THE EFFECTS OF DIET AND IONIZING RADIATION ON AZOXYMETHANE INDUCED COLON CARCINOGENESIS

A Thesis

by

JOHN CLIFFORD MANN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2005

Major Subject: Nutrition

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Approved by:

Chair of Committee,	Joanne R. Lupton
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ABSTRACT

The Effects of Diet and Ionizing Radiation on Azoxymethane-Induced Colon Carcinogenesis. (August 2005)

John Clifford Mann, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Joanne Lupton

The ability of ionizing radiation to enhance colon carcinogenesis and the role of diet in this process has not been documented. We hypothesized that radiation would enhance the formation of aberrant crypt foci, ACF, known precursor lesions to colon cancer, by suppressing apoptosis and upregulating proliferation in colonocytes. Diets contained a combination of fish oil or corn oil and either pectin or cellulose. We exposed 40 male Sprague-Dawley rats to 1 Gy ionizing radiation (1 GeV Fe) 10 d prior to injection with AOM. Colons were resected at the promotion stage of carcinogenesis (7 wk post initial injection) and assayed for ACF and apoptosis. Radiation treatment increased ($P=0.0327$) the incidence of high multiplicity ACF (foci with four or more aberrant crypts) and decreased ($P=0.0340$) the apoptotic index compared to non-irradiated rats. Radiation also resulted in an increase ($P<0.0001$) in the proliferative index compared to the nonirradiated rats. The fish oil containing diets resulted in fewer ($P=0.0002$) high-multiplicity ACF compared to the corn oil treatment. Dietary pectin significantly increased ($P=0.0204$) the apoptotic index compared to cellulose treatment. These data suggest that ionizing radiation can work synergistically with AOM and increase the formation of high-multiplicity ACF, upregulate cellular proliferation and decrease

apoptosis in colonocytes. The data also suggest that diets containing fish oil and pectin may protect against colon cancer by increasing apoptosis and reducing the formation of high multiplicity ACF.

ACKNOWLEDGEMENTS

I first would like to thank my committee chair, Dr. Joanne Lupton, for her guidance, advice, encouragement and mentoring throughout this thesis process. The work was made easier by having a mentor who was always willing to share her expertise and advice throughout all stages of this work. I also need to thank Dr. Nancy Turner for her help in the laboratory and in writing this thesis, as well as Mary Murphy for providing the statistical analysis of our data. I appreciate Dr. Robert Chapkin and Dr. Bani Mallick for serving as willing committee members. I am very grateful to Stella Taddeo for her technical assistance with the experiments, as well as Natasa Popovic for her extensive work on this project. Additionally I would like to thank all of the undergraduates who have worked in the lab and assisted with many aspects of this project.

I also owe a huge thank you to my wife, Amanda, for her incredible support and encouragement during the past few years. I would not have been able to accomplish this without you, Amanda. This research has been funded by NSBRI NASA NCC 9-58, NIH CA61750, CA82907, NIEHS P30-ES09106.

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CHAPTER I

INTRODUCTION

Epidemiology. Cancer is the second leading cause of death in the United States behind cardiovascular diseases. Specifically, colon cancer is the second leading cause of death due to cancer and affects men and women equally. The American Cancer Society estimates that there will be 106,370 new cases of colon cancer this year (1). While surgery and radiation therapy are obvious modalities of treatment once cancer has developed, epidemiological evidence indicates that certain dietary changes may prevent as much as 80% of colon cancers (2). Dietary prevention of colon cancer is thought to be effective partly due to the well-documented multi-step nature of colon carcinogenesis and the long period of time required for development (3). The environmental factor of dietary intake has been implicated in the development of colon cancer due to the increase in incidence found in migrant studies, as well as the increased incidence noted in populations previously considered to be at low risk for the disease (4). Several reviews of epidemiological studies have concluded that there may be an increased risk for developing colon cancer associated with a low intake of dietary fiber, which was associated with a low intake of fruits and vegetables (5-7). Additionally, recent reviews have noted a protective effect of fish consumption on colon cancer development believed to be related to the high content of n-3 polyunsaturated fatty acids present in fish (7-8). Due to these findings in human epidemiological studies much of the current research involving colon

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This thesis follows the style and format of the Journal of Nutrition.

cancer prevention seeks to elucidate mechanisms by which these potentially protective nutrients elicit their effects.

Fat and fiber. Dietary fat has been regarded as one of the primary factors in colon cancer development (9). Epidemiological evidence suggests that fish consumption may be protective against colon carcinogenesis due to the high content of the omega-3 polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (7). Omega-3 fatty acids found in fish oil and omega-6 fatty acids found in corn oil, play vital roles within the body as structural phospholipids of the cell membrane and as modulators of cell signaling. Omega-6 fatty acids are metabolized into precursors of inflammatory molecules such as eicosanoids and prostaglandins that can stimulate tumor growth and metastasis. Omega-3 fatty acids are metabolized into precursors of a different class of eicosanoids and prostaglandins that are known to decrease tumor growth and increase apoptosis (8).

High intakes of dietary fiber in epidemiological studies have been shown to decrease the risk of colon cancer development (5-7). Fibers are polysaccharides that leave the small intestine undigested but can be fermented to some degree by the bacteria of the large intestine (10). Soluble fibers are fermented in the large intestine and are found in foods such as apples and oranges. Insoluble fibers are those that avoid fermentation such as cellulose, a primary component of plant cell walls found in vegetables (11).

Cell cycle kinetics. Cancer development is a stepwise process that causes the formation of altered cell populations (12) via the activation of oncogenes or the inactivation of tumor suppressor genes (13). Altered gene expression usually leads to a

loss of control of the mechanisms that regulate cell growth (14). Colonic cells are produced by stem cells located at the base of the crypt (15) and proliferate primarily in the lower two-thirds of the crypt (16). While moving up the crypt toward the lumen, cells lose their ability to divide, and are exfoliated into the lumen of the intestine upon reaching the surface (17). An alteration of the proliferative pattern of colonic epithelial cells (18) has been noted as an early event in colon carcinogenesis. Dietary intake has been shown to alter the proliferative pattern of colonic epithelium as well (18). Additionally, impairment or loss of control in the process of programmed cell death, termed apoptosis, has been found to be a contributing factor in the development of colon cancer (19). Apoptosis is a controlled process that results in the activation of enzymes termed endonucleases that cleave chromatin material causing the generation of DNA fragments. Cells undergoing apoptosis tend to shrink away from adjacent cells. Chromatin condensation at the nuclear membrane is common and results in the formation of crescent shaped bodies. Condensation of cytoplasmic contents also occurs and these cell fragments along with the condensed nuclear material are eventually engulfed by macrophages (15). Hong et al. (20) measured apoptosis in the colons of rats 6 wk after a series of two carcinogen injections. Compared to the rats that received saline injections, carcinogen injected rats had less than one-third the number of apoptotic cells per crypt colmn. Measuring the levels of apoptosis and proliferation in histological sections of colonic tissue are well-documented as biomarkers in determining alterations in cell cycle kinetics at various stages of the carcinogenic process.

Colonic cancer development. The carcinogenic process is commonly divided into three stages, initiation, promotion and progression. Initiation involves genetic mutations induced by chemicals or radiation. In the promotion phase, cells containing genetic mutations gain a selective growth advantage and become an expanded population that may be visibly altered compared to the normal cell population. The progression phase is characterized by tumor formation (12). While the carcinogenic process can be divided into three relatively simple phases, tumors that stem from genetic mutations may take 20-40 yr to develop (3). Research has identified various biomarkers that allow for the detection of alterations to the colonic mucosa prior to tumor development.

Aberrant crypt foci. Expanded populations of cells termed aberrant crypts were first identified by Bird in unsectioned colons of mice that had received the carcinogen azoxymethane (AOM). Bird described these aberrant crypts as being larger and having a thicker lining of epithelial cells compared to normal crypts and noted that they were easily identified under the light microscope after staining with methylene blue (21). Aberrant crypts are formed following injections with known colon carcinogens such as AOM (22). McLellan et al. (23) treated rats with increasing doses of the carcinogen 1,2-dimethylhydrazine and examined the colons for the presence of aberrant crypts. They found that the number of aberrant crypts increased with increasing doses of carcinogen and noted that they were sometimes present in clusters, or foci, of two or more. McLellan et al. (24) found that aberrant crypts were not present in control animals injected with saline, and that carcinogen treated rats developed aberrant crypts between 2 and 4 wk following injection.

Pretlow et al. (25) found that the formation of aberrant crypt foci containing four or more aberrant crypts was greater in AOM-injected rats with tumors compared to animals that did not develop tumors. They also noted that the incidence of foci with four or more aberrant crypts increased over time, leading them to the hypothesis that foci containing four or more aberrant crypts may be more likely to form tumors. Multiple studies identified ACF in human colon cancer patients using the same characteristics used for their identification in animal models (26-28). Pretlow et al. (29) measured proliferation in ACF that developed 4 wk after AOM injection in rats and found a 3-fold increase in proliferative activity compared to normal adjacent crypts. Corpet et al. (30) also measured proliferative activity in rats 5 wk after receiving a single AOM injection. They noted that aberrant crypts had higher proliferative indices and larger proliferative zones compared to normal crypts. These studies lend support to the hypothesis that ACF are indeed precursor lesions of colon cancer development (22-31). Common methods of studying the effects of diet on colon cancer development rely on tumor formation as the sole endpoint (32). ACF are excellent biomarkers of the promotion phase of cancer development that can be used in studies attempting to identify dietary modulators of the carcinogenic process. They allow research efforts to be focused on alterations that occur early in cancer development. Additionally, they provide a quantifiable biomarker that may be used to identify the underlying cellular mechanisms that lead to eventual tumor development (33-34).

Beta-catenin. Within the cell, several proteins are known to play roles in signal transduction pathways implicated in the carcinogenic process (35). Beta-catenin is known

to function as a part of the Wnt signaling pathway, a well-documented pathway implicated in colon carcinogenesis (36). Under homeostatic conditions beta-catenin functions as a cadherin-binding protein and is located at the plasma membrane (37). However, during carcinogenesis, a decrease in the degradation of beta-catenin by the proteosome results in its accumulation in the cytoplasm and eventual translocation to the nucleus where it has been shown to activate several growth promoting genes (38-39). Hao et al. (40) examined localization of the beta-catenin protein in colonic crypts of human colon cancer patients and found that 54% of dysplastic ACF stained positively for beta-catenin in the cytoplasm. Additionally, 13% of ACF stained positively for beta-catenin in the nucleus. Furihata et al. (41) induced ACF formation in rats with DMH injections and found that most ACF had normal levels of beta-catenin at the cell membrane. However, positive beta-catenin staining was identified in the cytoplasm of 9% of the ACF. The authors also noted that they did not observe any nuclear staining for beta-catenin in the ACF. Takahashi et al. (42) used an AOM-induced colon cancer model in the rat to study beta-catenin expression and localization in ACF via immunohistochemistry. All dysplastic ACF were found to contain beta-catenin in the cytoplasm and nucleus while a decrease in membrane staining was noted. Yamada's group has also described cytoplasmic and nuclear immunostaining for beta-catenin in dysplastic crypts found in AOM-injected rats (43-44). The beta-catenin protein appears to be readily detectable in normal colonic tissue as well as in ACF. Evidence suggests that altered localization of the protein may be a characteristic of the promotion phase of carcinogenesis. While several studies have

highlighted the effects of AOM injection on beta-catenin, to date no evidence exists as to how ionizing radiation may affect its localization.

Dietary studies. While diets high in fat are generally thought to be cancer promoting, recently attention has been given to the type of fat included in the diet. Latham et al. (45) found that animals fed diets containing fish oil for 48 h after DMH injection had fewer ACF than animals fed diets containing corn oil and given similar injections. Rao et al. (46) found that AOM-injected rats fed diets containing 17% fish oil had fewer high-multiplicity ACF compared to injected rats consuming a mixed lipid diet. Coleman et al. (47) found that AOM-injected rats fed diets containing fish oil had fewer high-multiplicity ACF compared to injected rats fed diets containing sunflower oil, which is high in n-6 polyunsaturated fatty acids. Fish oil's ability to prevent ACF formation may be due in part to the fact that fish oil has been shown to upregulate apoptosis in colonocytes (48). Chang et al. found that fish oil treatment resulted in a 33% increase in apoptosis compared to corn oil treatment in rats terminated 15 wk after receiving their second AOM or saline injection (48). The same group had previously reported (49) that fish oil treated rats had a larger apoptotic index than corn oil treated rats, regardless of carcinogen treatment, however this difference did not reach significance ($P=0.0751$). They reported in this same study an apparent synergistic effect of fish oil and pectin on apoptosis. Analysis of the apoptotic index showed that diets containing fish oil/pectin had a greater ($P=0.0215$) apoptotic index compared to diets containing corn oil/pectin (49).

Fiber has also received attention as a dietary chemopreventive agent due to epidemiological evidence suggesting a decreased risk of colon cancer with increased fiber

intake (5-7). Shivapurkar et al. (50) found that AOM-injected rats fed a high-fiber diet had fewer aberrant crypts compared to injected rats fed a low-fiber diet. Zoran et al. (51) found that AOM-injected rats fed a diet containing wheat bran as the fiber source had less tumors than rats fed a diet containing oat bran as the fiber source. Thorup et al. (52) have also found that high fiber diets prevented ACF formation in DMH-injected rats. Hong et al. (20) compared the effects of cellulose and pectin fiber on proliferation and apoptosis in rats that received 2 AOM or saline injections and were terminated 6 wk after the final injection. They found that cellulose treatment resulted in an expansion of the proliferative zone compared to pectin, yet there was no difference in the proliferative index. However, contrary to other works, they did not find any effect of fiber type on apoptosis (20). While it is appears that fiber intake is protective against colon cancer development in general, it remains to be determined which type of fiber is the most protective and whether it depends on the stage of cancer development.

Ionizing radiation. Ionizing radiation is present in outer space in a variety of forms, including high-energy protons, heavy ions as well as neutrons that are released from atomic nuclei by nuclear reactions occurring in outer space. Many of these particles attain extremely high velocities in outer space and are known as high linear energy transfer (LET) radiation (53-54). It is not possible to completely shield astronauts from this high LET radiation due to limitations on the weight of the shuttle (54). High LET radiation is carcinogenic because of its ability to penetrate cell barriers and deposit energy within cell nuclei, where it can cause DNA damage. The oxidative damage to DNA caused by radiation can result in single- or double-strand breaks. If cells containing this

damage continue to proliferate, subsequent generations of cells will likely contain mutations; a major initiating event in the carcinogenic process (55-56). It is known that astronauts are exposed to much higher doses of ionizing radiation than terrestrial workers and thus may have an increased risk for developing cancer (53).

A review of the risks associated with long-term space flight by Turner et al. (54) highlighted areas where potential health risks may be lowered via dietary modification. The specific risk of colon cancer was noted as a target for nutritional countermeasures because of the extensive epidemiological evidence correlating dietary habits with cancer incidence (54). One study on tumor formation in rats exposed to radiation and carcinogen injection found a greater number of tumors in rats given both injection and radiation as opposed to injection only (57). However, this study did not have a diet component and examined the tumor phase of carcinogenesis (57). There are currently no studies examining the effects of diet and irradiation on the promotion phase of chemically induced colon carcinogenesis.

Summary. Diet has been shown to play a key role in the development of colon cancer. Experimental studies have illustrated the protective effects of dietary components such as fish oil and fiber on the colon carcinogenesis. Studying biomarkers such as aberrant crypts, proliferation rates and apoptosis in experimentally induced colon cancer allows us to learn more about the pathogenesis of colon cancer as well as the role of diet can play in slowing or halting the process. The irradiation component of this project will help us understand the potential synergistic effects irradiation may have on these variables as well as identifying potential nutritional countermeasures to radiation exposure.

Hypothesis. Ionizing radiation will act synergistically with the colon-specific carcinogen AOM and lead to an increase in aberrant crypt foci formation by increasing proliferation and decreasing apoptosis compared to the non-irradiated animals. Colonic crypts of irradiated animals will also have more beta-catenin protein localized to the nucleus compared to the crypts of non-irradiated animals.

Specific Aims

1. Determine the effects of diet and irradiation on the formation of high-multiplicity aberrant crypt foci.
2. Determine the effects of diet and irradiation on the levels of apoptosis and cell proliferation.
3. Determine if the increase in aberrant crypt formation in irradiated animals may be due to an accumulation of beta-catenin protein in the nucleus.

CHAPTER II

MATERIALS AND METHODS

Experimental design. Animal protocols used for this study were approved by the Institutional Animal Care Committees of Texas A&M University and Brookhaven National Laboratory and conform to the guidelines of the National Institutes of Health. Eighty, 21 d old Sprague-Dawley rats (Harlan Sprague-Dawley, Houston, TX) were housed in a temperature and humidity controlled facility with a 12 h light-dark cycle. After a 1 wk acclimatization period, the rats were stratified by body weight and assigned to one of four experimental diets consisting of either fish or corn oil as the fat source, and either cellulose or pectin as the fiber source. After 3 wk of receiving the experimental diet 40 rats were exposed to 1 Gy (1 GeV/nucleon Fe) of ionizing radiation at the Alternating Gradient Synchrotron facility of the Brookhaven National Laboratory (Upton, NY). The other 40 rats served as radiation controls. All of the rats were injected with the colon specific carcinogen Azoxymethane (AOM, Midwest Research Institute, Kansas City, MO, 15 mg/kg) 10 d after irradiation. A second injection was given 1 wk later. Animals were terminated 6 wk after receiving the second injection. Rat weights were recorded prior to each injection and the day before termination. Food intake was also measured for a 48 h period before the first injection and again prior to termination. Rats had free access to food and deionized water throughout the study, and health checks were performed daily. The four diet groups (fish oil or corn oil and cellulose or pectin) and two radiation treatments (irradiated and nonirradiated rats) represent a 4X2 factorial design.

Radiation. After receiving the experimental diets for 3 wk, 40 rats were exposed to a single dose of approximately 1 Gy, 1GeV/nucleon Fe-56 ions at the Alternating Gradient Synchrotron/Relativistic Heavy Ion Collider (AGS/RHIC) facility at Brookhaven National Laboratory (Upton, NY). Each rat was immobilized in a poly-methyl-methacrylate cylindrical holder with walls 3 mm thick during the procedure. The holders were clear and had ventilating holes surrounding the rats. Prior to irradiation, rats were placed in the individual holders and mounted on a supporting plexi-glass block for stabilization. The beam of radiation was directed at the abdomen of the rats, which were treated in pairs. Rats were returned to their cages immediately following radiation.

Diets. All diets contained dextrose as the carbohydrate source with equal amounts of either pectin or cellulose as the fiber source. The oils used in the diets were 15% by weight and 30% of the caloric content of the diet. The fiber used in the diets was 6% by weight and corresponds to a 30-g/d intake in humans. Corn oil diets used only corn oil as the source of fatty acids. Fish oil diets used 11.5 g of fish oil per 100 g of diet with the remaining fat content made up of 3.5 g of corn oil in order to meet the essential fatty acid requirements of the animals. Vitamin E, although present endogenously in corn oil, was added to fish oil diets to ensure equality throughout all diets. Antioxidants were added to all diets equally as well. Table 1 gives the complete composition of the experimental diets.

Table 1

Composition of experimental diets.

Component	Quantity – g/100g	
Dextrose[1]	51.06	
Casein[1]	22.35	
Cellulose/Pectin[1]	6.00	
Mineral mix[1]	3.91	
Vitamin mix[1]	1.12	
DL-Methionine[1]	0.34	
Choline Bitartate[1]	0.22	
Fat	Corn oil diet	Fish oil diet
Corn oil[2]	15.00	3.50
Fish oil[2]	0.00	11.50
Antioxidants		
MT-70[3]	0.00	0.015
Tenox 20A[4]	0.005	0.005

[1] Harlan Teklad (Madison, WI)
[2] Degussa (Waukesha, WI)
[3] Archer-Daniels Midland (Decatur, IL)
[4] Gillco (Vista, CA)

Tissue sample collection. Six weeks after the second AOM injection rats were euthanized by CO_2 asphyxiation and cervical dislocation. The entire colon was resected and rinsed with RNase free PBS. One centimeter of the proximal and distal colon was taken for fixation in 4% PFA. An additional 1 cm was taken from the proximal and distal portion for ethanol fixation. Tissues fixed in EtOH and PFA were used for immunohistochemistry. The remaining colon was then cut in half longitudinally. One half was used for the ACF assay and the other half was scraped for RNA isolation (Figure 1).

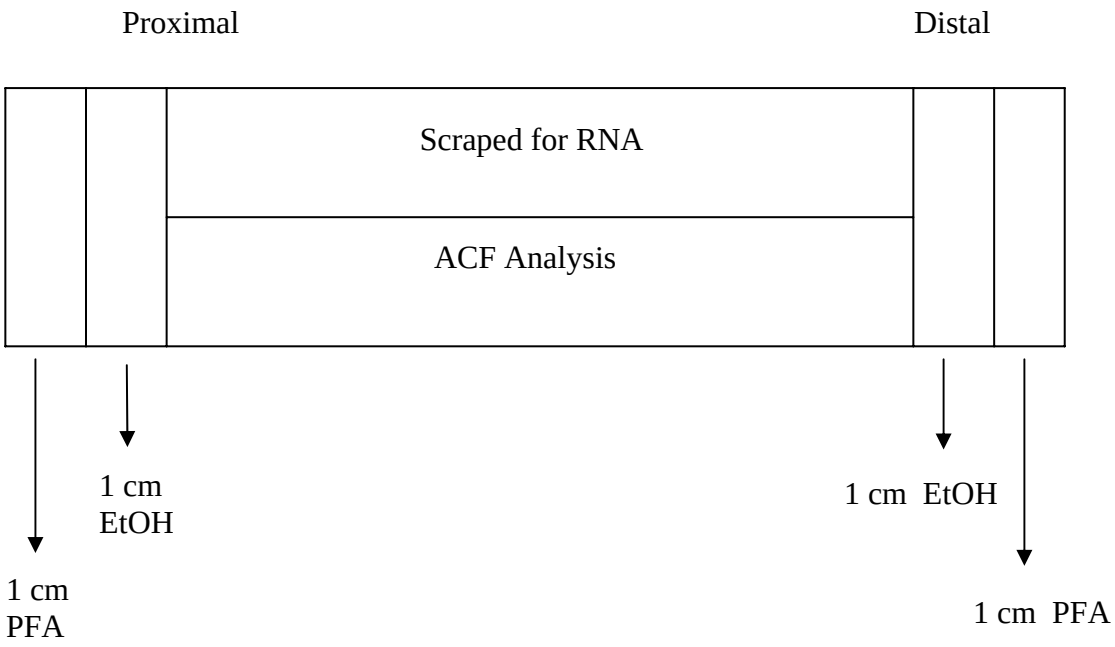

Figure 1 Illustration of tissue sample collection.

Aberrant crypt foci assay. Aberrant crypts are larger and have irregular openings when compared to normal surrounding crypts and are present in rats that have been given the colon specific carcinogen AOM. For determination of ACF, the colon was removed and cut longitudinally and placed in a folded sheet of Whatman #1 filter paper and fixed in 70% ethanol for 24 h. Colons were then dipped into 0.5% methylene blue for 45 s and viewed under the light microscope at 40X magnification. PBS was dabbed onto the colons periodically during scoring to prevent the tissue from becoming dry and stiff. Total number of aberrant crypts as well as the number of high multiplicity aberrant crypt foci (ACF with four or more aberrant crypts per foci) was determined for each animal.

Apoptosis assay. Apoptosis was measured by the binding of TdT-mediated dUTP –biotin nick end labeling (TUNEL assay) (Intergen, Norcross, GA) to fragmented pieces of DNA. Paraformaldehyde-fixed, paraffin embedded tissue sections were deparaffinized and rehydrated. Tissues were incubated in Proteinase K (10 μg/ml PBS solution) (Ambion, Austin, TX) at 37°C in order to break any crosslinks and retrieve the antigen binding sites. Endogenous peroxidase activity was quenched by immersing sections in 0.3% H_2O_2 in methanol for 30 min. Tissues treated with DNase 1 (Ambion) instead of TdT enzyme were used as positive controls. Equilibration buffer was applied to each section for 15 min at room temperature. About 50 μl of TdT:PBS (1:50 v/v) was applied to each section, and then incubated at 37°C for 1 h. Negative control sections received only the reaction buffer. Anti-digoxigenin peroxidase was then applied to all sections for 30 min at room temperature. Tissues were stained with 0.05% DAB (Sigma, St. Louis, MO) for 30 s and counterstained by dipping quickly into 0.05% methyl green. Following

dehydration in ethanol, slides were wet mounted with 80% Permount (Fisher, Fair Lawn, NJ). Apoptotic cells were identified by the brown stain of their nuclei as well as the morphological characteristics described previously (15). Negative controls were viewed under the microscope and had no brown staining. Positive control sections incubated with DNase 1 were viewed and found to be stained completely brown as expected. Crypt column height in number of cells as well as the number and position of apoptotic cells were recorded. The apoptotic index was determined by dividing the number of apoptotic cells per column by the total number of cells in the column and multiplying by 100 (58). Figure 2 shows apoptotic cells identified by a brown stain against the background stain of methyl green.

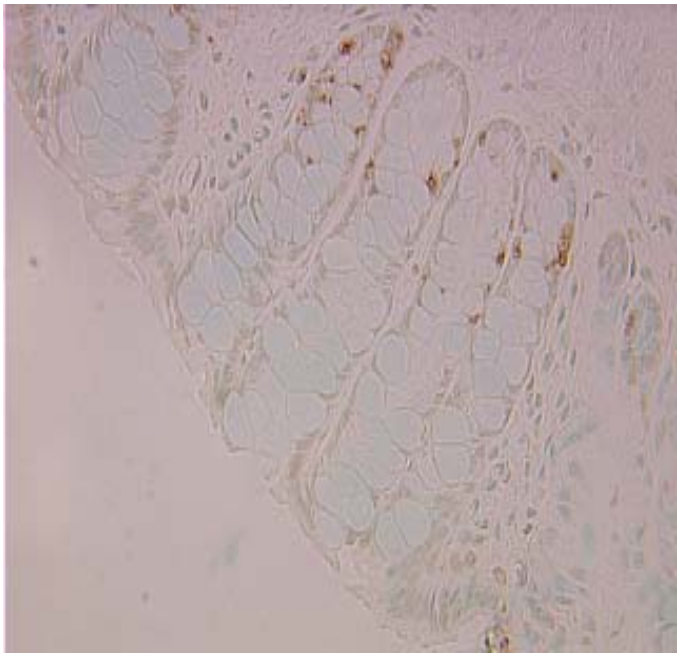

Figure 2 Apoptosis.

Proliferating cell nuclear antigen (PCNA) assay. Tissue taken from the proximal and distal colon of the rat was fixed in 70% ethanol, embedded in paraffin and mounted onto glass slides in 4 μm sections. The deparaffinized and rehydrated tissue was incubated with 150 μl PCNA monoclonal antibody, anti-PC – 10:PBS (1:10 v:v) (Signet Laboratories, Inc., Dedham, MA). Subsequently, tissue sections were incubated with biotinylated antimouse IgG using a Vectastain ABC Elite kit (Vector Lab, Burlingame CA). Negative control tissues were prepared by incubation with PBS instead of the anti-PC-10 antibody. Sections were then stained with diaminobenzidine tetrehydrochloride (DAB; Sigma Chemical) and counterstained with hematoxylin (Sigma Chemical). PCNA containing nuclei show up as brown spots within crypt columns, indicating a proliferating cell. When viewed under the light microscope, negative control sections lacked any brown staining. Twenty-five crypt columns were counted per tissue and the number and proportion of cells per crypt column and proliferating cells per crypt column was determined (59). Figure 3 shows colonic cells stained for PCNA, which have brown nuclei.

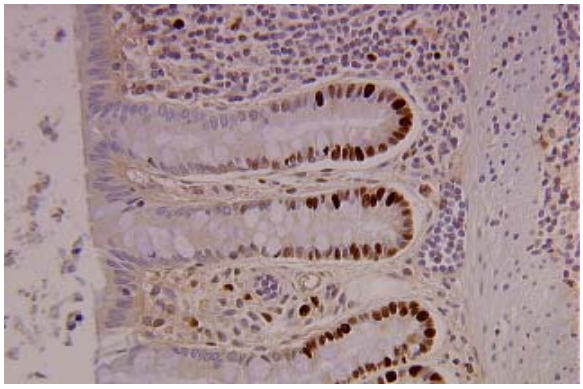

Figure 3 PCNA.

Beta-catenin assay. Beta-catenin staining was performed in a manner similar to the procedure outlined by Takahashi et al. (42). A 1 cm section of the distal portion of the colon was removed from the whole-mount colon and embedded in paraffin. Slides of fixed tissue were then deparaffinized and rehydrated in xylene and graded ethanol washes. Endogenous peroxidase was quenched by incubation in H_2O_2 and methanol for 30 min. Endogenous avidin and biotin were blocked by incubation with an Avidin/Biotin blocking kit (Vector Lab). Each section was then incubated overnight with 50 μl of mouse anti-beta-catenin antibody:PBS (1:100 v:v) (BD Pharmingen, San Diego, California) in a cold room at 4˚C. PBS was used instead of the primary antibody on negative control sections. Tissues were then incubated with a biotinylated anti-mouse IgG using a Vectastain ABC Elite kit (Vector Lab). Visualization of target staining was done by DAB (Sigma Chemical). Negative control sections did not have any staining when viewed under the light microscope. Tissues from nonirradiated and irradiated animals were assayed at a variety of antibody dilutions until a consistent staining pattern was observed under the light microscope. The staining intensity of each nucleus in 25 crypt columns was measured using NIH Image Analysis software. Figure 4 shows colonic cells stained for Beta-catenin.

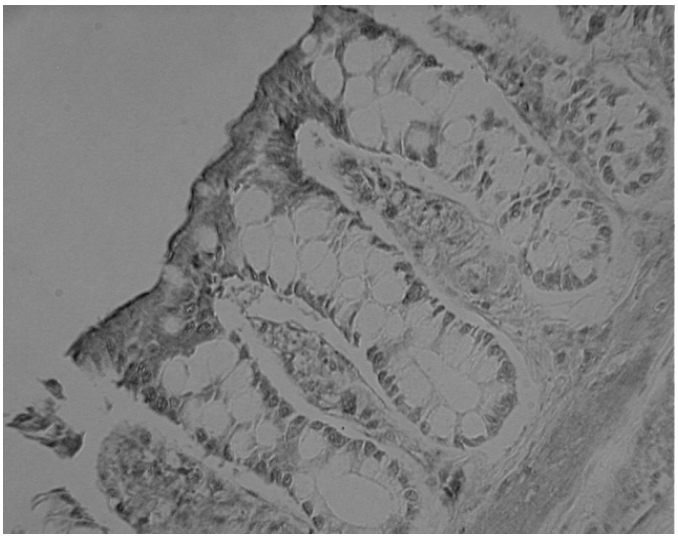

Figure 4 Beta-catenin.

Statistical analysis. The four diet groups (fish oil or corn oil and cellulose or pectin) and two radiation treatments (irradiated and nonirradiated rats) represent a 4X2 factorial design. Aberrant crypt foci data was analyzed by an ANOVA in SAS using proc glm. Apoptosis data was analyzed by an ANOVA in SAS using the proc mixed procedure. Proliferation data was analyzed using a mixed model is SAS. Beta-catenin data was analyzed using the mixed procedure in SAS (SAS Institute Inc., Cary, NC).

CHAPTER III

RESULTS

Food intake and weight gain. Food intakes were measured and recorded twice during the study. Food intake was measured over a 48 h period prior to the first AOM injection and again prior to termination. No significant differences were found between any of the diet or radiation groups during the first or second intake measurement periods, and there was no significant difference between the first and second intake measurements for any group. Actual intake values are available in the Appendix A.

An analysis of the weight gain data revealed an interaction between fiber and radiation, thus these variables will only be discussed together. Radiation had no effect on weight gain in rats consuming cellulose. However, nonirradiated rats consuming diets containing pectin gained more weight than the irradiated rats receiving pectin diets (Table 2). Data comparing all diet groups and radiation treatment are included in the Appendix A.

Table 2

Effect of radiation and dietary fiber on weight gain (g)[1].

	Irradiated	Nonirradiated
Cellulose	$340.87 \pm 7.02^{a,b}$	$348.84 \pm 7.02^{a,b}$
Pectin	326.75 ± 7.02^{a}	368.01 ± 7.21^{b}

[1]Effect of radiation and fiber on weight gain. Values are LS means ± SEM, $n = 79$. Irradiated pectin group and nonirradiated pectin group are different at $P < 0.05$.

High multiplicity aberrant crypt foci. Ionizing radiation increased (P=0.0327) the incidence of high-multiplicity (HM) ACF (foci containing four or more aberrant crypts) compared to the nonirradiated rats. Irradiated rats had about 30% more HM ACF compared to the nonirradiated rats (Figure 5). Animals receiving the corn oil/cellulose diets had the greatest number of HM ACF, which was higher (P=0.0007) than fish oil/cellulose and fish oil/pectin (P=0.0002) but not different from corn oil/pectin. Additionally, rats fed corn oil/pectin diets had more HM ACF than rats fed fish oil/pectin (Figure 6).

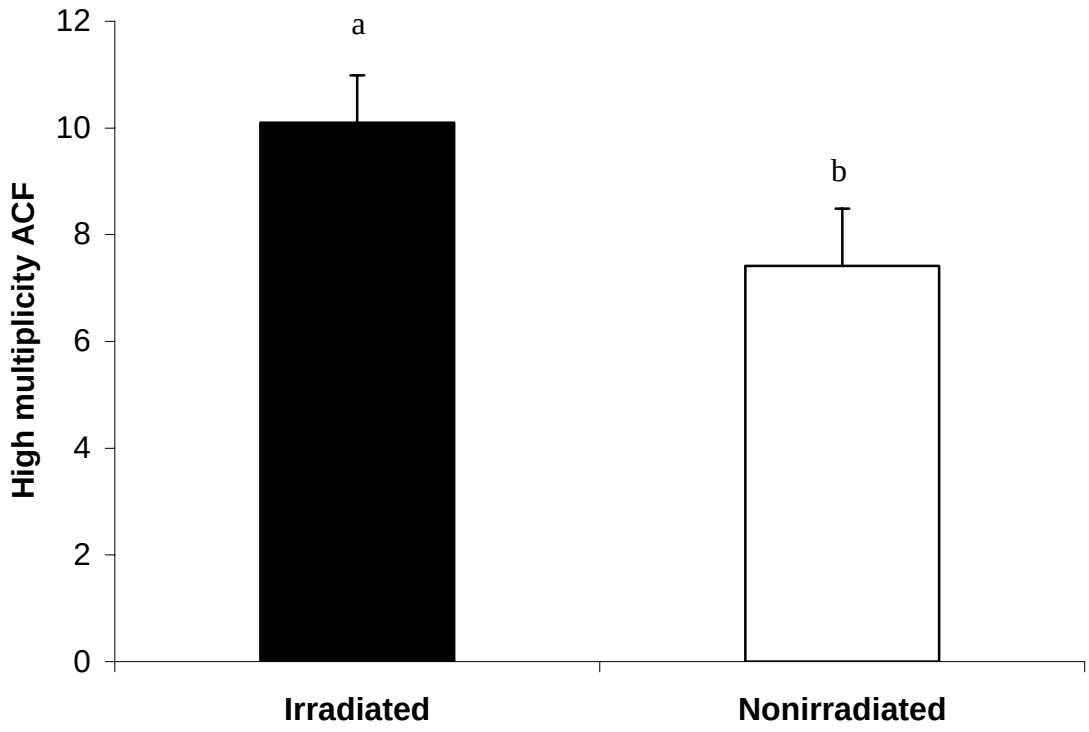

Figure 5 Effect of ionizing radiation on the formation of high-multiplicity ACF. Values are expressed as LS means ± SEM, $n = 60$. Irradiated and nonirradiated are different at $P < 0.05$.

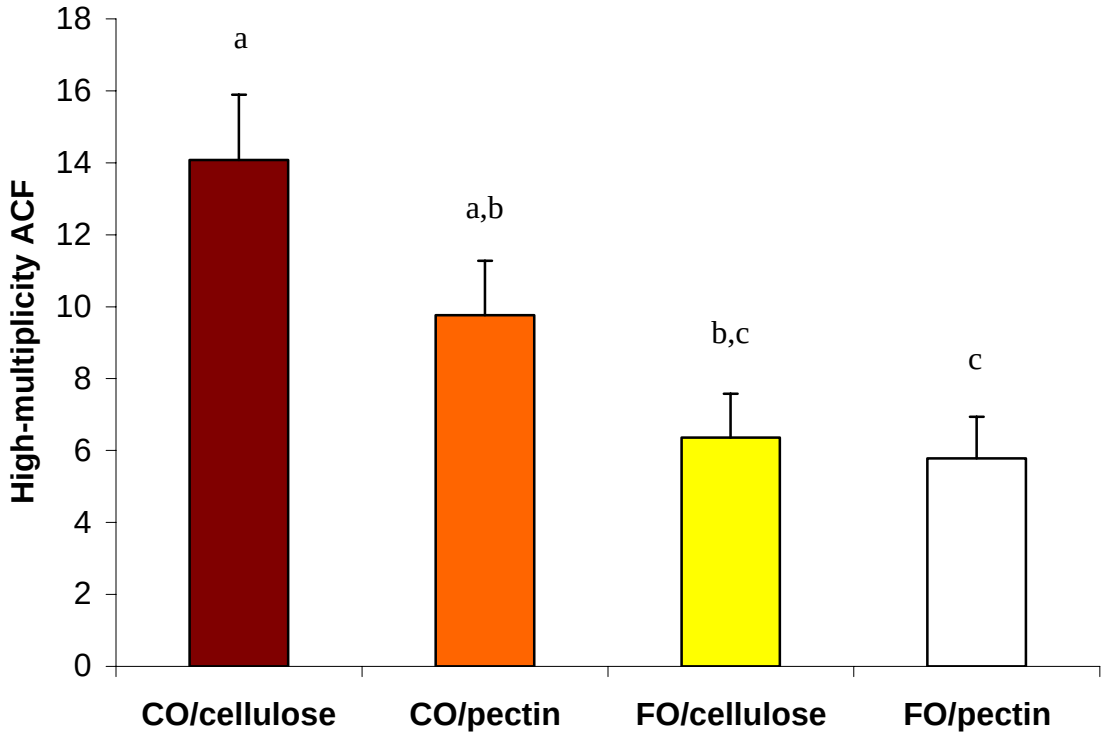

Figure 6 Effect of diet on the formation of high-multiplicity ACF. Values are expressed as LS means ± SEM, $n = 60$. Means without a common superscript differ at $P < 0.005$.

When the individual components of the diets were analyzed, animals fed diets containing corn oil had almost twice as many HM ACF as animals fed diets containing fish oil (P=0.0002) (Figure 7). Although the difference was not significant, rats fed diets containing pectin had a mean number of HM ACF that was 20% lower than rats fed diets containing cellulose (see Appendix).

Within the irradiated group, rats receiving the corn oil/cellulose diets had a mean HM ACF that was more than twice the mean of rats fed diets containing fish oil/cellulose (P=0.0004) and rats fed diets containing fish oil/pectin (P=0.0002) (Figure 8). Within the

nonirradiated group, rats receiving fish oil/pectin diets had a lower (P=0.0478) mean than rats receiving corn oil/cellulose diets (Figure 8). Radiation treatment did not result in a significant increase in HM ACF within any of the diet groups and there was no interaction between the variables diet and radiation. No other comparisons within the nonirradiated group resulted in significant differences, and there was not a significant effect of oil or fiber alone in the nonirradiated group.

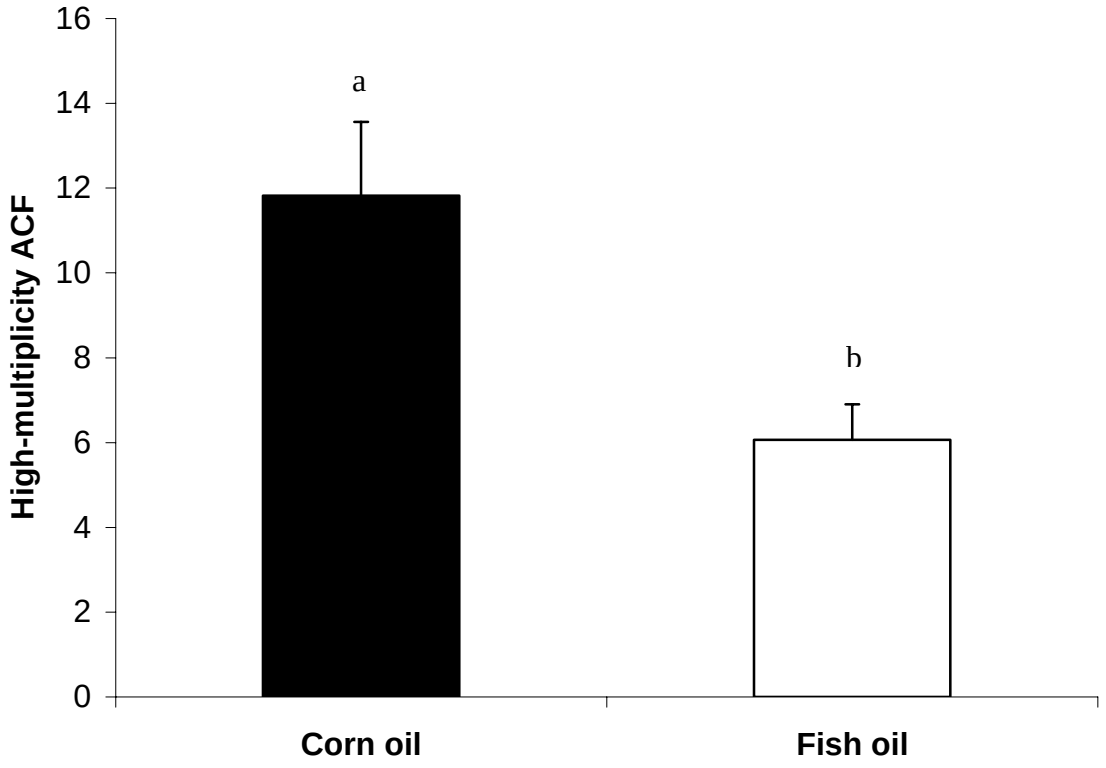

Figure 7 Effect of dietary fat on the formation of high-multiplicity ACF. Values are expressed as LS means ± SEM, $n = 60$. Corn oil is different from fish oil at $P < 0.05$.

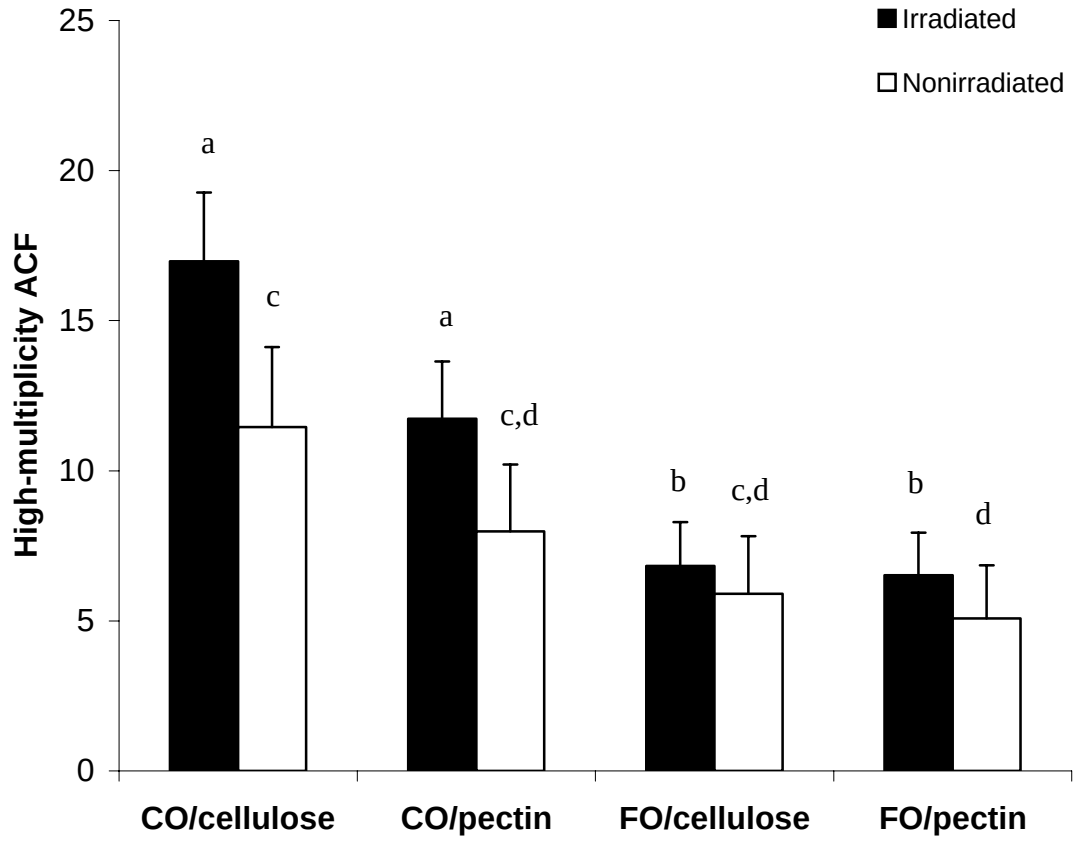

Figure 8 Effect of diet and radiation on the formation of high-multiplicity ACF. Values are expressed as LS means ± SEM, $n = 60$. Different sets of letters are used to show significant effects at $P < 0.05$ of the diets within respective radiation treatments. There were no significant differences between radiation treatments within any diet group, and no interaction between any of the variables displayed in the graph.

Apoptosis. Ionizing radiation caused a 50% decrease (P=0.0340) in the apoptotic index compared to the nonirradiated animals (Figure 9). Animals fed fish oil/pectin and corn oil/pectin both had apoptotic indices that were higher (P=0.0274 and 0.0451, respectively) than animals fed corn oil/cellulose diets (Figure 10). The apoptotic index of animals fed fish oil/cellulose did not differ from any of the other three diets. The

differences in apoptotic index was primarily in response dietary pectin (P=0.0204) (Figure 11). Rats receiving corn oil/cellulose diets consistently had the lowest apoptotic index within each radiation group, although the differences were not significant. Additionally, rats receiving the fish oil/pectin diets had the highest overall apoptotic index in both radiation treatment groups (actual values included in the Appendix A).

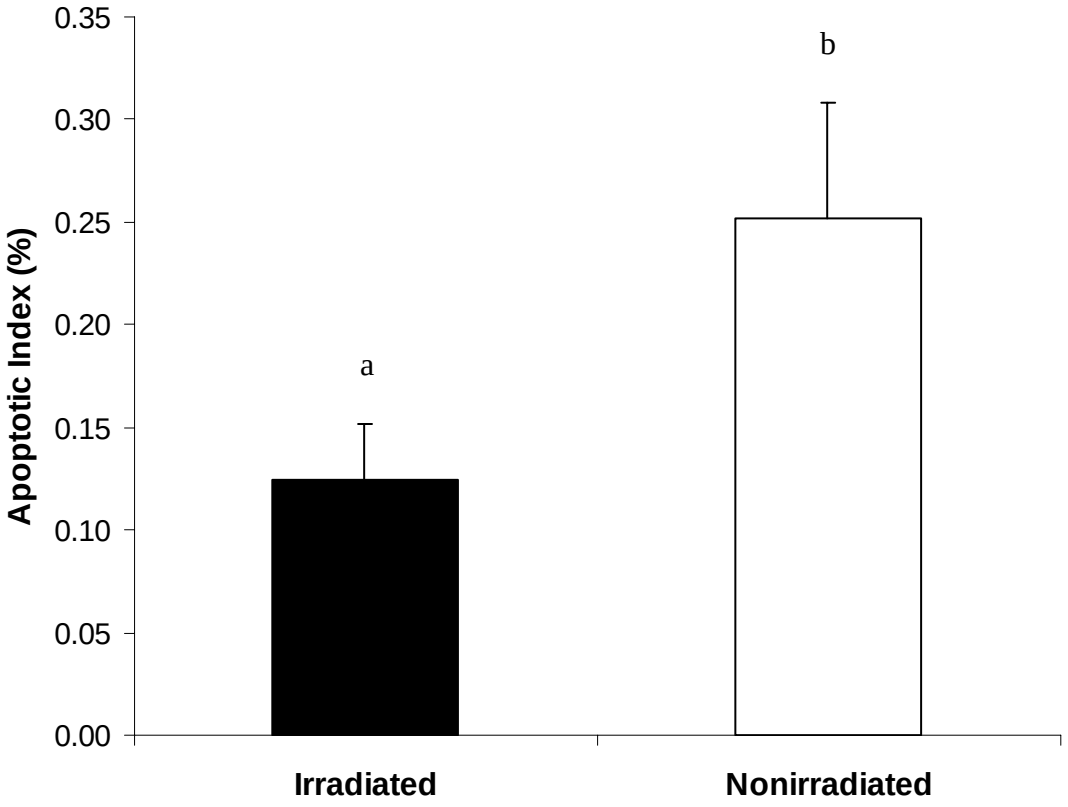

Figure 9 Effect of ionizing radiation on apoptotic index. Values are expressed as LS means ± SEM, $n = 60$. Irradiated and nonirradiated are different at $P < 0.05$.

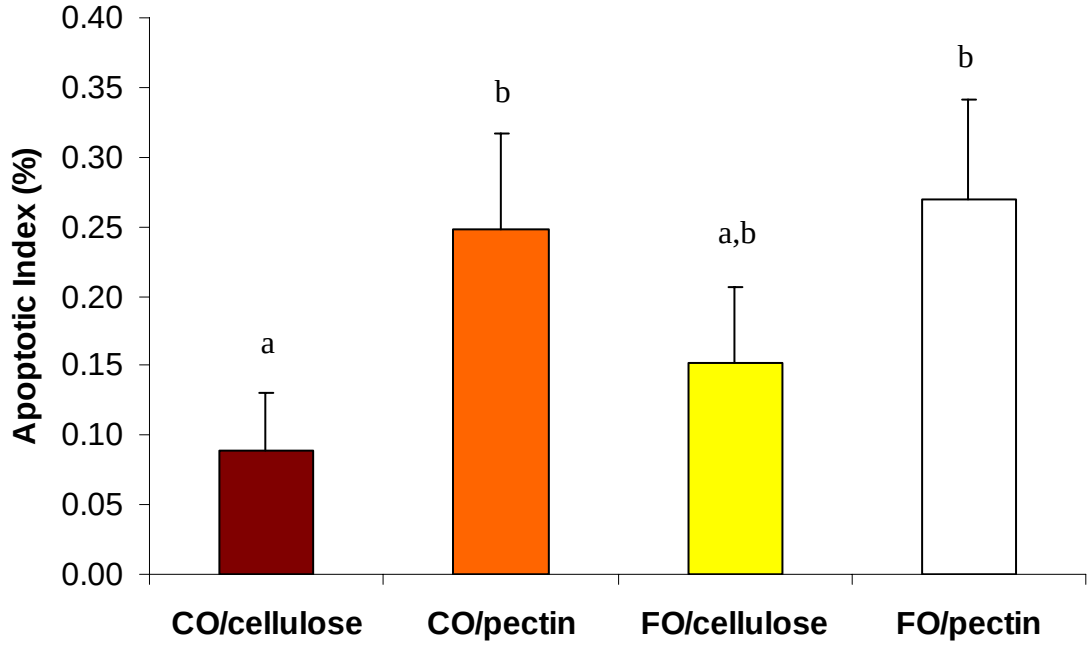

Figure 10 Effects of diet on apoptotic index. Values are expressed as LS means ± SEM, $n = 60$. CO/pectin and FO/pectin are different form CO/cellulose at $P < 0.05$.

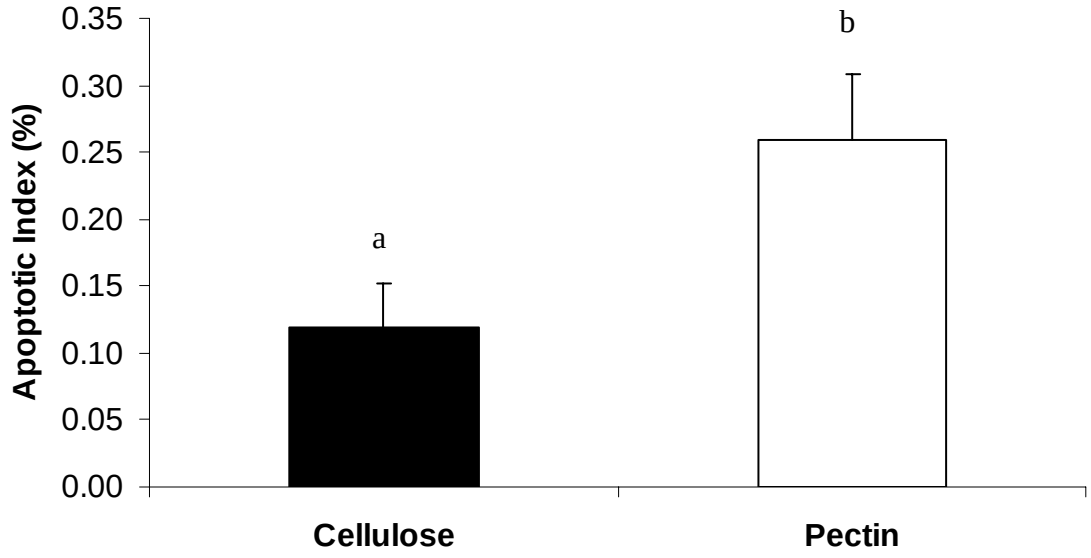

Figure 11 Effect of dietary fiber on apoptotic index. Values are expressed as LS means ± SEM, $n = 60$. Cellulose and pectin are different at $P < 0.05$.

Proliferative index. Radiation caused an increase (P=0.0001) in the proliferative index compared to nonirradiated rats (Figure 12). Diets had no significant effect on the proliferative index, although animals receiving diets containing fish oil had a lower mean index compared to the animals receiving corn oil diets (see Appendix A).

Extent of the proliferative zone. A significant interaction was found between radiation and oil (P=0.0253) when the extent of the proliferative zone was analyzed, therefore these two factors can only be considered together. Figure 10 illustrates the extent of the proliferative zone with the variables radiation and fat type. Radiation treatment resulted in an expansion of the proliferative zone in rats consuming corn oil and fish oil diets, such that they were not different from each other, but different from the nonirradiated rats (Figure 13). Nonirradiated rats consuming corn oil or fish oil diets had a smaller proliferative zone compared to irradiated rats consuming corn oil (P=0.004) or fish oil (P=0.0022). Additionally, nonirradiated rats consuming fish oil diets had a smaller (P<0.0001) proliferative zone compared to irradiated rats consuming either oil. Fish oil diets also resulted in a smaller (P=0.0109) proliferative zone compared to corn oil diets among rats not receiving radiation. Actual values are available in Appendix A.

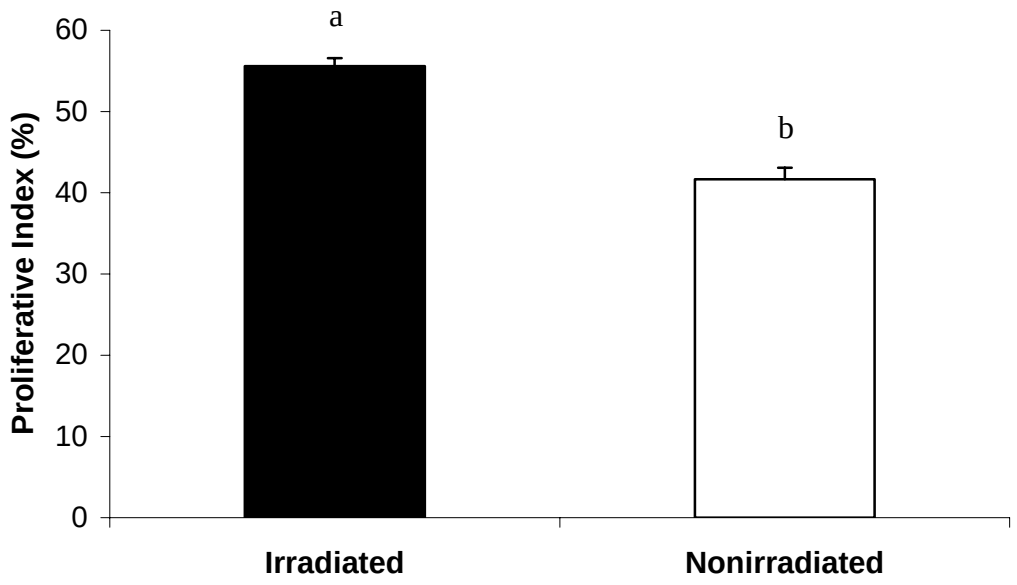

Figure 12 Effects of ionizing radiation on proliferative index. Values are expressed as LS means ± SEM, $n = 60$. Irradiated and nonirradiated are different at $P < 0.05$.

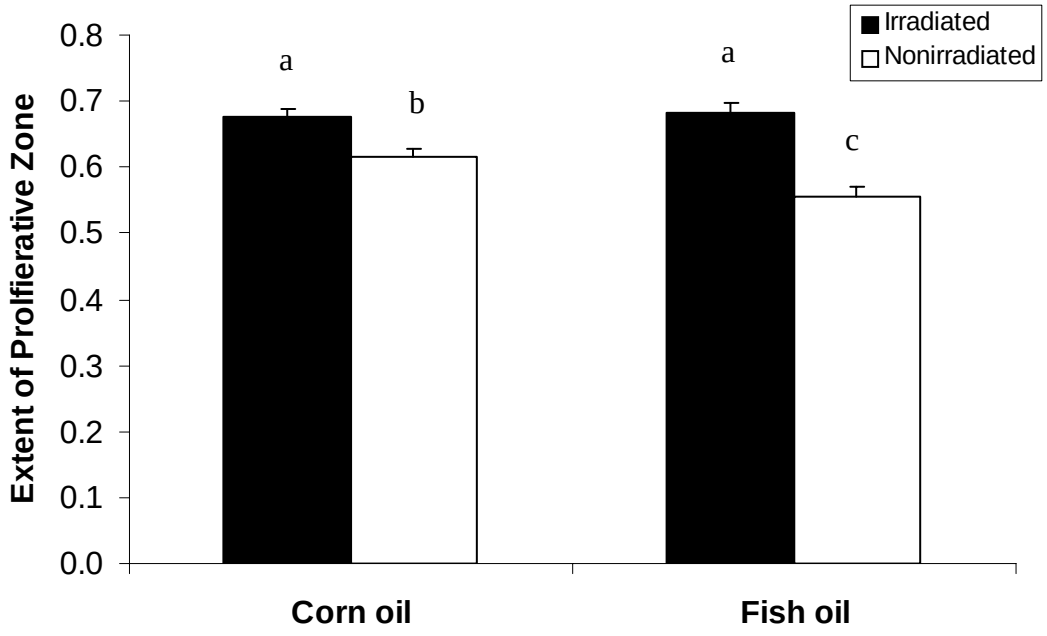

Figure 13 Effect of radiation and type of fat on the extent of the proliferative zone. Values are expressed as LS means ± SEM, $n = 60$. Columns not sharing superscripts differ at $P < 0.05$.

Beta-catenin. Colonic crypt columns were divided into tertiles for this analysis, with tertile one starting at the base of the crypt column. A significant (P = 0.0004) interaction was found between the factors tertile, radiation and oil, therefore these factors cannot be analyzed in isolation. Figure 14 shows the effects of all three factors. In the first two tertiles of the colonic crypt, nonirradiated rats fed diets containing corn oil had the highest staining intensity. However, no comparisons within either of these two tertiles yielded significance. Within the third tertile, nonirradiated rats fed diets containing fish oil had the highest staining intensity, although none of the comparisons within the third tertile yielded significance. Regardless of radiation treatment or type of dietary fat, there was a significant increase in the staining intensity within each group moving from tertile one to tertile three. This same effect was found for each treatment group when comparing tertile two to tertile three. There was not a consistent increase in Beta-catenin staining intensity across the experimental groups when the first tertile was compared to the second. Actual values are listed in Appendix A.

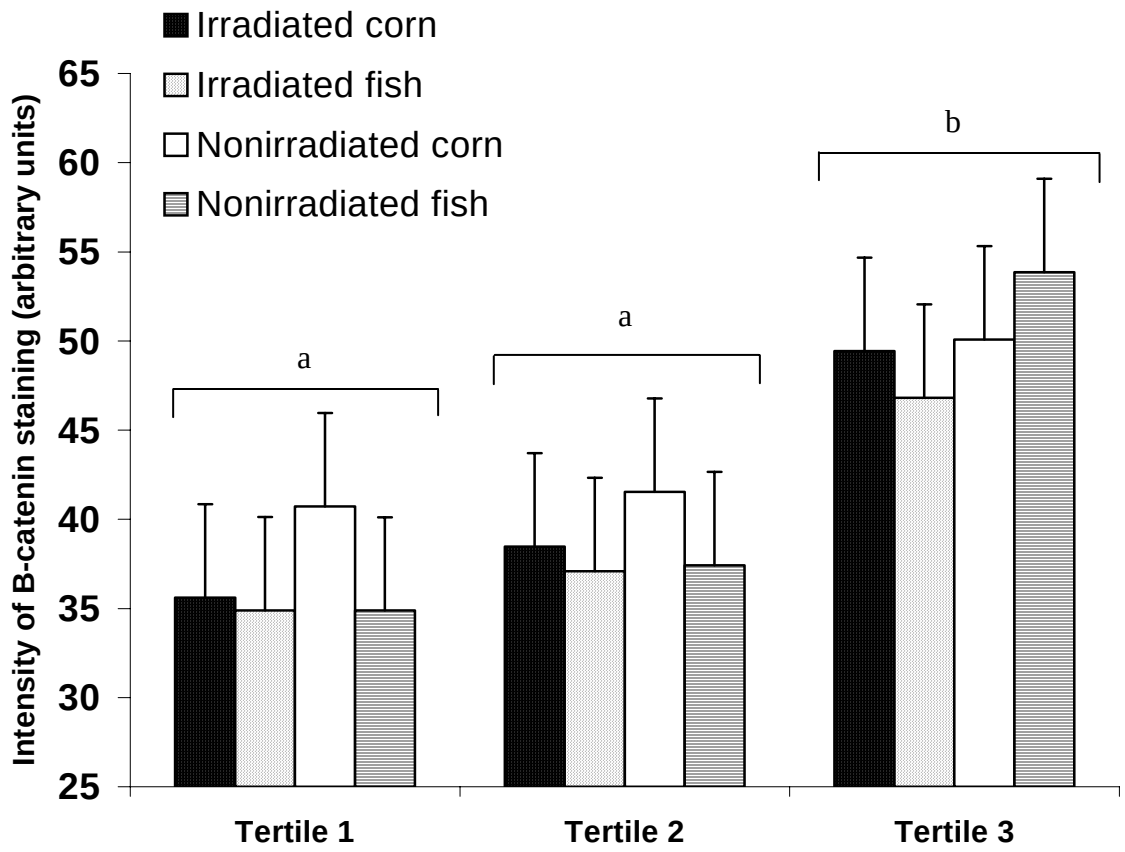

Figure 14 Effects of tertile, radiation and dietary fat on Beta-catenin staining intensity. Values are expressed as LS means ± SEM, $n = 40$. Columns not sharing superscripts differ at $P < 0.05$.

CHAPTER IV

SUMMARY AND DISCUSSION

One of the primary objectives of this study was to determine the effects of ionizing radiation on the formation of high-multiplicity aberrant crypt foci induced by a carcinogen injection. Our results support the hypothesis that radiation would lead to an increase in the formation of high-multiplicity aberrant crypt foci compared to animals that only received AOM injections. It is likely that this was caused by the increase in proliferation and decrease in apoptosis that resulted from the radiation treatment. It is commonly known that an imbalance between these two factors favors an increase in proliferation over apoptosis can lead to cancer development (14, 18, 19).

Effects of ionizing radiation on carcinogenesis. Sharp et al. (57) found that rats developed more colon tumors when treated with the carcinogen dimethylhydrazine (DMH) and 9 Gy of Co-60 radiation compared to rats that received either a single DMH injection or a single 9 Gy dose of radiation. Only 7% of the rats that received a single dose of radiation developed colon tumors, while 28% of the rats that received a single DMH injection developed tumors. They found a much higher incidence of tumor formation, 57%, when the treatments were combined. These results led them to the conclusion that though radiation may be a weaker carcinogen, it seemed to work synergistically with more potent carcinogens. Our study found that high-multiplicity ACF formation was increased by about 25% as a result of the radiation treatment before AOM injection. This implies that the majority of the lesions identified in rats that received both treatments may have been primarily the result of the carcinogen AOM.

The reason for the varied effects of these two treatments is likely due to the fact that they are carcinogenic in different ways. Radiation is known to cause oxidative damage to DNA via the formation of free radicals (53). Injected carcinogens such as AOM are known as alkylating agents, which lead to DNA adduct formation. DNA adducts cause base pair transitions during DNA replication and lead to mutations (58). Due to the differences found in high-multiplicity ACF formation, it would seem that AOM is a more potent carcinogen than ionizing radiation at the doses used in this study. Our study did not quantify the level of DNA damage caused by AOM or radiation, so it is not possible to discern which carcinogen was actually more harmful at the cellular level.

Two differences between our study and the work by Sharp et al. (57) were the radiation dose given and end point that was studied. Our rats were treated with 1 Gy 1 GeV/nucleon Fe-56 of radiation and were sacrificed at the promotion phase of carcinogenesis, 6 wk after receiving the second AOM injection. Sharp et al. (57) treated their rats with 9 Gy Co-60 radiation and terminated them at the tumor phase, 8 mo after receiving the final injection. Sharp et al. (57) also noted that tumors were only found in the colon. While it is not possible to directly compare high-multiplicity ACF and tumor formation because they are entirely different endpoints, it should be noted that the formation of high-multiplicity ACF has been found to be a good predictive indicator of future tumor development in the rat model (25).

While the increase in formation of high-multiplicity ACF in irradiated rats was due to a combination of the increased proliferation and decreased apoptosis, it appears that apoptosis may have had a more profound effect on this outcome. Nonirradiated rats had

an apoptotic index that was twice as high as the irradiated rats, while the proliferative index was about 50% higher in irradiated rats compared to nonirradiated rats. Hong et al. (20) found that the apoptotic index in the colons of rats receiving AOM injections was 40% lower than the index in rats that did not receive AOM injections. Additionally, Corpet et al. (30) have shown that rats treated with AOM exhibit higher levels of colonocyte proliferation when compared to rats that were not treated with AOM. This previous work, when taken together with our data, indicate that the alterations in cell cycle kinetics caused by carcinogen injection could be exacerbated by ionizing radiation. Our work supports the hypothesis that ionizing radiation would cause a decrease in apoptosis and increase in proliferation when compared to animals that only received AOM injections.

Effects of diet on colon carcinogenesis. The effects of dietary fat on colon carcinogenesis in experimental models have been discussed briefly in a review by Bird et al. (32). They noted that in several studies done in the 1970's, dietary corn oil was found to promote colon tumorigenesis, while dietary fish oil did not exhibit a promoting effect. The dietary component of our study yielded results that were similar to those found in previous work. We found that fish oil treatment was effective at significantly reducing the formation of high-multiplicity ACF compared to corn oil treatment, while the type of dietary fiber did not have a significant effect. Rao et al. (46) found that rats consuming a diet containing 17% fish oil and treated with two AOM injections developed fewer high-multiplicity ACF compared to injected rats that consumed a high-fat mixed lipid diet that contained no fish oil. Coleman et al. (47) also found that AOM-injected rats that

consumed diets containing 10% fish oil by weight developed fewer high-multiplicity ACF compared to rats that consumed diets that contained 10% sunflower seed oil by weight (47). This previous work supports the results of our study with regards to the effects of dietary fish oil and the formation of high-multiplicity ACF. When viewed collectively, these studies imply that fish oil may be one of the most protective lipid sources against colon carcinogenesis, as it has proven more effective at preventing colon cancer development than a variety of types of fats.

The fish oil/pectin diet resulted in the lowest number of high-multiplicity ACF, which was significantly lower than diets containing corn oil/cellulose and corn oil/pectin. Rats consuming the corn oil/cellulose diet had the highest incidence of high-multiplicity ACF in our study. These results are similar to those found previously in our laboratory. Chang et al. (49) studied the effects of the same diets on colon tumor formation in rats that received AOM injections using the same protocol used in the present study. They found that rats that consumed diets containing fish oil developed significantly fewer tumors when compared to rats that consumed diets containing corn oil. Although the type of dietary fiber did not have a significant effect on tumor formation, rats consuming the pectin diets had a lower tumor incidence compared to the rats consuming cellulose diets (49). When all of the diets were compared, they found that the corn oil/cellulose diet resulted in a highest tumor incidence, while the rats that consumed the fish oil/pectin diets developed the least number of tumors (49). Again it should be noted that the formation of high-multiplicity ACF has been shown to be predictive of tumor formation in the rat model (25). The work by Chang et al. (49) supports our results because in both studies the

rats received two AOM injections, 1 wk apart, and the experimental diets provided were identical with regards to the type and proportion of fat and fiber. The results from these two studies indicate that the type of dietary fat has a more profound impact on end point outcome compared to the type of dietary fiber. Our results have also shown that specific combinations of nutrients in the diet may confer a greater amount of protection than either nutrient alone. Both studies found that the type of fiber used did not have a significant effect on the formation of either high-multiplicity ACF or tumors.

Several studies have found fish oil to be protective of colon carcinogenesis due to its ability to upregulate apoptosis in the colonocyte (48, 49, 60). Two studies by Chang et al. (48-49) found that rats consuming diets containing fish oil and the fermentable fiber pectin had a higher level of apoptosis when compared to the individual effects of either nutrient alone. The data from these studies suggested that there may be a synergistic relationship between these two nutrients that allows them to be more effective inducers of apoptosis when combined in the diet. However, our results showed that fish oil did not have a significant effect on the apoptotic index, though the apoptotic index from rats fed fish oil diets was slightly higher than the index of rats fed corn oil diets. We did find that pectin fiber resulted in a higher apoptotic index compared to cellulose, regardless of the type of oil in the diet. Similar to the studies cited previously, diets containing fish oil and pectin had the highest apoptotic index, however this was only significantly higher than the apoptotic index of rats that consumed the corn oil/cellulose diet. It should be noted that rats consuming the fish oil/pectin diets had significantly fewer high-multiplicity ACF and a significantly higher apoptotic index than rats consuming the corn oil/cellulose diets and

this same dietary effect has been found in rats at the tumor phase of carcinogenesis (48). Therefore, one mechanism by which fish oil/pectin diets protect against the development of high-multiplicity ACF may be through an increase in apoptosis. It would seem that this effect is continuous into the tumor phase of carcinogenesis as well.

Effects of radiation and diet on colon carcinogenesis. Irradiated rats consuming either the fish oil or corn oil diet had an expanded proliferative zone, compared to nonirradiated rats consuming either type of fat. Within the nonirradiated group, rats consuming fish oil diets had a significantly smaller proliferative zone compared to rats consuming corn oil diets. However, the protective effect of the fish oil diets was lost if the rats received the radiation treatment, as the extent of the proliferative zone was nearly identical in irradiated rats consuming either type of oil. One previous study from our lab found that there was an expansion of the proliferative zone with pectin diets (20). However, they did not find an effect due to dietary fat, and there was no radiation treatment, therefore these previous results are hardly comparable.

Our results suggest that the diets used in our study did not confer any significant protection from the radiation-induced increase in proliferative activity. We did find that the fish oil diets were more protective than the corn oil diets in rats receiving only the AOM injections. These data suggest that the protective effect of the fish oil/pectin diets in nonirradiated rats could be due to the increase in apoptosis caused by pectin treatment, and the smaller proliferative zone that resulted from fish oil treatment compared to corn oil treatment. In the irradiated rats, those consuming fish oil/pectin diets had the lowest number of high-multiplicity ACF, which was only statistically different from rats

consuming corn oil/cellulose diets. The proliferative zone in rats consuming either fish or corn oil was not different in irradiated rats, and the proliferative index among the four diets varied little within the irradiated group. While there was a definite increase in proliferation and expansion of the proliferative zone in our irradiated rats regardless of diet, the data do not correlate well with our end point marker of high-multiplicity ACF.

Effects of radiation and diet on beta-catenin levels and localization. Beta-catenin has been implicated in colon carcinogenesis as part of the Wnt pathway (36). While no effects of diet or radiation alone were observed, we did find an interaction between radiation, fat type and crypt tertile. Crypts were divided into three tertiles of roughly equal numbers of cells with the first tertile designated as the one which began at the base of the crypt, and the third tertile being one-third of the cells at the lumen of the crypt. An analysis of the staining intensity of Beta-catenin found that tertile, radiation and fat type were involved in a three way interaction. We noted a significantly higher staining intensity in the third tertile when it was compared to the first tertile, regardless of radiation treatment or type of oil used in the diet. The same effect was observed for each group when we compared the third tertile to the second tertile. This implies that as cells are progressing up the crypt wall, Beta-catenin protein is present in increasing amounts in the cell nucleus. Nonirradiated rats consuming either diet had the highest staining intensity in the third tertile of cells, while the irradiated rats had the lowest intensity in this same tertile, though these differences were not significant. Our diets did not appear to have conferred protection from Beta-catenin accumulation in the nucleus. It seems that this increase in nuclear localization did not have a measurable effect on the end point used in

the study, as animals with the highest measured intensity at the top of the crypt had the lowest numbers of high-multiplicity ACF. Additional work needs to be done to determine the role of Beta-catenin in radiation enhanced carcinogenesis.

Several previous studies in the rat model have found that carcinogen injections cause Beta-catenin to be translocated from its usual location in the cytoplasmic membrane to the nucleus of cells (41-42). These studies found that the location of Beta-catenin was altered in ACF but not in normal colonic crypts (41-42). Additionally, neither of these two studies mentioned whether the presence of beta-catenin was altered based on location within individual crypts (41-42). Although our results are very different from those found previously, there were multiple differences in the way each study was conducted. Furihata et al. (41) used DMH injections at a dose of 20 mg/kg of body weight. Their rats were given 4 DMH injections over a period of 10 d. Our rats received 2 injections over a period of 7 d at a dose of 15 mg/kg of body weight. Thus some of the differences could likely be due to the dose of carcinogen the animals received. Takahashi et al. (42) sacrificed the animals in their study 36 wk after the final AOM injection, and only reported data on ACF tissue and tumors. These are several differences between previous studies and our work that could lead to very different staining patterns.

Overall, this study showed that diets containing fish oil and pectin are the most protective of high-multiplicity ACF development in rats that have received radiation treatment along with AOM injections. The fish oil/pectin diet resulted in significantly lower high-multiplicity ACF formation compared to corn oil/cellulose diets and this finding is supported by prior research using the tumor endpoint (49). However it is not

clear how these diets conferred this protection in the irradiated rats, as cell proliferation was markedly higher in the irradiated rats, regardless of diet. Pectin fiber was found to increase levels of apoptosis in nonirradiated animals, while irradiated rats consuming pectin diets did not have the same increases in apoptosis. Beta-catenin staining intensity increased as cells moved up the crypt column, and did not seem to be affected by diet or radiation in a manner that correlated with any of the other biomarkers.

Ionizing radiation has been shown to exacerbate the action of the colon-specific carcinogen AOM in this study. It also appears that certain dietary components may slow the promotion of colon carcinogenesis. These dietary components are well-studied in the rodent model of colon carcinogenesis. Our study shows that fish oil and pectin, which have been shown to be protective at various phases of colon carcinogenesis in previous studies, may help protect against radiation enhanced colon cancer development. Future work should focus on cellular mechanisms of radiation induced damage as well as how the potentially protective components of the diet inhibit colon carcinogenesis.

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APPENDIX A

Table 3

Effect of diet on food intake prior to first AOM injection[1].

Food Intake (g)	
Corn oil/cellulose	18.35 ± 1.00[a]
Corn oil/pectin	19.14 ± 1.03[a]
Fish oil/cellulose	19.97 ± 1.00[a]
Fish oil/pectin	20.88 ± 1.00[a]

[1]Values expressed LS means ± SEM, n = 79.

Table 4

Effect of radiation on food intake prior to first AOM injection[1].

Food Intake (g)	
Irradiated	19.46 ± 0.72[a]
Nonirradiated	19.70 ± 0.07[a]

[1]Values expressed as LS means ± SEM, n = 79.

Table 5

Effect of diet on food intake prior to termination[1].

	Food Intake (g)
Corn oil/cellulose	21.06 ± 0.66^{a}
Corn oil/pectin	20.17 ± 0.66^{a}
Fish oil/cellulose	20.49 ± 0.66^{a}
Fish oil/pectin	21.47 ± 0.66^{a}

[1] Values expressed as LS means ± SEM, $n = 59$.

Table 6

Effect of radiation on food intake prior to termination[1].

	Food Intake (g)
Irradiated	21.38 ± 0.38^{a}
Nonirradiated	20.21 ± 0.54^{a}

[1]Values expressed as LS means ± SEM, $n = 59$.

Table 7

Effect of radiation and diet on weight gain[1].

Weight Gain (g)		
	Irradiated	Nonirradiated
Corn oil/cellulose	333.70 ± 9.92^{a}	$353.08 \pm 9.92^{a,b}$
Corn oil/pectin	324.98 ± 9.92^{a}	$361.90 \pm 10.46^{a,b}$
Fish oil/cellulose	348.04 ± 9.92^{a}	344.60 ± 9.92^{a}
Fish oil/pectin	328.53 ± 9.92^{a}	374.12 ± 9.92^{b}

[1]Values expressed as LS means ± SEM, $n = 79$. Means not sharing a common superscript are significantly different at $P < 0.05$

Table 8

Effect of radiation on the formation of high-multiplicity ACF[1].

High-multiplicity ACF	
Irradiated	10.098 ± 0.886^{a}
Nonirradiated	7.416 ± 1.074^{b}

[1]Values are expressed as LS means ± SEM, $n = 60$. Means not sharing a common superscript are significantly different at $P < 0.05$.

Table 9

Effect of diet on the formation of high-multiplicity ACF[1].

	High-multiplicity ACF
Corn oil/Cellulose	14.08 ± 1.81^{a}
Corn oil/Pectin	$9.77 \pm 1.51^{a,b}$
Fish oil/Cellulose	$6.36 \pm 1.22^{b,c}$
Fish oil/Pectin	5.78 ± 1.16^{c}

[1]Values are expressed as LS means ± SEM, $n = 60$. Means not sharing a common superscript are significantly different at $P < 0.05$.

Table 10

Effect of dietary fat on the formation of high-multiplicity ACF[1].

	High-multiplicity ACF
Corn oil	11.82 ± 1.74^{a}
Fish oil	6.06 ± 0.84^{b}

[1]Values are expressed as LS means ± SEM, $n = 60$. Means not sharing a common superscript are significantly different at $P < 0.05$.

Table 11

Effect of dietary fiber on the formation of high-multiplicity ACF[1].

	High-multiplicity ACF
Cellulose	9.84 ± 1.07^{a}
Pectin	7.64 ± 0.94^{a}

[1]Values are expressed as LS means ± SEM, $n = 60$.

Table 12

Effect of radiation and diet on the formation of high-multiplicity ACF[1]**.**

	High-multiplicity ACF	
	Irradiated	Nonirradiated
Corn oil/cellulose	16.97 ± 2.30^{a}	11.45 ± 2.67^{c}
Corn oil/pectin	11.73 ± 1.91^{a}	$7.98 \pm 2.23^{c,d}$
Fish oil/cellulose	6.83 ± 1.46^{b}	$5.90 \pm 1.92^{c,d}$
Fish oil/pectin	6.52 ± 1.42^{b}	5.08 ± 1.78^{d}

[1]Values are expressed as LS means ± SEM, $n = 60$. Means not sharing a common superscript are significantly different at $P < 0.05$.

Table 13

Effect of radiation on apoptotic index[1].

	Apoptotic Index
Irradiated	0.12 ± 0.03^{a}
Nonirradiated	0.25 ± 0.06^{b}

[1]Values are expressed as LS means ± SEM, $n = 60$. Means not sharing a common superscript are significantly different at $P < 0.05$.

Table 14

Effect of diet on apoptotic index[1].

	Apoptotic Index
Corn oil/cellulose	0.089 ± 0.041^{a}
Corn oil/pectin	0.25 ± 0.07^{b}
Fish oil/cellulose	$0.15 \pm 0.05^{a,b}$
Fish oil/pectin	0.27 ± 0.07^{b}

[1]Values are expressed as LS means ± SEM, $n = 60$. Means not sharing a common superscript are significantly different at $P < 0.05$.

Table 15

Effect of dietary fat on apoptotic index[1].

	Apoptotic Index
Corn oil	0.159 ± 0.039^{a}
Fish oil	0.20 ± 0.04^{a}

[1]Values are expressed as LS means ± SEM, $n = 60$.

Table 16

Effect of dietary fiber on apoptotic index[1].

	Apoptotic Index
Cellulose	0.119 ± 0.034^{a}
Pectin	0.26 ± 0.05^{b}

[1]Values are expressed as LS means ± SEM, $n = 60$. Means not sharing a common superscript are significantly different at $P < 0.05$.

Table 17

Effect of radiation and diet on apoptotic index[1].

	Irradiated	Nonirradiated
Corn oil/cellulose	0.049 ± 0.085^{a}	0.14 ± 0.04^{c}
Corn oil/pectin	0.15 ± 0.14^{a}	0.37 ± 0.06^{c}
Fish oil/cellulose	0.15 ± 0.09^{a}	0.15 ± 0.06^{c}
Fish oil/pectin	0.17 ± 0.14^{a}	0.40 ± 0.06^{c}

[1]Values are expressed as LS means ± SEM, $n = 60$.

Table 18

Effect of diet on proliferative index[1].

	Proliferative Index
Corn oil/cellulose	49 ± 1.7^{a}
Corn oil/pectin	50 ± 1.7^{a}
Fish oil/cellulose	47 ± 1.7^{a}
Fish oil/pectin	49 ± 1.7^{a}

[1]Values are expressed as LS means ± SEM, $n = 60$.

Table 19

Effect of dietary fat on proliferative index[1].

	Proliferative Index
Corn oil	49 ± 1.2^{a}
Fish oil	48 ± 1.2^{a}

[1]Values are expressed as LS means ± SEM, $n = 60$.

Table 20

Effect of radiation on proliferative index[1].

	Proliferative Index
Irradiated	56 ± 1.0^{a}
Nonirradiated	42 ± 1.4^{b}

[1]Values are expressed as LS means ± SEM, $n = 60$. Means not sharing a common superscript are significantly different at $P < 0.05$.

Table 21

Effect of radiation and dietary fat on the extent of the proliferative zone[1].

	Extent of proliferative zone	
	Irradiated	Nonirradiated
Corn oil	0.68 ± 0.01^{a}	0.62 ± 0.02^{b}
Fish oil	0.68 ± 0.01^{a}	0.56 ± 0.02^{c}

[1]Values are expressed as LS means ± SEM, $n = 60$. Means not sharing a common superscript are significantly different at $P < 0.05$.

Table 22

ANOVA table of Beta-catenin data[1].

Effect	Num DF	Den DF	F Value	Pr > F
Radiation	1	32	0.27	0.6049
Oil	1	32	0.12	0.7272
Fiber	1	32	0.12	0.7266
Radiation x oil	1	32	0.00	0.9620
Radiation x fiber	1	32	0.04	0.8511
Oil x fiber	1	32	0.50	0.4843
Radiation x oil x fiber	1	32	1.63	0.2105
Tertile	2	64	205.01	<0.0001
Tertile x radiation	2	64	1.13	0.3302
Tertile x oil	2	64	4.23	0.0188
Tertile x fiber	2	64	0.20	0.8214
Tertile x radiation x oil	2	64	8.96	0.0004
Tertile x radiation x fiber	2	64	0.16	0.8487
Tertile x oil x fiber	2	64	0.15	0.8646
Tertile x radiation x oil x fiber	2	64	0.20	0.8164

[1]Statistical analysis of the effects of experimental variables. Pr > F values < 0.05 indicates significant interactions.

Table 23

Effect of tertile, radiation and dietary fat on Beta-catenin staining intensity[1].

	Irradiated		Nonirradiated	
	Fish oil	Corn oil	Fish oil	Corn oil
Tertile 1	34.9 ± 5.24^{a}	35.6 ± 5.24^{a}	34.9 ± 5.24^{a}	40.7 ± 5.24^{a}
Tertile 2	37.1 ± 5.24^{a}	38.5 ± 5.24^{a}	37.4 ± 5.24^{a}	41.5 ± 5.24^{a}
Tertile 3	46.8 ± 5.24^{b}	49.4 ± 5.24^{b}	53.9 ± 5.24^{b}	50.1 ± 5.24^{b}

[1]Values are expressed as LS means ± SEM, $n = 40$. Means not sharing a common superscript are significantly different at $P < 0.05$.

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