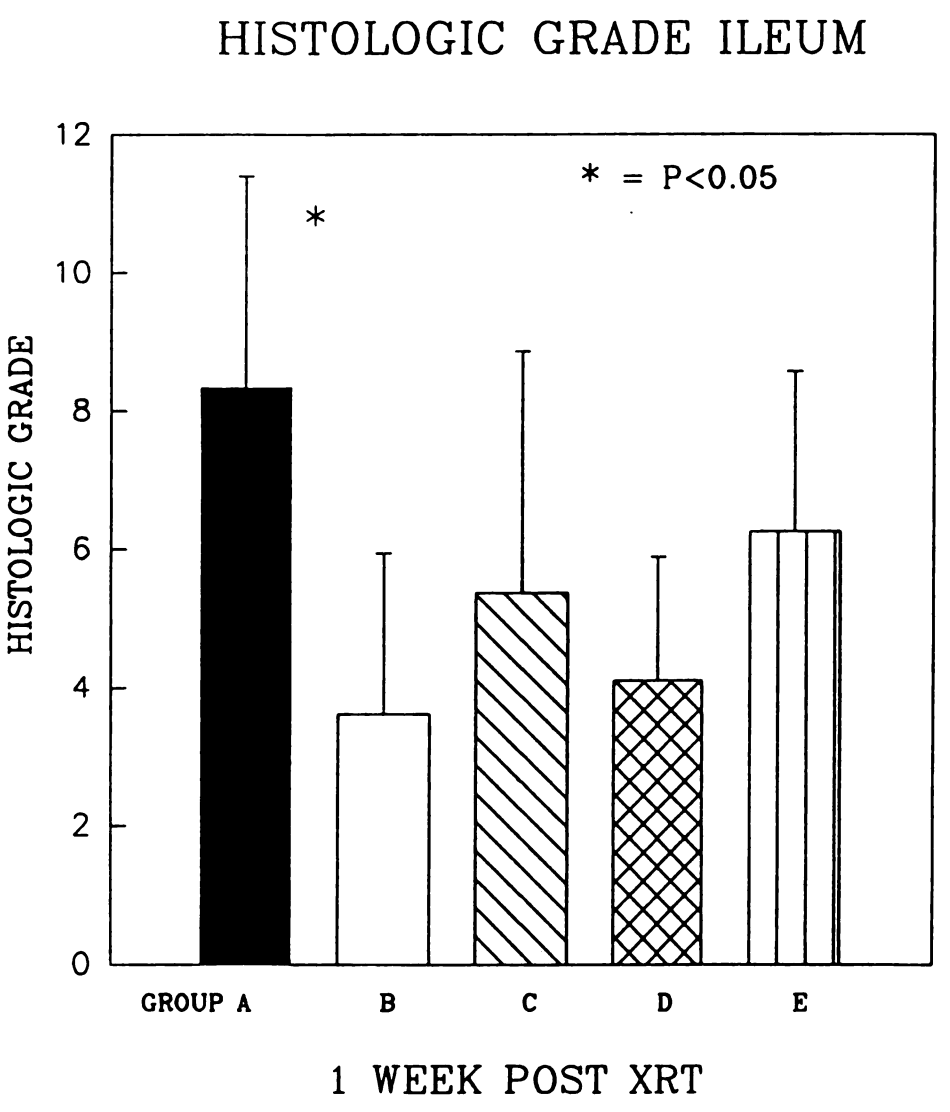

BLOOD FLOW ILEUM
*=P<0.05
GROUP A
GROUP B
GROUP C
GROUP D
GROUP E
2.500
2.000
1.500
1.000
0.500
0.000
ML/MIN/GM
5 WEEKS POST XRT

GRAPH 28

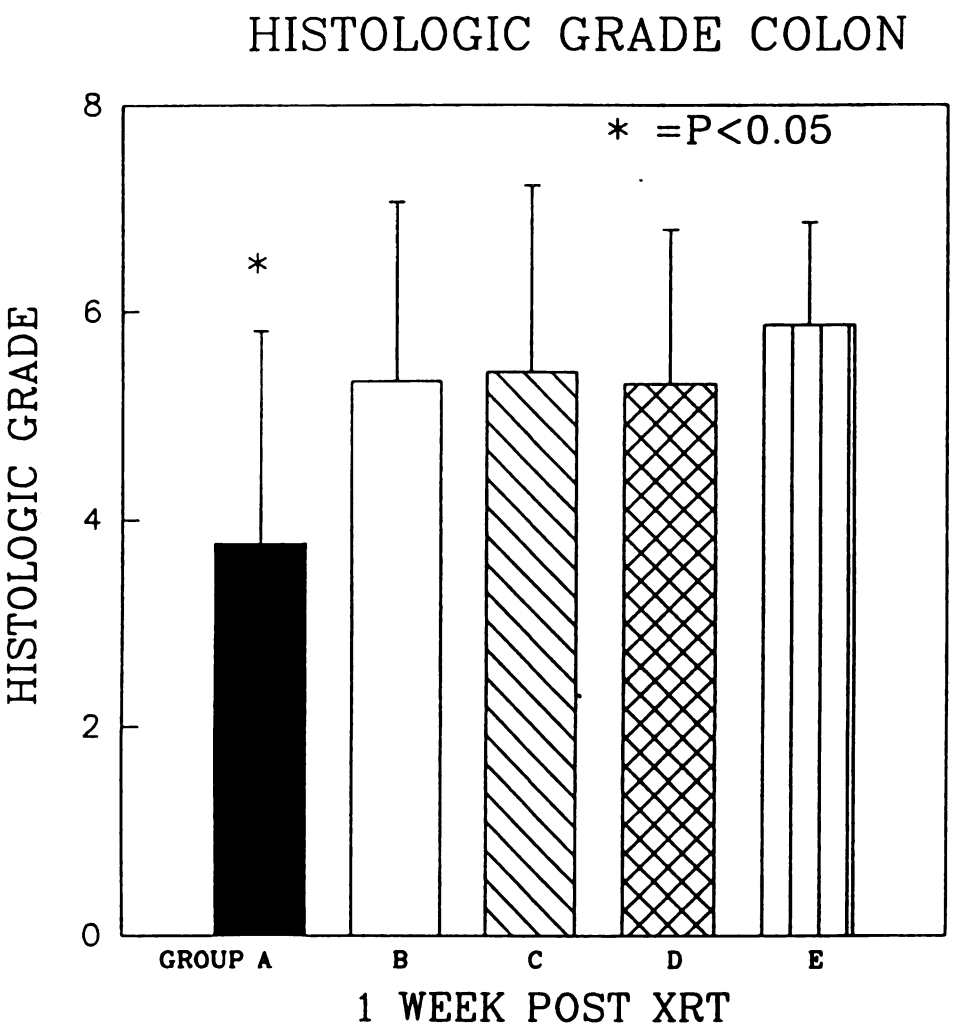

BLOOD FLOW COLON
GROUP A
GROUP B
GROUP C
GROUP D
GROUP E
2.000
1.500
1.000
0.500
0.000
ML/MIN/GM
5 WEEKS POST XRT

GRAPH 29

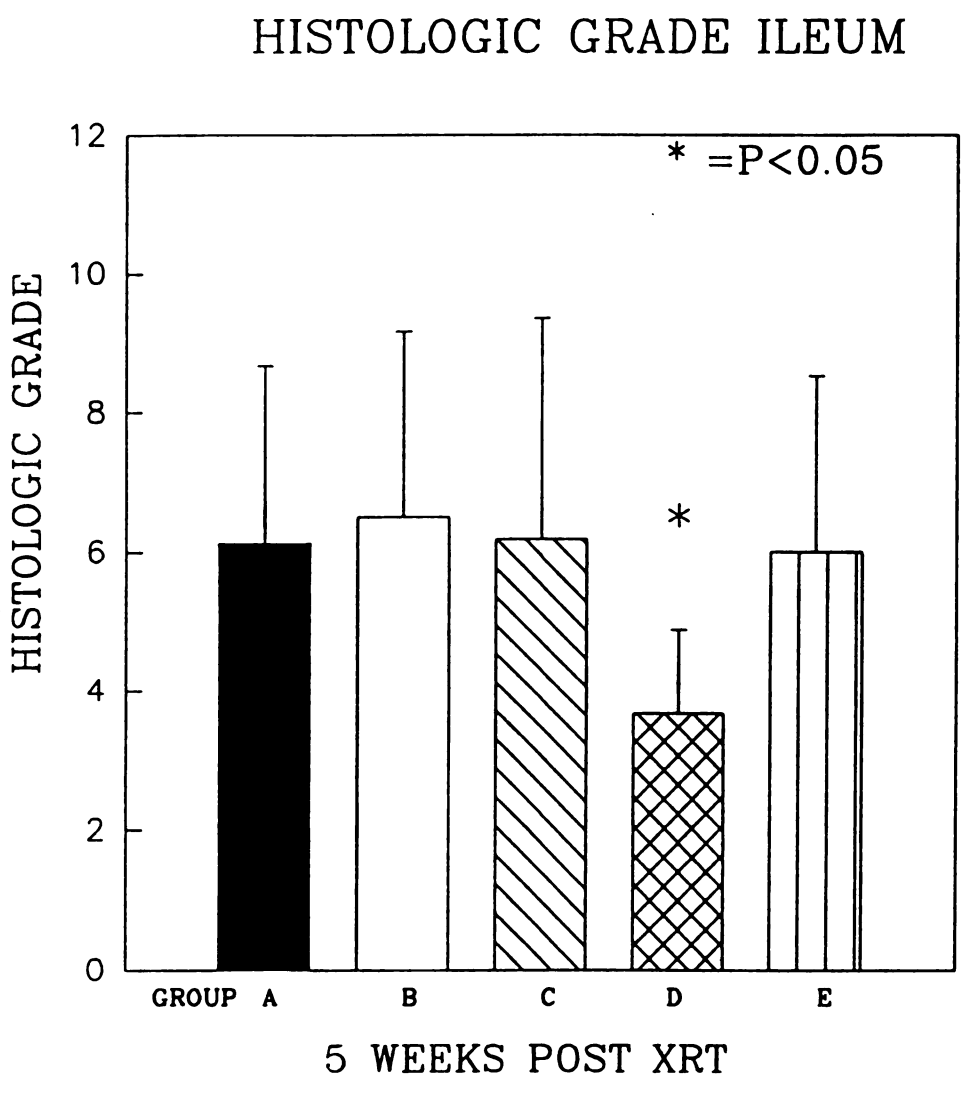
GRAPH 30


HISTOLOGIC GRADE ILEUM
* = P<0.05
*
HISTOLOGIC GRADE
12
10
8
6
4
2
0
GROUP A
B
C
D
E
1 WEEK POST XRT

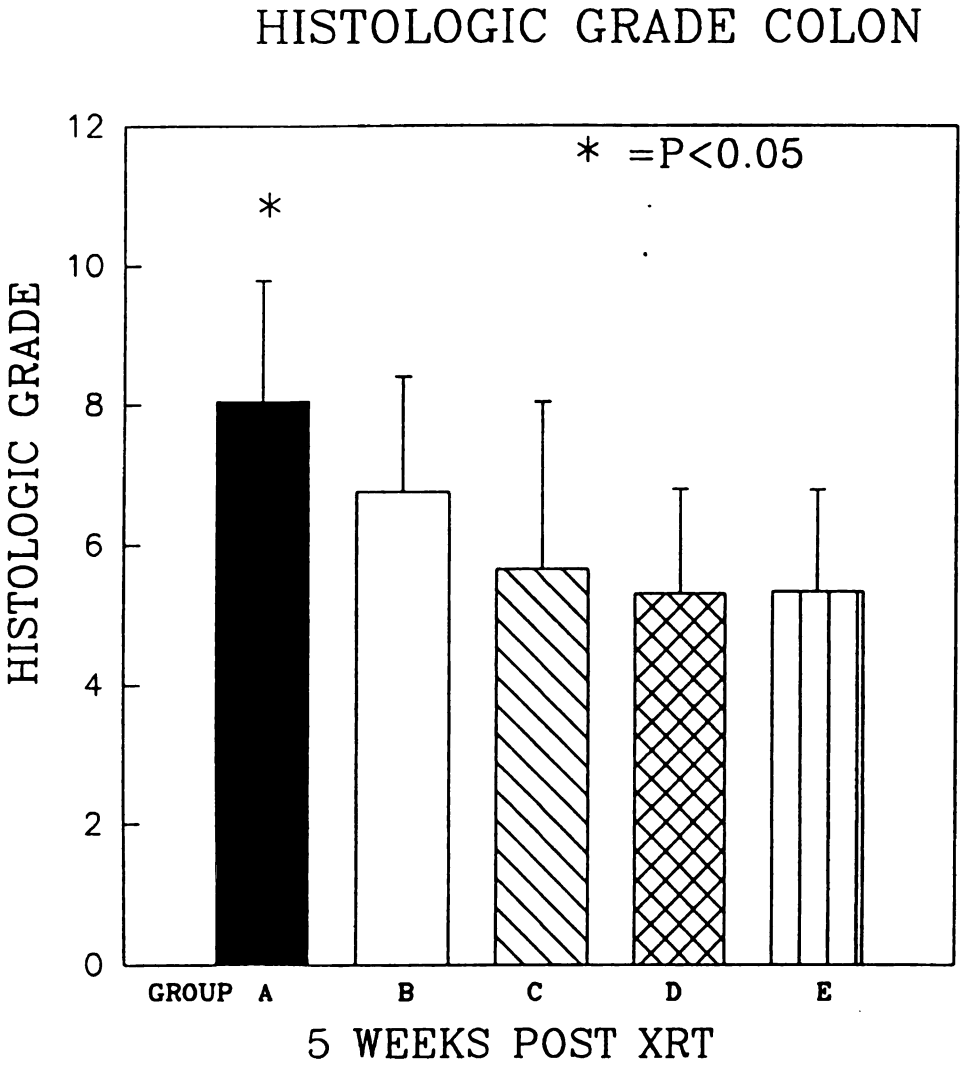
GRAPH 31


HISTOLOGIC GRADE COLON
* =P<0.05
HISTOLOGIC GRADE
8
6
4
2
0
*
GROUP A
B
C
D
E
1 WEEK POST XRT

GRAPH 32


HISTOLOGIC GRADE ILEUM
* =P<0.05
HISTOLOGIC GRADE
12
10
8
6
4
2
0
*
GROUP A
B
C
D
E
5 WEEKS POST XRT

GRAPH 33


HISTOLOGIC GRADE COLON
* =P<0.05
HISTOLOGIC GRADE
12
10
8
6
4
2
0
*
GROUP A
B
C
D
E
5 WEEKS POST XRT

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BIBLIOGRAPHY

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