EFFECTS OF DIETARY CARBOHYDRATE ON THE EARLY STAGES OF COLON CARCINOGENESIS

by

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A thesis submitted to the Faculty of Graduate Studies in partial fulfillment of the requirements for the degree of

Master of Science

Department of Foods and Nutrition

University of Manitoba

Winnipeg, Manitoba

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ABSTRACT

The effects of dietary carbohydrate on the induction and growth of aberrant crypt foci (ACF), and on the proliferative indices, ie. mitotic activity and labelling index, were investigated in the colons of Sprague-Dawley rats. This thesis consisted of three studies.

In Study I, eight Sprague-Dawley male rats were injected with 20 mg/kg AOM (sc). One week later rats were randomly allocated to experimental diets containing 50% (w/w) dextrose or 50% (w/w) sucrose with 4 rats/group for 4 weeks. The number, crypt multiplicity (number of crypts in each ACF) and the size of ACF were enumerated in the colons. Rats fed dextrose had a significantly higher average number of crypts per ACF than the sucrose group (2.2 versus 1.8 respectively) ($p < 0.05$). Dextrose appeared to support the growth of ACF compared to sucrose.

The effect of varying levels of cornstarch (CS) on ACF parameters and proliferative indices was investigated in Study II. In this study, dextrose served as the source of simple sugar. A total of 23 rats were injected with AOM (20mg/kg, sc) and one week later randomized into 3 experimental diets: 15% CS, 30% CS or 45% CS (w/w) for 8 weeks. Fewer ACF were observed in the colons of rats fed 30% (w/w) CS diet compared to those fed the 45% (w/w) CS diet ($p<0.05$).

Furthermore, the 30% CS diet was associated with greater mitotic activity, and 45% CS with a higher labelling index.

In Study III, rats were fed diets containing either raw or thermally-treated cornstarch (RCS or TCS respectively) at doses of 15% and 45% (w/w). In addition, a lactulose (l) group, 15% RCS + 1% l (w/w) was included as a control. Fifty male Sprague-Dawley rats were injected with AOM (20 mg/kg, sc). One week later, rats were randomized and assigned to five treatments of 10 rats/group for 8 weeks. Faecal parameters such as pH, weight, and water content were recorded. Using a generalized ANOVA test, it was evident that there were no significant differences in the total number, crypt multiplicity, and size of ACF due to dietary treatments. However, further testing with orthogonal statistical statements indicated that cooking had a significant impact on crypt multiplicity ($p=0.02$) and on size of the ACF ($p=0.04$). Rats fed 45% (w/w) TCS diets had a higher proportion of ACF with increased crypt multiplicity in their colons compared to the 45% RCS (w/w) group ($p<0.05$). Faecal parameters such as pH and weight were affected by diet specifically in the 45% TCS group. Findings on proliferative indices in the colonic epithelium were inconclusive. The results of Study III demonstrated that TCS which indeed contained some amount of resistant starch (RS) did not suppress the growth of ACF.

It was concluded that dietary carbohydrates are capable of modulating early events in colon cancer. Furthermore a distinct protective effect of diets containing high amounts of raw or thermally-treated starch was not demonstrated.

ACKNOWLEDGEMENTS

The author wishes to express her gratitude to the following persons and institutions:

Dr. Ranjana Bird, her thesis advisor, for her patience, encouragement and guidance.

Appreciation is extended to Dr. Beverley Watts (co-advisor) and Dr. Susan Arntfield for serving on the thesis committee and for their advice.

Special thanks to Dr. Arntfield, for the use of her laboratory in producing resistant starch. Also, my thanks to Dr. Biliaderis and Dale Prokopowich for their assistance with the Differential Scanning Calorimetry.

The laboratory of National Agri-Food Technology Centre, Portage la Prairie (Manitoba) analyzed the starch samples for resistant starch on a service basis.

The statistical advice of the Statistical Advisory Service, University of Manitoba was invaluable.

The funding for the project was provided by the NSERC operating grant to Ranjana Bird. The K.S. Middleton Studentship to the author is gratefully acknowledged.

A special gratitude to Ms. Dietlinde Tober, for her technical assistance and personal friendship throughout the entire study.

The support and encouragement of the Wednesday afternoon colon cancer group, staff and students of the Department of Foods and Nutrition is greatly appreciated.

A big thank you to my family and friends for their unconditional support and encouragement without which I could not have completed this thesis.

Lastly but not forgotten, my best friend and to whom I dedicate this thesis, Michael Yaw Ayisi. Thank you for everything.

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LIST OF ABBREVIATIONS

ACF	Aberrant crypt focus
AC	Aberrant crypt
AOM	Azoxymethane
BrdU	5'-Bromo-2'-deoxyuridine
CHO	Carbohydrate
CS	Cornstarch
CH	Crypt height
DAB	3,3'-Diaminobenzidine
1,2-DMH	1,2-Dimethylhydrazine
HCl	Hydrochloric acid
ip	Intraperitoneal
L, l	Lactulose
LC	Labelled cells
LI	Labelling index
MF	Mitotic figure
MI	Mitotic index
NSP	Non-starch polysaccharides
PBS	Phosphate buffered saline
PL	Preneoplastic lesions
PCNA	Proliferating cell nuclear antigen
RCS	Raw cornstarch
RS	Resistant starch

SCFA	Short-chain fatty acids
sc	Subcutaneous
TCS	Thermally-treated cornstarch

CHAPTER I

INTRODUCTION

Colorectal cancer is one of the major neoplastic diseases which afflicts a large portion of the population aged 40 and above in both North America and Europe (Fry et al, 1989). Approximately 60,000 deaths per year in North America can be attributed to cancer (Robbins and Kumar, 1987). Epidemiological and recent experimental studies suggest a strong correlation between the type of diet consumed and the incidence of colon cancer (McKeown-Eyssen, 1987; Wynder, 1987). Epidemiological findings have suggested that in industrialized nations the heavy consumption of dietary fats, proteins, and refined carbohydrates (CHO) contributes to increased pre-disposition to colon cancer compared with developing countries (Burkitt, 1971; Hill, 1975). Health professionals in developed nations are encouraging increased consumption of complex CHO such as non-starch polysaccharides (NSP) and starchy foods to help reduce risk of colon cancer (Canadian Cancer Society, 1992).

Dietary starch is proposed to have a protective role against the development of colon cancer. It has been postulated that starch could be a valuable substrate for microbial fermentation. Starch is hypothesized to be degraded and converted to short-chain fatty acids (SCFA) by microbial anaerobes in the colon. Essential SCFA such as butyric acids

are purported by investigators to enhance cell differentiation which has potential implications in retarding the development and growth of colonic tumours (Cummings and Engylst, 1987; Klurfeld, 1992). In addition, as a result of increased concentrations of SCFA in the colonic lumen, it has been suggested that this could result in a decrease pH in the lumen (Newmark and Lupton, 1990). It has been theorized that a lower colonic pH could lead to a significant physiological and biochemical changes in the colon, such as the suppression of the production and bioavailability of secondary bile acids which are suspected tumour promoters (Bartram et al, 1991).

The main objective of this thesis was to investigate the effects of feeding a complex carbohydrate, starch, on the pre-neoplastic and cytokinetic events in the colons of Sprague-Dawley male rats. The specific purposes were:

To investigate differences between dextrose or sucrose diets on Sprague-Dawley male rats with respect to the development of preneoplastic lesions and proliferative activity.

To determine the effects of feeding graded doses of raw cornstarch (15%, 30%, 45% w/w) on ACF development and proliferative status in the colons of Sprague-Dawley rats.

To compare effects of raw and thermally-treated cornstarch on the development and growth of ACF as well as on cytokinetic events in the colonic epithelium.

CHAPTER II

REVIEW OF LITERATURE

To put this thesis in proper perspective, this literature review provides a brief overview of cancer as a disease, the findings on epidemiological studies pertaining to diet and cancer association and experimental approaches available to assess the role of dietary components in the aetiology of colon cancer. Furthermore, this review includes a discussion on starch as a nutrient and its potential role in the development of colon cancer.

2.1 **CANCER**

Cancer develops through a multistep process involving several cellular and biochemical changes (Farber,1984). In each step, new cell populations develop with altered cytological appearances, and biochemistry (Farber and Sarma, 1987; Farber, 1984). In the past decade various organs have been used as models for the stepwise analysis of cancer. Attention has been focused on the liver, skin, and the gastrointestinal tract, especially changes that these tissues undergo during cancer (Farber and Sarma, 1987). Studies on skin and liver carcinogenesis have enabled investigators to divide the carcinogenic process into two main stages: initiation and promotion (Farber and Sarma, 1987). It is believed that a dynamic equilibrium between cell loss and

renewal exists, which must be controlled by a series of homeostatic mechanisms (Sprinz, 1971). The homeostatic balance is disrupted when the DNA of a cell becomes exposed and damaged by a carcinogen. The "exposed" cell is not an "initiated" cell, but contains a modified DNA. Initiation will occur only if the modified DNA is not repaired, or is repaired incorrectly. After cell division, cells containing altered DNA may exhibit properties of transformed cells. Following this critical replication, initiated or transformed cells are genotypically different but not yet phenotypically distinguishable from normal cells. Further growth of these cells depends on the post-initiation environment of the cell, which is important in the promotional stage.

During promotion, mitotic activity in the initiated cells is favoured. With further cell proliferation, these cells which are considered preneoplastic in nature could develop into focal proliferative lesions. The underlying mechanisms that triggers the progression of focal proliferative lesions into more advanced premalignant or malignant lesions remains unknown.

Histogenesis of colon cancer has been extensively investigated in animal and human models (Sunter, 1980; Pretlow et al, 1991; Tudek et al, 1989; Roncucci et al, 1991). It is believed that initiated cells originate in single crypt units, and that colon carcinogenesis involves several steps of preneoplastic events (Chang, 1980). These preneoplastic

events are accompanied by crypts with altered morphology, focal proliferative lesions, and microscopic and macroscopic lesions exhibiting dysplasia prior to the appearance of cancer (Farber and Sarma, 1987; McLellan et al, 1991b; McLellan et al, 1991c; Robbins and Kumar, 1987; Roncucci et al, 1991).

2.2 DIET AND COLON CANCER: EPIDEMIOLOGICAL STUDIES

Studies comparing international, migrant, and religious groups suggest that diet could be an important risk factor in the development of colon cancer (Burkitt, 1971; Hill, 1975; Reddy, 1980). It has been proposed that countries or population groups possessing high risk of colon cancer development have higher intakes of total fat and relatively low intakes of fibre (Canada, UK, USA) (Burkitt, 1971; Hill, 1975; Wynder, 1987; Reddy, 1987). A consistent association has been found between incidence of colon cancer and consumption of animal proteins (Bingham, 1988). Studies on groups have revealed that there is a higher incidence of bowel cancer in second generation Japanese migrants living in Hawaii compared to their counterparts living in Japan (Reddy, 1980). Differences between migrants and native Japanese populations were attributed to the adoption of western dietary habits by the migrant populations (Reddy, 1980). The Japanese migrants diet is high in animal protein and fat (40% of calories), while native Japanese consume a diet low in fat (10-15% of calories) but high in fibre and starch (Reddy, 1980; Wynder,

1987). Also, studies on religious groups revealed that certain lifestyle patterns could decrease the incidence of colon cancer. For instance, studies have reported low colon cancer incidence among Seventh Day Adventists (Reddy, 1980). These Adventists are known to consume highly fibrous diets including whole grain breads and cereals, and to abstain from meat.

High fat, low fibre diets lead to the mobilization of biliary steroids, which are required for the digestion and absorption of fat. Excess amounts of bile acids, and cholesterol, are excreted into the colon, where they undergo biotransformation by the action of the colonic microflora (Nair, 1988; Nigro et al., 1979; Bresalier and Kim, 1985). Evidence indicates that faecal secondary sterol and bile acids are potential tumour promoters (Bird, 1986; Bruce, 1987). Colonic perfusion studies have demonstrated that both deoxycholic and free fatty acids are able to damage the surface of the colonic mucosa (Bruce, 1987). In response to this injury, rapid cell proliferation is initiated, in which increased S-phase cells and mitotic activity can be found in the crypts (Deschner, 1987; Lipkin,1988). In this condition, hyperproliferation is generally regarded as a compensatory mechanism in which damaged epithelial cells are replaced with new ones (Bruce and Bird, 1986; Deschner, 1987). In the hyperproliferative state, epithelial cells are highly sensitive to carcinogenic agents, which increases the risk for

neoplastic transformation (Bruce and Bird, 1986). Based on these findings experimental approaches have been developed to examine the relationship between diet and colon cancer development.

2.3 CURRENT EXPERIMENTAL APPROACHES USED TO EXAMINE THE EFFECTS OF DIET ON COLON CANCER DEVELOPMENT IN AN ANIMAL MODEL

The following experimental approaches are currently employed to study the effects of diet on colon carcinogenesis:

1) Long-term tumorigenesis studies.
2) The examination of risk markers associated with the luminal environment and colonic tissues.
3) Short-term studies involving early precursor lesions as biological endpoints.

2.3.1 Long-Term Tumorigenic Studies

Tumour induction studies are frequently used to assess diet-cancer relationships, although these studies can be afforded by only a handful of investigators. In most cases, large numbers of animals are injected with a colon specific carcinogen using single or multiple injections. The animals are then assigned to specific diets. Some weeks after initiation (usually 30-40 weeks), the tumour incidence and multiplicity are measured, and histological examinations are

carried out.

Reddy (1980) reported that rats treated with 1,2-dimethylhydrazine (1,2-DMH) and fed diets consisting of 20% corn oil and 20% wheat bran had lower numbers of tumours than control groups (20% corn oil and 0% wheat bran). A similar inhibitory effect on tumour growth by fibre was demonstrated in rats fed 30% cellulose (1.95 tumours/rat) compared with rats placed on a fibre-free diet (4.0 tumours/rat) (Nigro et al, 1979). The promotional effects of bile acids on increasing tumour frequency were noted in rats given intrarectal instillation of methyl-N'-nitro-N-nitrosoguanidine (MNNG) and cholic or chenodeoxycholic acids (Reddy et al., 1977). Tumorigenesis studies have shown that diets containing bile acids enhanced tumour growth, while low fat, high fibre diets appeared to reduce tumour development.

Tumour induction studies do not allow investigators to assess the mode of action of the dietary components in the development of this disease. It is difficult to assess if the diet is acting on the early versus late stages. The inconveniences of tumour incidence studies have prompted investigators to search for new and economical alternatives, such as the use of risk markers as biological endpoints. Employing risk markers as biological endpoints enables investigators to assess the risk for developing the disease rather than conducting an examination which studies the development of the disease itself.

2.3.2 Risk Markers Associated With The Luminal Environment

Attempts have been made to quantitate the presence of genotoxic components in the faecal stream. It is suggested that genotoxic compounds are found in higher concentrations in the faeces of populations consuming high fat, low fibre diets than in the faeces of those consuming low fat, high fibre diets (Reddy et al, 1989; Bruce, 1987; Klurfeld, 1992). A few of these compounds: fecapentaenes, steroids, and heterocyclic amines have been identified by nuclear aberration and assay for mutagenic activity in bacterial systems (Wakabayashi et al, 1992; Bingham, 1988; Bruce, 1987).

Other studies have indicated that elevated levels of faecal secondary bile acids, particularly lithocholic and deoxycholic acids, in the colonic lumen are useful risk markers; secondary bile acids are reported to act as promoters in experimental tumorigenesis (Reddy et al, 1989; Nair, 1988).

Dietary influences in altering the colonic luminal pH could play a significant role in tumour development. It is proposed that certain dietary components such as undigested proteins could be used as substrate by facultative bacteria, resulting in production of ammonia, which in turn may increase the pH level of the colonic content (Newmark and Lupton, 1990; Englyst and Cummings, 1987). It is proposed that an alkaline pH in the lumen creates an environment which favours the

bioavailability of acidic lipids, ie. bile acids, and enhances their tumour promoting effect (Bruce, 1987; Newmark and Lupton, 1990;). Therefore, a few animal studies have focused on lowering the pH of the colonic mucosa by feeding high fibre and low fat diets, or by employing acidifying agents such as lactulose (van Berge Henegouwen et al, 1987).

2.3.3 The Examination Of Risk Markers In The Colonic Tissues

2.3.3.1 Enzymatic Risk Markers Associated With Colonic Neoplasia

Enzymatic changes in the colonic tissues associated with the risk of disease have been measured in experimental colon carcinogenesis. Thymidine kinase activity, which is essential for DNA synthesis was measured and found to be elevated in early neoplastic lesions and colonic adenocarcinomas compared with normal tissues (Lipkin, 1971). Bile salts are believed to increase proliferative activity of the colonic epithelium by stimulating activity of enzymes such as protein kinase C (PKC) and ornithine decarboxylase (ODC) (Klurfeld, 1992; DeRubertis and Craven, 1987). Both of these enzymes are proposed to be associated with increased cell growth and differentiation in normal and malignant cells (Pegg, 1986; O'Brien, 1976). Dietary intervention studies have demonstrated that the addition of 10% cellulose to a fibre-free diet caused a 50% decrease in ODC activity, and that this

inhibition of ODC was associated with a significant decrease in the induction of carcinogen-induced tumours (Kingsnorth et al, 1983; Klurfeld, 1992).

2.3.3.2 Measurement Of Colonic Cell Proliferation To Predict The Risk Of Colon Cancer Development

Assessment of colonic cell proliferation has been advocated to help quantify abnormal biological processes in the colonic crypts which could be associated with neoplasia. It is generally believed that increased proliferation will lead to enhanced tumour development. These measurements may encompass a variety of parameters employing different methodological approaches such as the determination of mitotic cells using the metaphase arrest technique. This technique employs agents such as colchicine or vincristine which allows for the identification and quantification of mitotic cells, specifically during the metaphase stage of the cell cycle. Another approach pertains to the determination of DNA synthesizing cells by immunohistochemical assessments using 5'-Bromo-2'-deoxyuridine (BrdU).

Although proliferative indices have been used by several investigators to assess risk, this approach has not been validated. For instant, Robblee and co-workers (1990) reported that mitotic figures (MF) and labelling index (LI) were affected by dietary fibre independent of each other, meaning that the two values did not corroborate each other.

Rats fed 5%(w/w) wheat bran, 10% (w/w) cellulose or 10% (w/w) pectin exhibited higher LI in the crypts but at the same time had lower MF (Robblee et al., 1990). Galloway and colleagues (1987) pointed out conflicting results between their tumour incidence studies and cell kinetic data. They reported that rats fed a low fat, high fibre diet developed lower numbers of tumours but this was associated with increased cell proliferation. The proposed positive link between colonic cell proliferation and colon cancer development has been widely-accepted by several investigators. However, the findings outlined above raise questions as to the validity and sensitivity of these biomarkers as risk markers in tumorigenesis studies.

Overall, it is important to identify reliable risk markers, because these can be employed using small number of animals and limited time studies. However, it is important to recognize that these proliferative biomarkers do not represent the actual disease, and should not be regarded as substitute for the disease process (Bird et al., 1989).

2.3.4 Short Term Studies Employing Early Precursor Lesions

A number of studies have been conducted to investigate histogenesis of colon cancer in animal models. In these studies, it is clearly demonstrated that very early on, following exposure to colon carcinogens, colonic crypts

exhibit altered morphological features (Chang, 1984; Richards, 1977; Wiebecke et al, 1973). Based on these findings, Bird (1987) developed a new method to identify and quantify early precursor lesions as early as two weeks in rodent colons. An unsectionned colon is stained with methylene blue and the topographical area of the mucosal surface is then viewed using a light microscope. Using this methodology, Bird (1987) reported the appearance of early microscopic lesions in colons of rodents after chemical induction. These lesions were termed aberrant crypts foci (ACF) and are hypothesized to represent putative pre-neoplastic lesions. This assumption was based on the following findings; firstly, that ACF are only present after carcinogen treatments (McLellan and Bird, 1988a). The appearance of ACF in the rodent colon was observed to be induced specifically by colon carcinogens, ie. 1, 2-DMH and AOM (McLellan and Bird, 1988b). A high fat diet promotes the growth of ACF (McLellan and Bird, 1988a). The induction and growth of ACF was observed to be inhibited by 1,2-disulfiram, an agent known to inhibit DMH-induced colon cancer (McLellan and Bird, 1991a). McLellan and co-workers (1991b) reported that a majority of ACF exhibited varying grade of nuclear atypia, indicative of dysplasia. Dysplasia is a critical histopathological marker showing that certain cell populations have the potential to become neoplastic. Some ACF exhibited marked increase in mitotic and DNA synthetic activity (McLellan et al, 1991c). It is proposed that the

examination of these preneoplastic lesions using parameters such as total number, crypt multiplicity and size of ACF may serve to help predict the disease outcome and this could be useful in identifying potential modulators in colon cancer.

2.4 COMPLEX CARBOHYDRATE AND ITS POSSIBLE ROLE IN THE DEVELOPMENT OF COLON CANCER

2.4.1 Starch

Starch is a major energy source provided by foods such as rice, corn and potatoes (Dreher et al, 1984). Complimentary to its nutritional benefits, starch has numerous functional applications, such as its use as a thickener or an emulsifier in various food systems (Light, 1990).

2.4.2 Chemical And Structural Compositions Of Starch

Starch is a homopolysaccharide, composed of a series of α-D-glucose units that are linked either as a straight-chain molecule known as amylose, or highly-branched structure called amylopectin. Amylose is composed of glucose units linked by α-D-(1→4) glycosidic bonds. Because of its small molecular weight (1.5×10^5-10^6), amylose can be found in different conformational forms (Biliaderis, 1991). The enzyme, α-amylase degrades amylose, releasing endproducts such as maltose, and glucose (Dreher et al, 1984; Biliaderis, 1991).

Amylopectin is a high molecular weight polymer (50-500 $x10^{6}$) (Biliaderis, 1991). Amylopectin is composed of glucose units linked by an α-D-(1→4) glycosidic bonds with interconnecting α-D-(1→6) branching units (Dreher et al, 1984). Hydrolysis of amylopectin occurs by α-amylase, followed by various debranching enzymes such as pullulanase or α-glucosidases (Aspinall, 1970; Gray et al, 1992). During starch hydrolysis, the endproducts α-limit dextrin, maltotriose, maltose, and glucose are released (Gray et al, 1992; Dreher et al, 1984; Aspinall, 1970). In contrast to amylose, amylopectin is stable in solution and rarely recrystallizes (Dreher et al, 1984; Biliaderis, 1990). According to Biliaderis (1991), most native starches contain between 20 to 35% amylose content. New technology has enabled plant breeders to produce plant strains with starch chemical composition containing between 50-80% amylose content compared to amylopectin, (ie. Amylomaize) (Biliaderis, 1991). Mutant strains composed primarily of amylopectin such as waxy maize can also be produced (Biliaderis, 1991). Amylose:amylopectin ratios of different starches influence their functional properties in food systems (Biliaderis, 1991; Light 1990).

Starch granules vary in size and shape. They range from 1 to 100 microns, and vary in shape from spheres to rods (Trimble, 1983; Biliaderis, 1991). Since the early 1930's, researchers have been able to examine the x-ray diffraction patterns of starch granules. Based on these studies, three

distinct crystalline patterns were identified in native starches. Cereal starches exhibit A-type patterns while B-type patterns are shown by tubers, high amylose, and retrograded starches (Biliaderis, 1991). Legume starches have a C-type arrangement, and possess an intermediate (between A and B-type) structure (Biliaderis, 1991). The causes of different crystalline structures are still unknown.

In this thesis, cornstarch, a cereal starch was used in all of the studies. Cornstarch is categorized as exhibiting a typical cereal crystalline A-type pattern, with a chemical composition consisting of 28% amylose: 72% amylopectin (Sievert and Pomeranz, 1990). In general, cornstarch is classified as a cereal starch and is considered to be a readily digestible starch (Englyst and Cummings, 1987).

2.4.3 **Digestion Of Starch**

The gastrointestinal tract is the site for initial hydrolysis of starch by salivary and pancreatic α-amylases (Dreher et al, 1984; Berry, 1986). End-products are α-limit dextrins, maltotriose, maltose, and some glucose molecules (Gray 1992; Dreher et al, 1984). The release of oligosaccharide molecules which consist of approximately five-to-nine glucose units causes the activation of enzymes from the intestinal brush border (Berry, 1986). Starch digestion ceases after the release of glucose as an endproduct. Recently, studies have suggested that a certain amount of

starch consumed, may escape digestion in the small intestine (Anderson et al, 1981; Dreher et al, 1984; Englyst and Cummings, 1987).

2.4.4 Factors Influencing Digestion Of Starch

Starch digestion is influenced by physical and chemical form, and cooking conditions (Dreher et.al., 1984; Biliaderis, 1991; Sievert and Pomeranz, 1989; Anderson et al, 1981). The rate of starch hydrolysis can be influenced by the physical state of the granules meaning whether they are unground or ground (O'Dea et al, 1980; Snow and O'Dea, 1981; Heaton et al, 1988). In-vitro studies demonstrated that the presence of whole grain wheat starch reduced the activity of pancreatic α-amylase compared to milled wheat flour (Heaton et al., 1988). Differences between unground and ground starch granules are reported to be caused by particle size. Large particle sizes, result in a less surface area for a given amount of starch (Snow and O'Dea, 1981; O'Dea et al., 1980).

The chemical composition of starch also affects the rate of digestion and absorption (Sievert and Pomeranz, 1989). In solution amylose molecules are capable of forming intermolecular linkages with each other through hydrogen bonds (Wolever, 1991). As a consequence, hydration of amylose-to-amylose linkages occurs slowly which makes it difficult for enzymatic hydrolysis. Therefore, legume starches which are naturally rich in amylose, are recommended for diabetics

rather than cereal starches (Goddard et al., 1984).

Different types of processing conditions may also affect the availability of starch to the enzymes of the gastrointestinal tract (Englyst and Cummings, 1987; Russell et al., 1989; Panlasigui et al., 1991). Processing conditions in this study refers to the degree of gelatinization (Holm et al., 1989). Gelatinization is the application of heat in which starch granules are weakened (Tovar et al., 1991). This procedure leads to the penetration of water molecules into the interior of the granules, eventually causing starch granules to swell (Bennion, 1980). As a result of swelling, the enzyme has access to the interior which allows for the subsequent cleavage of starch. Snow and O'Dea (1981) demonstrated an increase in starch hydrolysis with α-amylase for cooked oats compared to raw oats. Therefore physical, chemical and thermal factors that affect availability of starch to digestive enzymes may have profound implications for glucose metabolism and large bowel diseases. Based on these findings, Englyst and Cummings (1987) suggested that a nutritional classification for starch be established. The groupings are based on the rate of starch digestion in the small intestine.

2.4.5 Nutritional Classification Of Starch

2.4.5.1 Readily Digestible Starches

Readily digestible starches (RDS) are primarily consumed as freshly cooked foods or as ready-to-eat cereals like

breakfast cereals (Englyst and Cummings, 1987; Dreher et.al., 1984). Englyst and Cummings (1987) predicted that these starches can be easily digested by enzymes of the gut. Research has shown that glucose levels and insulin responses were greatly increased when RDS was incorporated into test diets (Dreher et.al, 1984). RDS gelatinizes at low temperatures and in the gelatinized form are susceptible to α-amylase attack (Englyst and Cummings, 1987). Although, these starches are readily digestible, whether indeed they are completely digested in the human gastrointestinal tract remains debatable.

2.4.5.2 **Partially Resistant Starches**

Partially resistant starches (PRS) are found in legumes, raw potatoes, and bananas (Englyst and Cummings, 1987). Their resistance to α-amylase attack is thought to be due to their B or C-type crystalline structures and to some extent to retrogradation of amylopectin (Englyst and Cummings, 1987). Digestibility of PRS improves with cooking.

2.4.5.3 **Resistant Starches**

Resistant starch (RS) is the product of hydrogen bonding between amylose molecules (Sievert and Pomeranz, 1989; Englyst and Cummings, 1987). It is unavailable for digestion by hydrolytic enzymes. However, in the colon, it can be used as a substrate for fermentation by anaerobic microbes.

Resistant starches are proposed to have a potential significant role in maintaining the health of the large intestine (Englyst and Cummings, 1987).

The physio-chemical properties of resistant starch, includes resistance to re-dispersion in boiling water and to enzymatic hydrolysis with pancreatic amylase (Englyst and Macfarlane, 1986). Resistant starches are soluble in potassium hydroxide (KOH) or dimethylsulfoxide (DMSO). X-ray diffraction patterns of RS reveal a B-type structure. These structures are generally known to be resistant to enzymatic digestion (Sievert and Pomeranz, 1989).

2.4.6 **Development Of Resistant Starch**

2.4.6.1 **Production Of Resistant Starch**

The production of RS occurs through starch retrogradation (Sievert and Pomeranz, 1989). Starch retrogradation occurs when gelatinized amylose molecules are slowly cooled, and certain portions of the molecules will spontaneously reassociate. This process leads to the formation of strong intermolecular hydrogen bonds among amylose molecules (Englyst and Cummings, 1985). Interactions between amylose and amylopectin are believed to be involved but to a lesser extent (Sievert and Pomeranz, 1989).

2.4.6.2 Factors Which Could Control Production Of Resistant Starch

A few factors that could affect the level of resistant starch (RS) in foods are autoclave temperatures, water content, number of heating and cooling cycles, and the type of starch (Russell et al, 1989). Sievert and Pomeranz (1989) demonstrated that a positive relationship exists between the level of amylose in starch and percent yield of RS. Amylomaize VII (70% amylose content) gave a higher yield of RS than starches with low amylose percentages, such as waxy maize (1% amylose), potato (20% amylose), wheat (25% amylose), and pea starches (33% amylose). Another factor is the autoclave temperature used in producing RS. Temperatures between 121 and 134 degrees Celsius seem to optimize the yield of RS while temperature such as 148 degree Celsius appeared to result a lower recovery of RS (Russell et al, 1989; Bjorck et al, 1987; Sievert and Pomeranz, 1990).

2.4.7 Proposed Role Of Starch Or Resistant Starch On The Intestinal Mucosa

Several investigators have proposed that starch or RS could modulate the growth of early preneoplastic lesions by altering physiological conditions in the colonic lumen (Englyst and Cummings, 1987; Ranhotra et al, 1991) . These researchers have suggested that the presence of starch or RS in the colon may help to stimulate bacterial fermentation, which in turn increases microbial growth and the production of short chain fatty acids (SCFA). Increased faecal bulk may help dilute and bind potential carcinogens or promoters as well as decrease transit time of faecal contents in the colon (Cummings and Englyst, 1987). In addition, high concentrations of SCFA, specifically butyric acids, could help enhance cell differentiation and maturation (Jacobs, 1987; Klurfeld, 1992). Furthermore, the presence of SCFA like butyrate, acetate, and propionate in the lumen could create an acidic environment which may not favour growth of transformed cells (Jacobs, 1987). Decrease in the colonic pH may help to inactivate microbial enzymes such as ß-glucuronidase and 7-α-dehydroxylase (Reddy, 1980). Both enzymes are proposed to be involved in the metabolism of potential initiators and promoters, respectively (Klurfeld, 1992; Reddy, 1980). 7-α-dehydroxylase is associated with the degradation of primary to secondary bile acids, while ß-glucuronidase is involved in the release of free unconjugated compounds, which are believed to

act as potential carcinogens (Bartram et al, 1992; Reddy, 1980).

2.5 RESEARCH PROPOSAL

Starch is a major component of the human diet. The low incidence of colon cancer observed in developing nations or less affluent countries has been attributed to higher levels of starch consumption in developing nations compared to affluent countries (Bright-See and Jazmaji, 1991). It is proposed that the increased availability of starch as a fermentable substrate in the colon could provide some type of protection against colon cancer (Jenkins et al, 1987; Reddy, 1987). Several investigators have suggested that diets containing starch or RS could be useful in the treatment and prevention of colon cancer (Englyst and Cummings, 1987; Ranhotra et al, 1991). If indeed this is the case, then with respect to experimental carcinogenesis, it would be most valuable to investigate the potential influence of dietary starch or RS on colon cancer development.

Therefore, the main purpose of this thesis is to investigate the effects of dietary starch on the development and growth of preneoplastic lesions in the rat colon in conjunction with potential risk markers of colon carcinogenesis.

CHAPTER III

GENERAL MATERIALS AND METHODS

3.1 ANIMALS

Sprague-Dawley male rats (Breeding Centre, University of Manitoba, Wpg., MB., Canada) were used for all studies. The rats were housed in a suspended metal cage with wire bottom grids with sawdust bedding. Room temperature was maintained at 24°C with 45% moisture and 12-hour alternating light and dark cycle. Rats were fed ad libitum and had free access to water.

3.2 CHEMICALS

Azoxymethane (AOM), colchicine, 5'-Bromo-2'-deoxyuridine (BrdU), and methylene blue were purchased from Sigma Chemical Co., St. Louis, MO. Primary antibody for BrdU was obtained from Becton Dickson, San Jose, CA., and immunoperoxidase staining kit supplied by ID Laboratory, London, Ont. All diet ingredients were purchased from US Biochemical Corp., (Cleveland, Ohio), with the exception of the following ingredients: cornstarch was obtained from Canada Starch Co., Pointe-Claire, Quebec; dextrose from Staley Mfg., Decatur, Ill.; sucrose and Mazola corn oil purchased from Canada Safeway (Canada Ltd.)

3.3 PREPARATION OF CARCINOGEN

Azoxymethane (AOM) was prepared as a solution in normal saline. Animals were injected once with AOM (20 mg/kg of body weight) prior to dietary treatments.

3.4 DIETS

Initially, rats were fed laboratory pellet chow (Agwai Prolab Animal Diet, rat-mouse-hamster 3000, Agwai Country Foods, Syracuse, New York). All experimental diets in our studies were based on the composition of AIN-76 semisynthetic diet (American Institute of Nutrition) (Table 4.0, 5.0, 6.0).

3.5 BODY WEIGHTS AND FOOD CONSUMPTION

Body weights were recorded biweekly and 24-hour food consumption was monitored periodically.

3.6 IDENTIFICATION And QUANTIFICATION OF ACF

Immediately after termination, the entire colon was excised and cleaned with normal saline. The colon was divided into three regions labelled; rectal, mid, and caecal. Individual sections were opened along the longitudinal axes and flattened out between two pieces of filter paper. Colonic pieces were fixed flat in a petri dish containing 10% buffered formalin and/or 70% ethanol. An ordinary microscope slide was placed over top of the filter paper, to ensure that the tissues remained flat. After a minimum of 24-hours in the

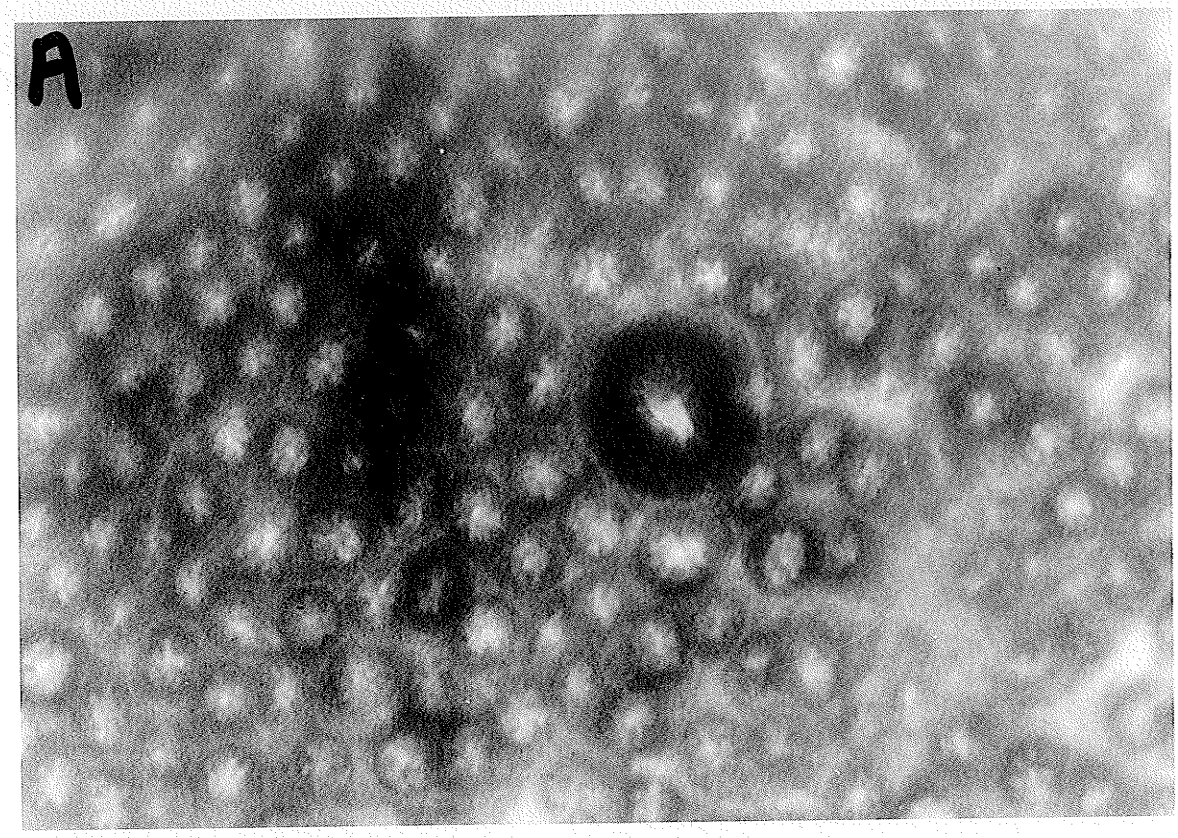
fixative, colonic pieces were stained with 0.2% methylene blue in phosphate buffered saline (PBS). After 30-45 minutes of staining, the tissues were placed on a glass slide with the mucosal side up. Specimens were viewed with a light microscope using 10X and 40X magnification power.

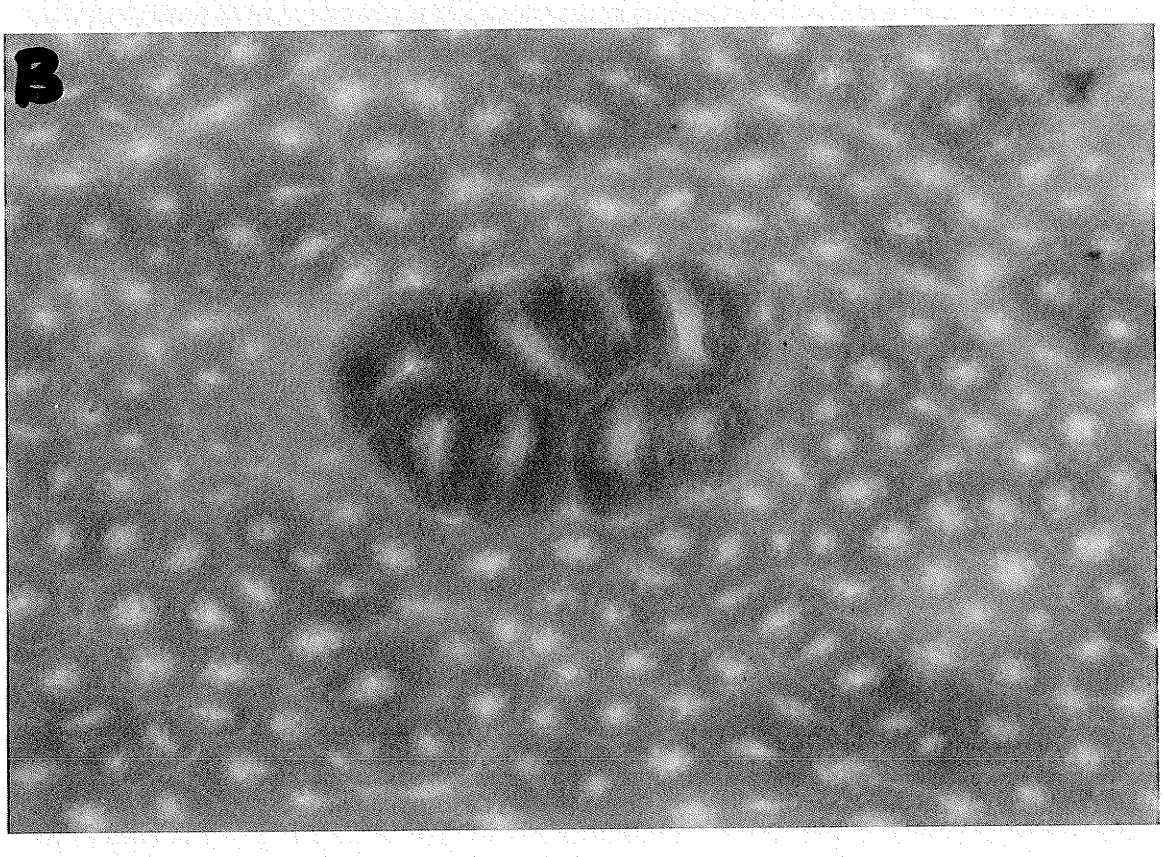
The criteria used in our laboratory to identify ACF with only one crypt are the following: (a) the area occupied by the crypt is at least two or three times greater than that of the normal surrounding crypts, (b) the luminal opening is elliptical shape and the lining is much thicker than those of the normal crypts, (c) the pericryptal zone separating the crypt from the normal surrounding crypts is greater than the pericryptal zone separating the normal crypts from one another, (d) the crypts have the appearance of forming a distinct focus, there are no normal-appearing crypts separating the crypts within the ACF (Figure 3.0) (Bird et al, 1989). This same criteria was used to define ACF consisting of more than one crypt (Figure 3.0).

The parameters used to quantify ACF in the colons were total of number ACF in the colon, number of aberrant crypts/focus (AC), size of ACF, and the distribution of ACF according to crypt multiplicity. The size of individual foci was recorded using a grid which had been placed in the eyepiece of the microscope. Ten times and 40X magnification lenses were used to count the number of squares on the grid occupied by the foci. Each square in the grid had an area of

FIGURE LEGEND

FIGURE 3.0 Topographical view of two ACF (A) with a single crypt; (B) ACF with eight crypt multiplicity. Note surrounding normal appearing crypts. Some surrounding crypts also exhibit abnormal staining (A), which may lead to ACF formation. Methylene Blue staining. 200X magnification.


A


B

$x10^{-2}mm^2$. To determine the distribution of ACF with respect to crypt multiplicity, the total number of ACF with one crypt for each rat, as well as those ACF having two or more crypts were recorded. The methods outlined above were used to identify and quantify ACF in all subsequent studies.

3.7 ASSESSMENT OF PROLIFERATIVE ACTIVITY IN THE COLONIC MUCOSA

3.7.1 Determination Of Mitotic Figure Employing The Metaphase Arrest Technique

Two hours before termination, rats were given a single i.p. injection of colchicine (1.0 mg/kg), prepared in saline. Colchicine is an agent which blocks dividing cells in the metaphase stage of the cell cycle. Colonic tissue of 0.5 cm were excised from the rectal and midsection and sent for histological sectioning at the Department of Pathology, St. Boniface Hospital, Wpg., MB. Sections were stained with haematoxylin and eosin. Five to ten completed crypts sections were examined on a light microscope for the presence of metaphase cells. The average mitotic figure (MF) and crypt height (CH) per crypt section was determined for each dietary group. Crypt height measures the number of epithelial cells in the mid-axial of the crypts in the colonic mucosa of Sprague-Dawley rats.

3.7.2 Measurement Of Labelled Cells By Immunohistochemical Technique

One hour before termination, rats were given a single i.p. injection of BrdU (30-50 mg/kg) dissolved in a mixture of normal saline:ethanol (70%). Bromodeoxyuridine is a thymidine analog, which is incorporated into the cells' DNA during the S-phase stage of DNA synthesis. This immunohistochemical procedure allows for the enumeration of cells engaged in DNA synthesis. Colonic tissues of 0.5 cm were excised from the rectal and midsection and fixed in 70% ethanol. Tissues sections were returned from St. Boniface, unstained and mounted on microscope slides. Unstained histological sections were then processed for immunohistochemistry using an anti-mouse immunoperoxidase staining kit. The steps for this procedure are: (1) Unstained tissues were deparaffinized in several changes of xylene and ethanol, followed by hydrolysis with hydrochloric acid (HCl). HCl is used to dissociate the DNA strands to make the antigenic site more accessible to the primary antibody. (2) Next, a blocking agent such as goat serum was added, in order to minimize non-specific binding of the antibody to cellular structures besides DNA. This was followed by the addition of mouse anti-BrdU antibody using a 1:40 dilution in PBS. (3) Ensuing steps include, the use of linking and peroxidase-labelled antibodies, and 3, 3' Diaminobenzidine Tetrahydrochloride (DAB), a chromogenic substrate solution, that allows for visualization and

quantification of the antibody-antigen complexes. The tissue sections were counter-stained with haematoxylin and dehydrated by processing through alcohol and xylene sequentially and then mounted permanently. Overall, this technique allows for the identification of BrdU-labelled cells in the crypts. Completed crypts that extended from the muscularis mucosa to the luminal surface were counted. Crypt height, labelled cells, and labelling index were determined for each treatment group.

3.8 STATISTICAL ANALYSIS

Generally, statistical analysis of the data was carried out by Statistical Analysis Software (SAS, 1988) using ANOVA and the Duncan's multiple range test for pairwise comparisons. A level of significance of $p < 0.05$ was considered significant.

CHAPTER IV

STUDY I

THE EFFECTS OF SIMPLE CARBOHYDRATE ON PRENEOPLASTIC LESIONS IN THE COLON: A PRELIMINARY STUDY

4.1 INTRODUCTION

The main objective of this study was to determine the effect of simple carbohydrates, specifically dextrose and sucrose on the induction and growth of ACF and on the proliferative activities in the colonic crypts.

In most animal studies sucrose or dextrose has been used as a source of simple sugars. Generally, rodent diet contains both simple and complex carbohydrates. The level of complex carbohydrate is altered by increasing or decreasing the amount of simple sugars, sucrose or dextrose. These simple sugars differ from each other with respect to their composition and physiological effects in the body (Mckeown-Eyssen, 1987; Wynder, 1987; Jenkins et al, 1987). Therefore, the purpose of this preliminary study was to determine if these refined sugars are different from each other in affecting the carcinogenic process in the colonic mucosa. This information was necessary in order to select one of these sugars as a source of simple sugar in future studies and in hopes of moderating its effect on ACF by employing higher levels of

complex carbohydrate, such as starch.

4.2 SPECIFIC MATERIALS AND METHODS

4.2.1 Diets

Modified AIN-76 semi-synthetic diets were used. These diets contained dextrose (50%, w/w), or sucrose (50%, w/w) as the source of simple sugar (Table 4.0).

4.2.2 Study Designs

Eight Sprague-Dawley rats were used. Each rat received a single s.c. injection of AOM. One week later, rats were randomized into 2 treatment groups consisting of 4 rats each. Rats were terminated by CO_2 asphyxiation after 30 days. Before termination individual rats were given a single i.p. injection of colchicine (1.0 mg/kg). The entire colon, caecal to rectal end was removed and examined for ACF and cell proliferation.

4.3 RESULTS

Overall, there were no significant differences in body weights of rats consuming different diets (Table 4.1; Appendix A). Rats in both dietary groups increased their body weights throughout the study (Figure 4.0). There appeared to be significantly higher daily food and energy intakes in rats fed the dextrose diet compared to the sucrose diet (Table 4.1) ($p<0.05$). The effects of simple carbohydrates on the induction and growth of ACF is presented in Table 4.2. The number of AC per focus was significantly lower in the group

TABLE 4.0: PERCENT WEIGHT COMPOSITION (g/100g) OF THE EXPERIMENTAL DIETS[1]

INGREDIENT	DEXTROSE	SUCROSE
CASEIN	20.0	20.0
DL-METHIONINE	0.3	0.3
DEXTROSE	50.0	-
SUCROSE	-	50.0
CORNSTARCH	15.0	15.0
CELLUFIL	5.0	5.0
AIN-76 MINERAL MIX	3.5	3.5
AIN-76 VITAMIN MIX	1.0	1.0
CHOLINE BITARTRATE	0.2	0.2
CORN OIL	5.0	5.0
ENERGY DENSITY KCAL/GRAM	3.86	3.86
% ENERGY FROM		
PROTEIN	21.0	
CHO	67.0	
FAT	12.0	

[1]AIN-76 SEMISYTHETIC DIET

TABLE 4.1: MEAN BODY WEIGHTS AND DAILY FOOD CONSUMPTION

TREATMENT GROUP	N	MEAN BODY[1,2] WEIGHT (GRAMS)	MEAN DAILY[3] FOOD INTAKE (GRAMS)	ENERGY (KCALS)
DEXTROSE	4	317.8 ± 10.0	21.8 ± 0.58[a]	84.0 ± 2.2[a]
SUCROSE	4	335.5 ± 17.0	18.8 ± 0.88[b]	72.5 ± 3.4[b]

[1]Weight determined on day of termination.
[2]All values shown are the mean ± SEM.
[3]Food consumption measured for week #4 of study.
Means in the same column with different letter are significantly different ($p < 0.05$).

FIGURE LEGEND

FIGURE 4.0 The effect of simple sugars on body weight of Sprague-Dawley male rats. 50% Dextrose, n=4; 50% Sucrose, n=4.

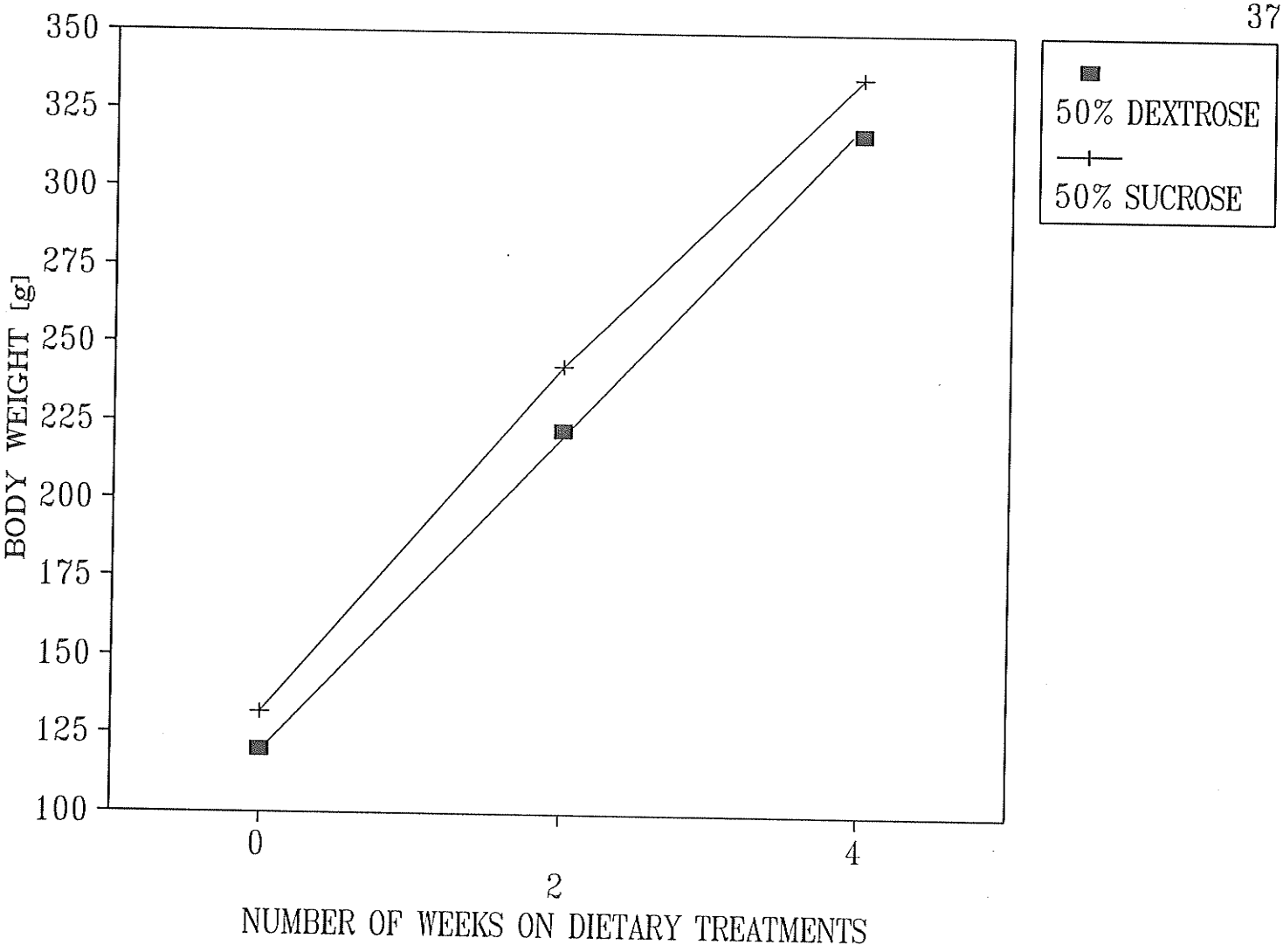

350
325
300
275
250
225
200
175
150
125
100
BODY WEIGHT [g]
0
2
4
NUMBER OF WEEKS ON DIETARY TREATMENTS
50% DEXTROSE
50% SUCROSE

TABLE 4.2: EFFECTS OF SIMPLE CARBOHYDRATES ON THE NUMBER, CRYPT MULTIPLICITY, AND SIZE OF AOM-INDUCED ACF IN SPRAGUE-DAWLEY RATS

TREATMENT GROUPS	MEAN ACF	MEAN AC/FOCUS	MEAN SIZE OF FOCUS $x10^{-2}mm^2$
	NUMBER OF ACF	CRYPT MULTIPLICITY	SIZE OF ACF
DEXTROSE	197.5 ± 18.1	2.2 ± 0.05[a]	3.0 ± 0.2
SUCROSE	187.3 ± 24.1	1.8 ± 0.14[b]	2.6 ± 0.2

All values shown are the mean ± SEM. Means in the same column with different letter are significantly different ($p < 0.05$). Dextrose, n=4; Sucrose, n=4.

fed sucrose diet compared to the group fed dextrose ($p<0.05$). The number and size of ACF were not significantly affected by sucrose or dextrose feeding. Despite these findings, trends indicated that a dextrose diet appeared to increase total number and size of ACF compared to the sucrose diet.

There were no significant differences on the distribution of ACF with different crypt multiplicity but generally, it was noted that a greater number of ACF consisted of 1 or 2 crypt/focus for both dietary groups (Table 4.3). Further trends indicated that rats fed dextrose diet had higher numbers of ACF consisting of 4 or more AC/focus compared to the group fed sucrose diet (21.0 versus 13.0).

Colonic cell proliferations were unaffected by dietary treatment (Table 4.4).

4.4 **DISCUSSION**

The present study was conducted to answer the key question: "Are there differences in diets containing dextrose or sucrose with respect to the development and growth of ACF?" Findings indicated that dextrose appeared to favour the growth of ACF more than sucrose, especially with respect to crypt multiplicity, ie. number of AC/focus. In addition, it was demonstrated that diet had no significant effect on cell proliferation expressed as mitotic activity in the colonic crypt.

It was reasoned that if dextrose were more conducive to ACF growth than sucrose, then the inhibitory effect of starch

TABLE 4.3: NUMBER AND CRYPT MULTIPLICITY OF ACF IN THE COLONIC MUCOSA OF SPRAGUE-DAWLEY MALE RATS

	NUMBER OF AC/ACF CRYPT MULTIPLICITY			
TREATMENT GROUPS	1	2	3	≥4
DEXTROSE	58.0 ± 5.0	84.0 ± 12.0	34.0 ± 4.1	21.0 ± 2.0
SUCROSE	81.0 ± 11.0	71.0 ± 16.0	23.0 ± 6.4	13.0 ± 6.0

All values shown are the mean ± SEM.
None of the values for each level of crypt multiplicity were found to be significantly different from each other ($p<0.05$).
Dextrose, n=4; Sucrose, n=4.

TABLE 4.4: EFFECTS OF SIMPLE CARBOHYDRATES ON CELL PROLIFERATION IN THE COLONIC MUCOSA OF SPRAGUE-DAWLEY MALE RATS

TREATMENT GROUPS	COLON SECTIONS	
	RECTAL	MID
	CRYPT HEIGHT/CRYPT SECTION[1]	
DEXTROSE	29.7 ± 2.6	33.8 ± 1.0
SUCROSE	34.6 ± 1.4	31.0 ± 2.1
	MITOTIC FIGURE/CRYPT SECTION[2]	
DEXTROSE	1.9 ± 0.7	2.3 ± 0.5
SUCROSE	2.4 ± 0.8	2.1 ± 0.6

All values shown mean ± SEM. Dextrose, n=4; Sucrose, n=4. None of the values within column for each parameter were found to be significantly different ($p<0.05$).
[1]Number of cells per mid-axial crypt.
[2]Number of mitotic figures per crypt section.

on ACF would be much more evident by comparing the effect of a diet containing dextrose as a source of simple sugar. Therefore, dextrose served as the source of simple sugar in all experimental diets in Study II and III.

CHAPTER V

STUDY II

THE EFFECTS OF VARYING LEVELS OF DIETARY STARCH ON AOM-INDUCED ABERRANT CRYPT FOCI

5.1 INTRODUCTION

The purpose of this study was to determine the effect of graded doses of dietary starch on the induction and growth of ACF. Study II was designed to query, "what are the effects of feeding rats high amounts of dietary starch on the induction and growth patterns of ACF?" The main hypothesis was that higher levels of raw cornstarch in a semi-synthetic diet would help reduce the development and growth of ACF with concomitant decrease in colonic cell proliferation.

This hypothesis was based on a handful of studies which have examined the effect of dietary starch on colon carcinogenesis (Bartram et al, 1991; Scheppach et al, 1988; Caderni et al, 1991a). Increased starch consumption has been reported to be associated with decreased risk of colon cancer development based on quantisation of biochemical risk markers, such as bile acids, SCFA and pH (Bartram et al, 1991; Scheppach et al, 1988; Kashtan et al, 1992).

5.2 SPECIFIC MATERIALS AND METHODS

5.2.1 Diets

Experimental diets consisted of three levels of cornstarch (CS): 15% (w/w), 30% (w/w), and 45% (w/w) (Table 5.0). Starch was added in place of dextrose. Dextrose served as the source of simple carbohydrate.

5.2.2 Study Designs

A total of 24 Sprague-Dawley rats were placed on laboratory chow. After 2-4 days of acclimatization, each rat received a single s.c. injection of 20 mg/kg of AOM. Then one week after AOM treatment, rats were randomized into dietary groups: 15% CS n=5; 30% CS n=9; and 45% CS n=9. These rats were fed the experimental diets for 60 days. Their colons were evaluated for ACF and proliferative indices.

5.3 RESULTS

In general, body weights were not significantly affected by diet (Figure 5.0 and Appendix B). Different levels of CS had no significant effect on food and energy intakes (Table 5.1).

The effect of CS on growth parameters of ACF are summarized in Table 5.2; Appendix C). Rats fed 45% CS diet had significantly higher numbers of ACF in their colons than the 30% CS group (369.8 versus 241.5 respectively) (p=0.007). This same group (45% CS) exhibited smaller ACF compared with rats fed the 30% CS diet ($2.1 \times 10^{-2} mm^2$ versus 2.7×10^{-2} mm^2

TABLE 5.0: PERCENT WEIGHT COMPOSITION (g/100g) OF THE EXPERIMENTAL DIETS

INGREDIENT	15% CS	30% CS[1]	45% CS[1]
CASEIN	20.0	20.0	20.0
DL-METHIONINE	0.3	0.3	0.3
DEXTROSE	50.0	35.0	20.0
CORNSTARCH	15.0	30.0	45.0
CELLUFIL	5.0	5.0	5.0
AIN-76 MINERAL MIX	3.5	3.5	3.5
AIN-76 VITAMIN MIX	1.0	1.0	1.0
CHOLINE BITARTRATE	0.2	0.2	0.2
CORN OIL	5.0	5.0	5.0

[1]Modified AIN-76 semisynthetic diet, CS=cornstarch.

FIGURE LEGEND

FIGURE 5.0 The effect of varying levels of cornstarch on body weights of Sprague-Dawley rats. 15% CS, n=5; 30% CS, n=8; 45% CS, n=9.

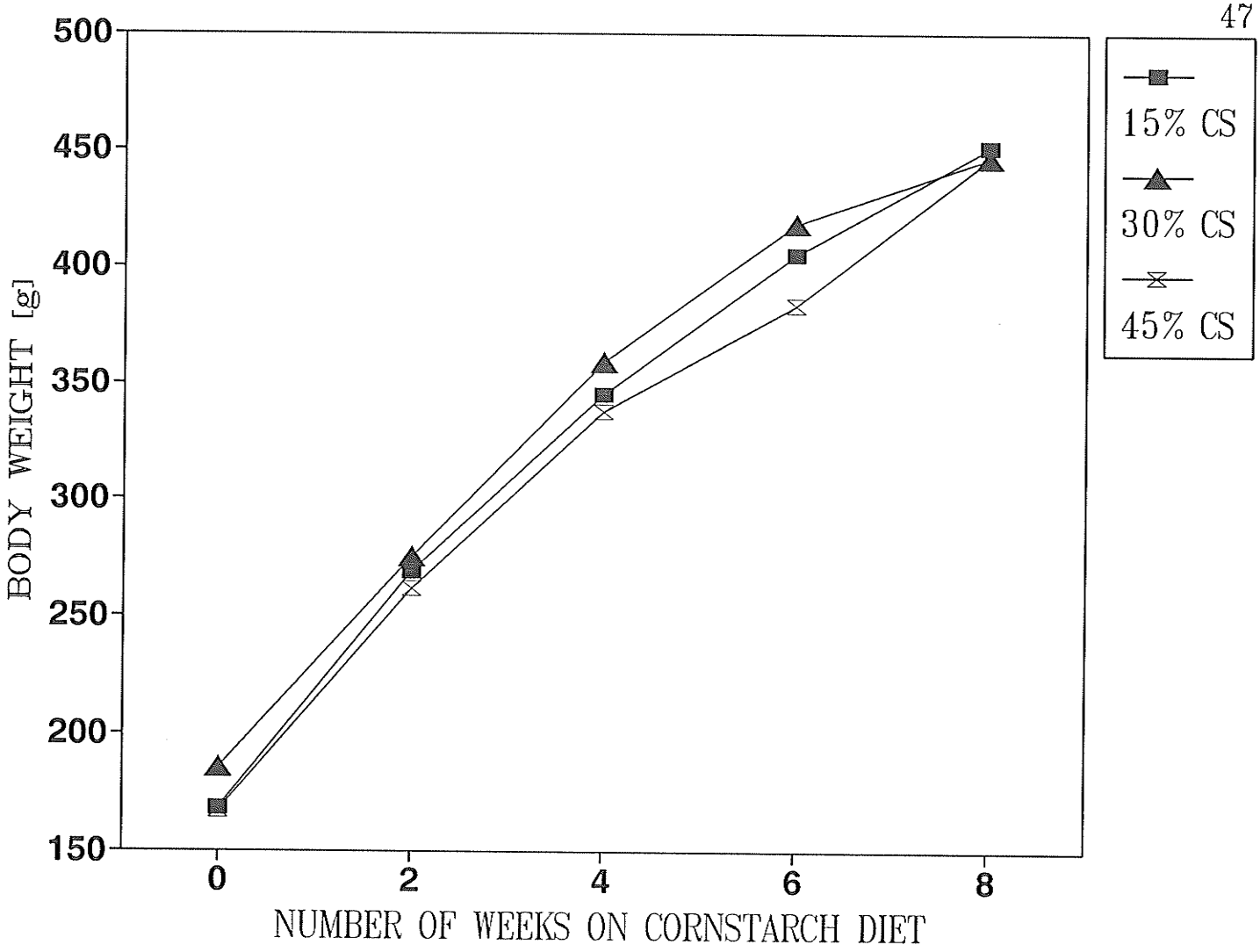

500
450
400
350
300
250
200
150
BODY WEIGHT [g]
0
2
4
6
8
NUMBER OF WEEKS ON CORNSTARCH DIET
15% CS
30% CS
45% CS

TABLE 5.1: MEAN BODY WEIGHTS (BW) AND ENERGY INTAKES

TREATMENT	N	BW[1]	MEAN DAILY FOOD INTAKE (GRAMS)	ENERGY (Kcal)	GROUP
15% CS	5	451.0 ± 24.0	26.4 ± 1.9	101.9 ± 7.2	
30% CS	8	446.6 ± 18.0	32.5 ± 4.5	125.5 ± 17.3	
45% CS	9	446.6 ± 13.0	24.6 ± 1.5	94.8 ± 5.8	

[1]The mean body weights determined on day of termination (week 8). All values shown are the mean ± SEM.
None of the values within each column for individual parameters were found to be significantly different ($p<0.05$).

TABLE 5.2: EFFECT OF VARYING LEVELS OF CORNSTARCH (CS) ON THE NUMBER, CRYPT MULTIPLICITY AND SIZE OF ACF IN THE COLONS OF RATS INJECTED WITH AZOXYMETHANE

TREATMENT GROUPS	MEAN ACF NUMBER OF ACF	MEAN AC/FOCUS CRYPT MULTIPLICITY	MEAN SIZE OF FOCI $x10^{-2}mm^2$ SIZE OF ACF
15% CS	313.8 ± 28.0[a,b]	1.9 ± 0.1	2.6 ± 0.2[a,b]
30% CS	241.5 ± 36.0[b]	1.9 ± 0.1	2.7 ± 0.2[a]
45% CS	369.8 ± 14.0[a]	1.9 ± 0.1	2.1 ± 0.1[b]

All values shown are the mean ± SEM. Means in the same column with same letter are not significantly different ($p < 0.05$). 15% CS, n=5; 30% CS, n=8; 45% CS, n=9.

respectively) ($p=0.05$). There were no significant differences in the mean number of AC/focus for all the treatment groups.

The effects of varying levels of CS on colonic cell proliferative indices were inconsistent. In the rectal region, CH was not affected by dietary treatments (Appendix D). Significant changes were observed in the mid-region, CH was found to be the highest in the colons of rats fed 30% CS diet compared to other dietary groups ($p=0.009$) (Figure 5.1 and Appendix D). Mitotic figures and LI were not significantly affected by dietary treatments (Appendix D and E). Nevertheless, some interesting trends were observed, the 30% CS group was associated with elevated mitotic activity and LI in the rectal region (2.7 and 11.3 respectively), the 45% CS group exhibited elevated mitotic and labelling index in the mid-region compared to the 15% or 30% CS groups (Figure 5.2 and 5.3).

5.4 **DISCUSSION**

The objective of Study II was to determine the effect of graded doses of raw cornstarch on the early stages of colon carcinogenesis. This study did not support the hypothesis that increasing levels of starch in the diet would reduce the risk of colon cancer by employing ACF as biological endpoints. Eight weeks of dietary treatments, employing higher levels of CS did not impart a distinct growth modulating effect on ACF in a dose-related manner.

FIGURE LEGEND

FIGURE 5.1 The effect of varying levels of cornstarch on crypt height (CH) in the colons of Sprague-Dawley rats. Bars with different superscript are significantly different ($p<0.05$). Bars represent mean number of cells per mid-axial crypt/crypt section/group. 15% CS, n=3, 30% CS, n=5; 45% CS, n=4.

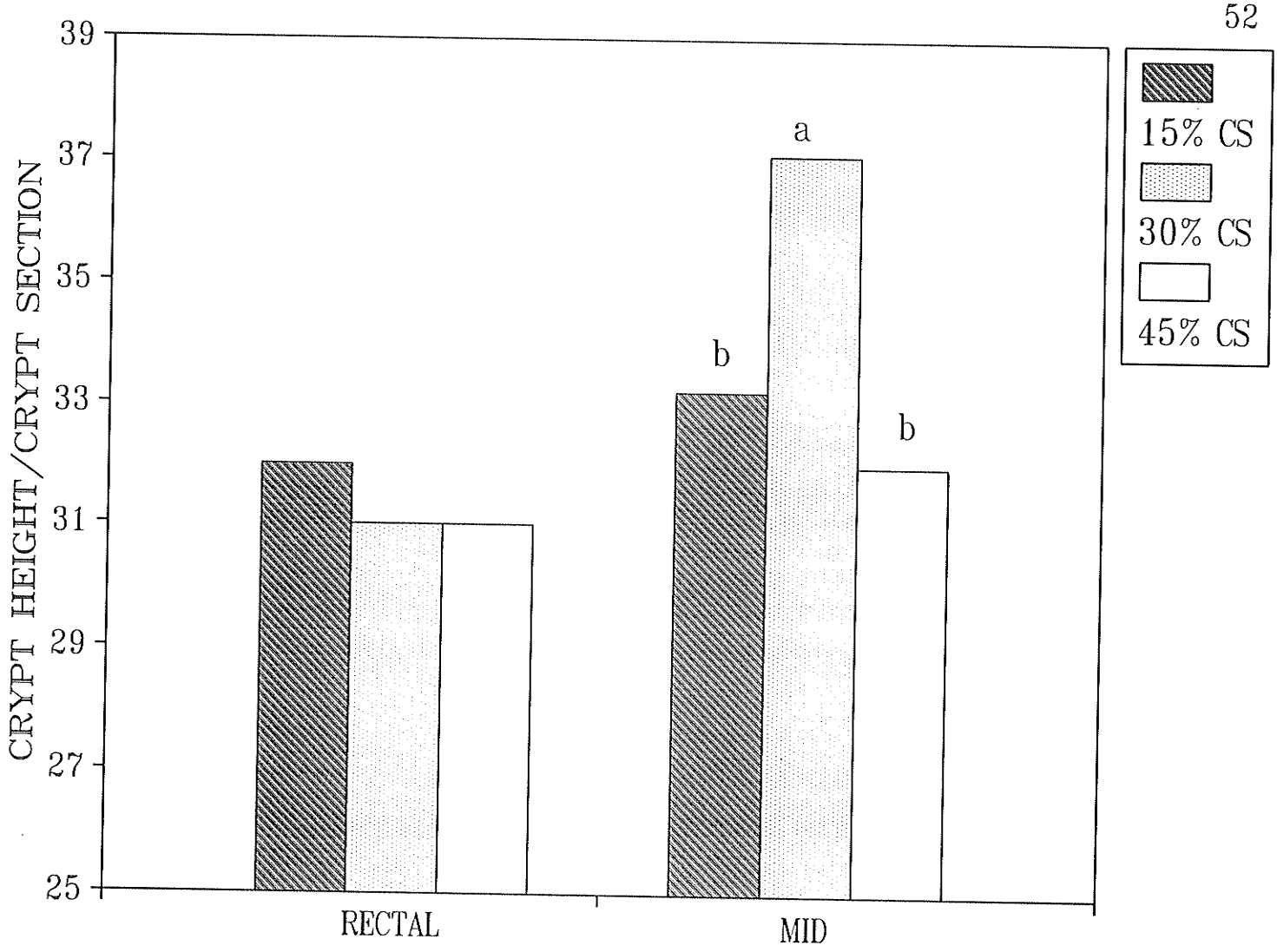

CRYPT HEIGHT/CRYPT SECTION
39
37
35
33
31
29
27
25
a
b
b
RECTAL
MID
15% CS
30% CS
45% CS

FIGURE LEGEND

FIGURE 5.2 Mitotic activity in 2 regions of the colons in rats fed varying levels of cornstarch. 15% CS, n=3; 30% CS, n=5; 45% CS, n=4. Bars represent mitotic figures/crypt section/group.

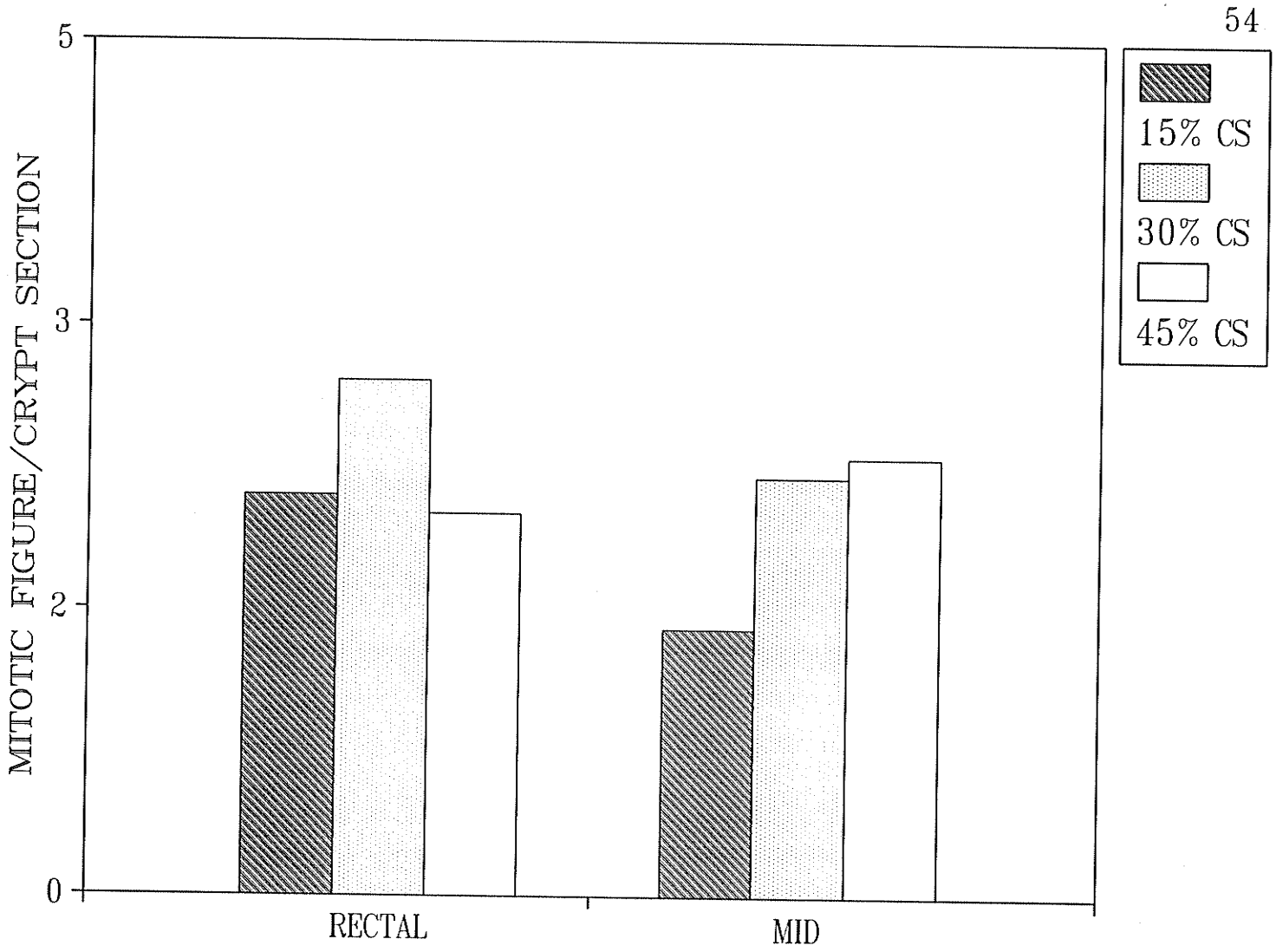
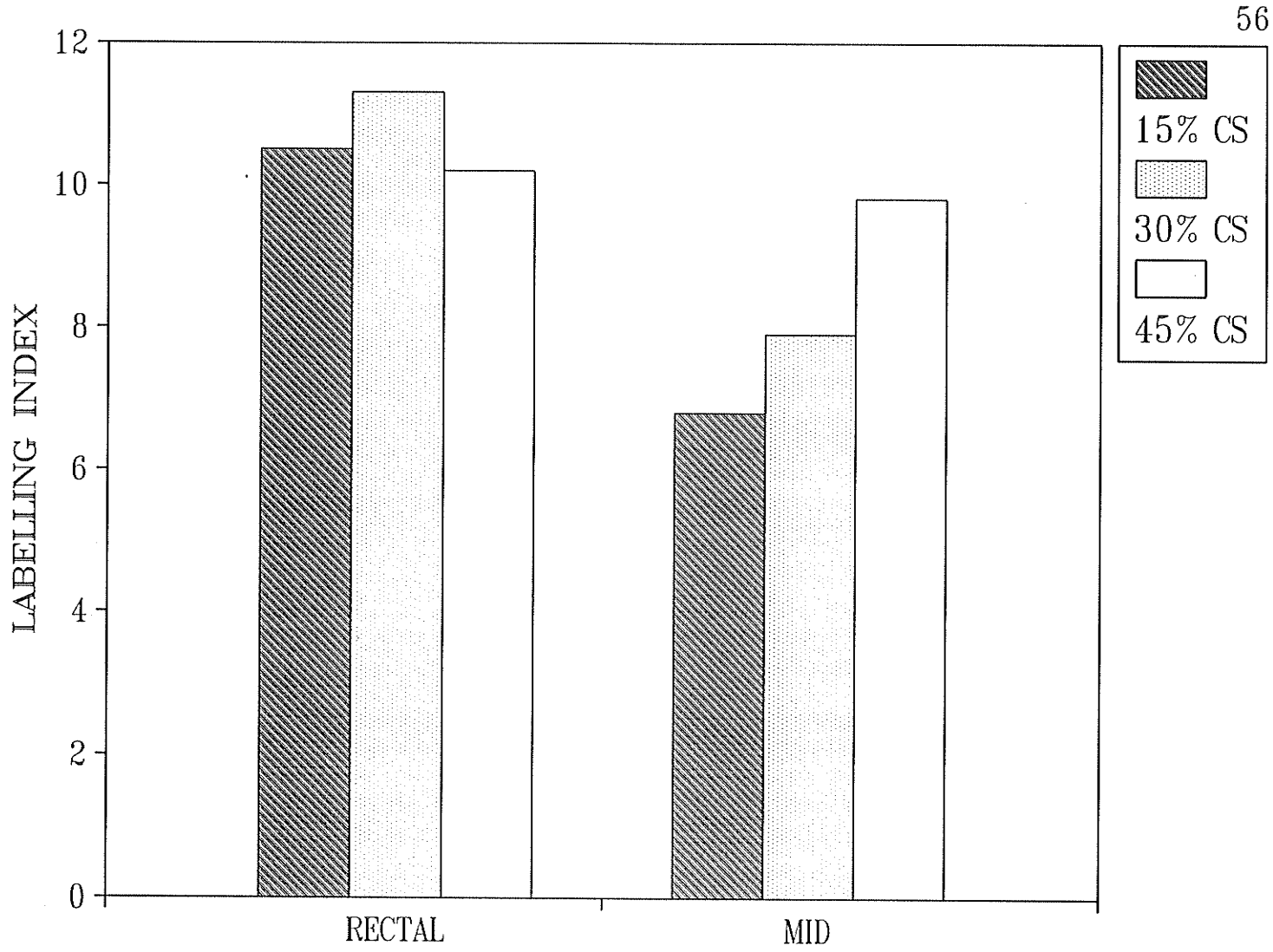

MITOTIC FIGURE/CRYPT SECTION
5
3
2
0
RECTAL
MID
15% CS
30% CS
45% CS

FIGURE LEGEND

FIGURE 5.3 Labelling index in 2 regions of the colons in rats fed varying levels of cornstarch. 15% CS, n=2; 30% CS, n=3; 45% CS, n=5. Bars represent mean labelling index/crypt section/group.


12
10
8
6
4
2
0
LABELLING INDEX
RECTAL
MID
15% CS
30% CS
45% CS

The risk of colon cancer development was assessed by using number and growth characteristics of ACF as the biological endpoints. Based on these criteria ,it would appear that 30% CS diet was associated with suppressing the formation of new ACF as reflected by a decrease in the total number of ACF in the colons. To ascertain the validity of this finding another study was conducted for 4 weeks in which rats were fed 15% or 30% CS diets. In this study, the trend suggested that the 15% CS group possessed higher number of ACF with increased crypt multiplicity, especially of those ACF consisting of 4 or more crypts/focus than the 30% CS group (Appendix F). These findings suggest and strengthens the contention that there could exist a threshold level, in this case 30% CS (w/w) at which starch may exert a protective role in colon carcinogenesis.

It was apparent that in the 45% CS group an increase in the total number of ACF was accompanied by reduced mean size of ACF compared to the 30% CS groups. Whether larger aberrant crypts are biologically different than smaller aberrant crypts with respect to carcinogenic potential remains to be established. A preliminary study in our laboratory suggest that the larger dilated aberrant crypts consist of larger number of goblet cells (Bird, personal communication, 1993). Therefore, larger ACF may indicate their state of differentiation. Further studies are in progress to evaluate this proposal.

Proliferative indices of the colonic epithelium belonging to various diet groups were unable to predict the risk as shown by employing ACF as the biological endpoints. Bird (personal communication, 1993) reported that the colons of rats fed high fat, low fibre diets contained larger number of lesions but were accompanied by decreased mitotic activity. Galloway and co-workers (1987) reported that a low risk diet, low fat, high fibre induced lower numbers of colonic tumours. However, this diet was associated with increased colonic cell proliferation as measured by mitotic activity. In Study II, this parameter was not significantly affected by dietary interventions but trends indicated that mitotic activity was highest in the rectal regions of the 30% CS group compared to 15% or 45% CS groups (Figure 5.2; Appendix D).

Caderni and co-workers (1991a) investigated the effect of a low starch diet (0% w/w; 46% w/w sucrose) versus a high starch diet (46% w/w; 0% w/w sucrose) on the induction and growth of ACF (which was referred to as foci of dysplastic crypts). These investigators demonstrated (after 12 weeks of feeding) that ACF with higher crypt multiplicity were less frequently observed in the high starch group. In spite of a marked difference in the starch content of the diets, (high starch, 46% w/w; 0% w/w sucrose versus low starch, 0% w/w; 46% w/w sucrose) the differences reported in the number of ACF among the two groups were minor and not statistically significant. Furthermore, the experimental diets used by the

investigators were formulated to reflect the so-called "western-stress" diet, of high fat (23% corn oil, w/w)/low calcium (0.1%, w/w)/low cellulose (2.0%, w/w). This diet included many variables and did not assess the effect of starch per se. Therefore, it is possible that effects of diet reported by Caderni and co-workers (1991a) maybe due to an interaction between starch and fat.

In summary, this study has demonstrated that the 30% CS diet had an inhibitory effect on the development and growth of ACF compared to the 45% CS diet. Also, it would appear that the starch content of the diet may influence colonic cell proliferation but a distinct association between proliferative indices and disease development was not established.

CHAPTER VI

STUDY III

THE EFFECTS OF RAW AND THERMALLY-TREATED CORNSTARCH ON ABERRANT CRYPT FOCI AND CELL PROLIFERATION IN THE COLONS OF SPRAGUE-DAWLEY MALE RATS

6.1 INTRODUCTION

The main objective of this study was to determine the effects of two different starch forms, raw (RCS) and thermally-treated cornstarch (TCS) at two different dose levels 15% and 45% (w/w), on the carcinogenic process and cytokinetic events in the colons of Sprague-Dawley male rats.

The thermal processing of starch via extreme cooking conditions, ie autoclaving, is known to generate modified starches referred to as *resistant starch* (RS) (Englyst and Cummings, 1987; Sievert and Pomeranz, 1989). It is believed that RS may be a useful substrate for bacterial fermentation in the large bowel, and this has potential implications in the prevention and treatment of colon cancer (Englyst and Cummings, 1987; Ranhotra et al, 1991). It is also believed that TCS which contains RS may impart protection in the colon against the development and growth of ACF compared to raw starch which contains low amounts of RS (Englyst and Cummings, 1987). The hypothesis for this study was thermally-treated

cornstarch which contains RS when incorporated into a semisynthetic diet would modulate the early stages of colon cancer by reducing the appearance and growth of ACF in the colons of Sprague-Dawley rats.

In the present study, a lactulose group was included as a positive control in order to compare diets containing raw or thermally-treated cornstarch with respect to fermentation in the colon. Lactulose is a non-digestible carbohydrate, which has been demonstrated to act as a fermentable substrate in the colon (Jenkins, 1991; Florent et al, 1985). In addition to measuring ACF, colonic cell proliferation, faecal output, water and pH were also determined.

6.2 SPECIFIC MATERIALS AND METHODS

6.2.1 Chemicals

4-O-ß-D-galactopyranosyl-D-fructofuranose (Lactulose) was purchased from Sigma Chemical Co. (St. Louis, MO.)

6.2.2 Production Of Resistant Starch

The methodology used for producing RS was adapted from Sievert and Pomeranz (1989). One kilogram of cornstarch (CS) was mixed with deionized water. The starch to water ratio used was 1:2. Starch and water were mixed for 5 minutes to ensure adequate suspension. The mixture was then autoclaved for 1 hour at 121°C (using a thermostatically-controlled autoclave AMSCO, USA). After a single autoclave step, samples were allowed to cool for 30 minutes, and then chilled in a

refrigerator at 4°C for 24 hours. Afterwards, chilled starch samples were dehydrated using a fan oven at 80°C for 24 hours. Dried starch samples were ground on a mill. Further re-grinding into powder was done using a Magic Mill III(Milltown International, Wpg., MB.). Thermally-treated cornstarch (TCS) were stored in the refrigerator prior to use in the study.

6.2.3 Detection and Quantisation of Resistant Starch with Differential Scanning Calorimetry and Enzymatic Gravimetric Assay

RS was qualitatively and quantitatively estimated by Differential Scanning Calorimetry (DSC) and Enzymatic-Gravimetric assay respectively.

6.2.3.1 Differential Scanning Calorimetry (DSC)

The purpose of the DSC was to qualitatively determine the presence of retrograded amylose crystallites. Differential Scanning Calorimetry allows the identification of a specific endothermic peak that distinctively corresponds to the disassociation of amylose crystallites. DSC measurements were performed with a DuPont 9900 thermal analyzer, which is equipped with a 910 DSC cell base and a high pressure cell. An indium standard was used for temperature and energy calibration. A pressure of 1400 kPa (200 psi) was maintained with nitrogen gas. This helps to eliminate pan failure because of moisture loss at temperatures above 120°C. Samples of 3.0-3.3 mg were weighed into DuPont coated aluminum pans. Water was added making a final 30% w/w solid in the pans.

The pans were hermetically sealed. An empty pan was used as a reference. All samples were heated from 20-180°C with a 10°C/minute increase in heating rate.

6.2.3.2 **Enzymatic-Gravimetric Assay**

Analysis for RS was carried out according to the Official Method of Analysis of the Association of Official Analytical Chemists (AOAC) method 991.43 (Lee et al, 1992) (National Agri-Food Technology Centre, Portage la Prairie, MB). The AOAC method is an enzymatic-gravimetric assay which is applicable to processed foods, cereal, fruit, and vegetable products (Lee et al, 1992). Briefly, starch samples had undergone sequential enzymatic digestion by heat stable α-amylase, protease, and amyloglucosidase. The residue was then treated with various washing of alcohol and acetone, dried and weighed. Residue values were adjusted for protein and ash content. Resistant Starch was considered as the residue remaining after these steps.

6.2.4 **Diets**

Five different diets were prepared based on AIN-76 diet (Table 6.0):

a) Diet #1: This diet contained 15% RCS + 1% lactulose (l) by weight.

b) Diet #2 and #3: Modified AIN-76 diets containing 15% and 45% by weight raw corn starch (RCS).

TABLE 6.0: PERCENT WEIGHT COMPOSITION (g/100g) OF THE EXPERIMENTAL DIETS

INGREDIENTS	15% RCS[1] + 1% l	15% RCS	45% RCS	15% TCS	45% TCS
CASEIN	20.0	20.0	20.0	20.0	20.0
DL-METHIONINE	0.3	0.3	0.3	0.3	0.3
DEXTROSE	49.0	50.0	20.0	50.0	20.0
CORNSTARCH	15.0	15.0	45.0	15.0	45.0
CELLUFIL	5.0	5.0	5.0	5.0	5.0
AIN-76 MINERAL MIX	3.5	3.5	3.5	3.5	3.5
AIN-76 VITAMIN MIX	1.0	1.0	1.0	1.0	1.0
CHOLINE BITARTRATE	0.2	0.2	0.2	0.2	0.2
CORN OIL	5.0	5.0	5.0	5.0	5.0
LACTULOSE	1.0	-	-	-	-
RS[2]	0.2	0.2	0.2	4.2	4.2
TOTAL % INDIGESTIBLE CHO[3]	6.03	5.03	5.09	5.63	6.89

[1]l, L=lactulose; RCS=raw cornstarch; TCS= thermally-treated cornstarch.
[2]RS expressed as % total dietary fibre (TDF) (g/100g)(AOAC METHOD, 991.43) (Lee et al, 1992.
[3]Example in 15% RCS diet: amount of RS as % TDF = 0.2%. therefore, 0.2% in 15% RCS = 0.0002 x 0.15 = 0.003 (x100)= 0.03. Therefore, total % indigestible CHO in 15% RCS diet = CELLUFIL + RS = 5.00 + 0.03 = 5.03%.

c) Diet #4 and #5: Modified AIN-76 diets containing 15% and 45% by weight thermally-treated cornstarch (TCS). In all diets dextrose served as the source of simple carbohydrate.

6.2.5 **Study Designs**

Fifty male Sprague-Dawley rats were used in this study. During the period of acclimatization, rats were fed laboratory chow (Chapter III). Immediately after acclimatization, individual rats were given a single injection of AOM (20 mg/kg, sc). One week after carcinogen treatment, rats were randomly allocated to 5 treatment of 10 rats/group. The length of treatment was 8 weeks. Prior to termination, rats were injected with both colchicine (1.0 mg/kg) and BrdU (30 mg/kg). The entire length of the colon was examined for ACF and cell proliferative parameters as described previously (Chapter III).

6.2.6 **Body Weight, Food Consumption, And Organ Weight**

Body weights and 24-hour food consumption were monitored. Immediately after termination, liver, kidney, and heart were excised, weighed, and examined for the presence of any macroscopic abnormalities. After removal of faecal material, individual colons were measured for colonic weight and lengths prior to sectioning into caecal, mid, and rectal regions.

6.2.7 **Determination of Faecal pH**

Faecal pellets were collected from individual cages.

Each cage housed 2-3 rats. Paper towels were placed underneath the cages to help collect the faeces. Immediately after collection, 3-5 faecal pellets were ground, mixed with deionized water, and poured into a 20 ml beaker. The aqueous phase was used to determine pH using a Fisher Accumet pH meter, model 810.

6.2.8 **24-hour Faecal Collection**

A 24-hour faecal collection was conducted using metabolic cages. Each metabolic cage was equipped with a water and food dispenser. Individual rats were housed in the metabolic cages until the 24-hour collection period expired. The wet weight of the faecal samples was recorded (Mettler AE-200). Faeces were stored at -80°C prior to analysis.

6.2.9 **Faecal Water**

Faecal samples were analyzed for faecal water. Five faecal pellets were removed from the freezer, weighed then heated in a Fisher IsoTemp Oven at 60°C for 24 hours. The dehydrated samples were re-weighed and the dry/wet ratio was determined. This ratios represents dry mass remaining per gram of wet weight of faeces.

6.3 **Statistical Analysis**

Statistical evaluation was carried out using the Statistical Analysis Software (SAS, 1988) package. SAS orthogonal contrast statements were used to examine main

effects and interactions. Comparison using paired t-tests on least square means was utilized to assess differences between the rectal and mid-end of the colon with respect to proliferative activity. The Pearson Product Moment was used to investigate the relationship between LI and MF.

6.4 RESULTS

Resistant starch in the TCS samples were analyzed using the Differential Scanning Calorimetry and the enzymatic assay. Four endothermic transition peaks were observed (Figure 6.0). The peak at 47.13°C was attributed to retrograded amylopectin while those at 90.14 and 119.05°C were due to amylose-lipid interactions (Sievert and Pomeranz, 1990). The endothermic transition peak at 144.93°C in the TCS sample was indicative of the presence of resistant starch.

A greater amount of RS expressed as percent total dietary fibre residue was noted in TCS than in RCS samples. Raw starch samples containing 1% w/w cellufil also demonstrated the presence of dietary fibre residue (Table 6.1).

6.4.1 Body Weight, Food And Energy Intakes

In Figure 6.1, significant differences in body weights at Week 8 were apparent between the 15% and 45% RCS groups (395.6g versus 452.9g) ($p=0.03$) (Figure 6.1; Appendix G). Further analysis revealed that a main effect of dose and interaction (dose levels and starch forms: RCS versus TCS) on

FIGURE LEGEND

FIGURE 6.0 Differential Scanning Calorimetry (DSC) profile for thermally-treated starch (TCS), autoclaved for 1 hour at 121°C.

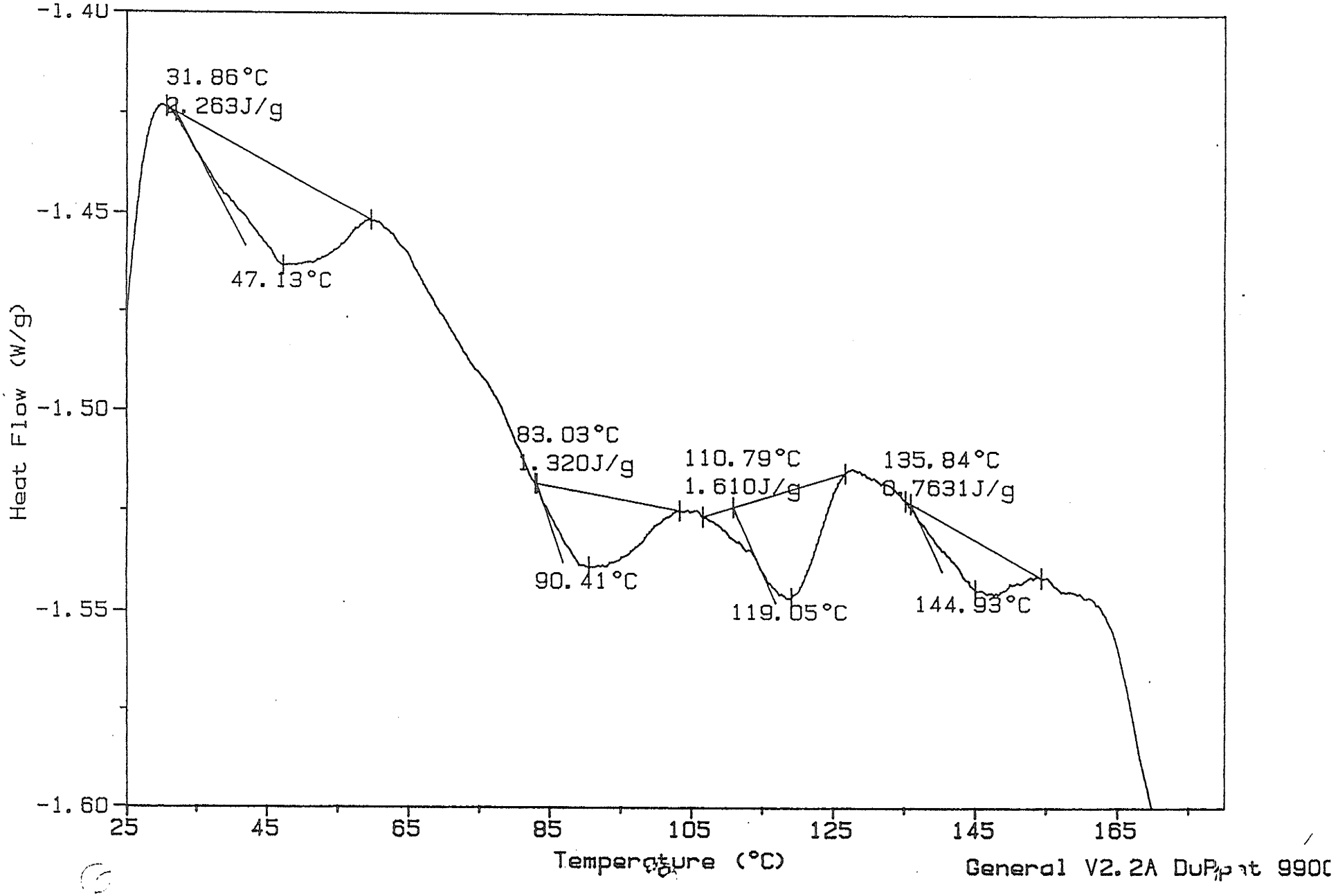

-1.40
-1.45
-1.50
-1.55
-1.60
Heat Flow (W/g)
31.86°C
8.263J/g
47.13°C
83.03°C
1.320J/g
90.41°C
110.79°C
1.610J/g
119.05°C
135.84°C
0.7631J/g
144.93°C
25
45
65
85
105
125
145
165
Temperature (°C)
General V2.2A DuPont 990C

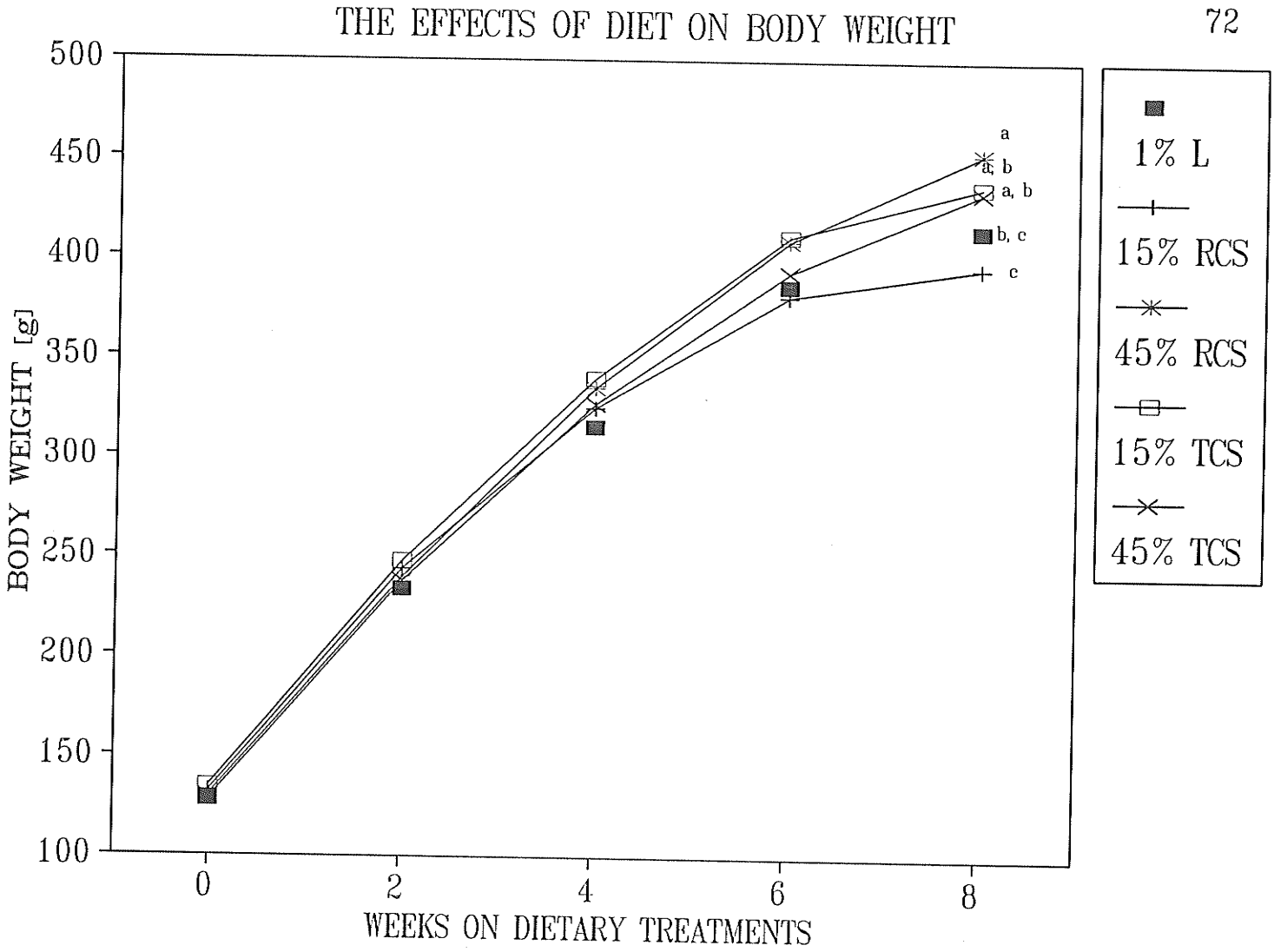
TABLE 6.1: ENZYMATIC-GRAVIMETRIC ANALYSIS FOR TOTAL DIETARY FIBRE

STARCH SAMPLES	% TOTAL DIETARY FIBRE[1]
RAW CORNSTARCH (RCS) (A)	0.2
THERMALLY-TREATED CORNSTARCH (TCS) (B)	4.2
1% (w/w) CELLUFIL + RAW CORNSTARCH (C)	1.6

[1]Determination of total dietary fibre (g/100g)(AOAC METHOD, 991.43)(Lee et al, 1992)
A + C were included in the analysis to establish the validity and senstivity of the procedure. All samples were coded and analysized blindly.

FIGURE LEGEND

Figure 6.1 The effect of dietary treatment on body weight. Line graph with the same letters are not significantly different ($p<0.05$). L=lactulose; RCS=raw cornstarch; TCS=thermally-treated cornstarch. 1% L=15% RCS + 1%L, n=9; 15% RCS, n=9; 45% RCS, n=9; 15% TCS, n=10; 45% TCS, n=10.


THE EFFECTS OF DIET ON BODY WEIGHT
BODY WEIGHT [g]
500
450
400
350
300
250
200
150
100
0
2
4
6
8
WEEKS ON DIETARY TREATMENTS
a
a, b
a, b
b, c
c
1% L
15% RCS
45% RCS
15% TCS
45% TCS

body weights were shown (Appendix H). Rats fed 15% RCS demonstrated higher food and energy intakes than all other dietary groups (p=0.0001) (Table 6.2).

6.4.2 Organ Weights

Generally, the liver, kidney, and heart were not significantly affected by diet (Table 6.3). Despite these findings, differences between dietary groups were evident by employing SAS orthogonal statements (Appendix I). It was revealed that the liver, kidney, and heart were significantly affected by interactions between dose levels and cooking (RCS versus TCS) (p=0.05; 0.04; 0.02 respectively) (Appendix I). Rats fed 45% TCS diet had significantly longer colons than those which were fed 15% RCS + 1% l or 15% RCS (23.4cm versus 21.5cm and 21.2cm respectively) (p=0.02) (Table 6.4). Concomitantly, colonic weight was unaffected by dietary treatments. Further investigations using SAS orthogonal statements indicated effects of starch forms (RCS, TCS) on colonic length (p=0.004) and weight (p=0.04) (Appendix J).

6.4.3 The Effects Of Diet On The Induction And Growth Of Aberrant Crypt Foci

Despite no apparent significant differences between dietary groups with respect to ACF, trends indicated that rats fed 15% RCS + 1% l, 15% or 45% TCS diets had larger ACF and a higher number of AC/focus in their colons compared to rats on

TABLE 6.2: FOOD AND ENERGY INTAKES IN SPRAGUE-DAWLEY MALE RATS

TREATMENT GROUP	N	DAILY FOOD INTAKE (g)	ENERGY Kcal/day
15% RCS + 1% l	9	32.5 ± 1.0[b]	125.3 ± 4.0[b]
15% RCS	9	36.1 ± 1.5[a]	139.3 ± 5.7[a]
45% RCS	9	28.2 ± 0.5[d]	108.8 ± 1.9[d]
15% TCS	10	31.3 ± 1.1[b,c]	120.9 ± 4.1[b,c]
45% TCS	10	28.9 ± 0.5[c,d]	111.4 ± 1.8[c,d]

All values shown are mean ± SEM. Means in the same column with the same letter are not signifcantly different ($p < 0.05$).
Length of treatments was for 8 weeks. Food consumption obtained on week 8 of study.
l=lactulose
RCS=raw cornstarch
TCS=thermally-treated cornstarch

TABLE 6.3: ORGAN WEIGHTS IN SPRAGUE-DAWLEY RATS

TREATMENT GROUPS	N	ORGAN
		LIVER (g)
15% RCS + 1% l	9	14.1 ± 0.8
15% RCS	9	13.8 ± 0.8
45% RCS	9	16.9 ± 0.9
15% TCS	10	15.8 ± 1.2
45% TCS	10	15.1 ± 1.0
		KIDNEY (g)
15% RCS + 1% l	9	1.5 ± 0.05
15% RCS	9	1.4 ± 0.04
45% RCS	9	1.6 ± 0.08
15% TCS	10	1.6 ± 0.08
45% TCS	10	1.5 ± 0.05
		HEART (g)
15% RCS + 1% l	9	1.3 ± 0.02
15% RCS	9	1.3 ± 0.04
45% RCS	9	1.4 ± 0.06
15% TCS	10	1.5 ± 0.07
45% TCS	10	1.3 ± 0.05

All values shown are the mean ± SEM.
Organ weights obtained on week 8.
l=lactulose
RCS=raw cornstarch
TCS=thermally-treated cornstarch
None of the values within each row for each parameter were found to be significantly different ($p<0.05$).

TABLE 6.4: LENGTH AND WEIGHT OF COLONS IN SPRAGUE-DAWLEY RATS

TREATMENT GROUPS	N	*COLON LENGTH (cm)*	*COLON WEIGHT (g)*
15% RCS + 1% l	9	21.5 ± 0.7[b]	1.8 ± 0.08
15% RCS	9	21.2 ± 0.5[b]	1.8 ± 0.05
45% RCS	9	21.9 ± 0.5[a,b]	1.6 ± 0.09
15% TCS	10	23.0 ± 0.6[a,b]	1.8 ± 0.09
45% TCS	10	23.4 ± 0.3[a]	1.9 ± 0.06

All values shown are mean ± SEM. Means in the same column with the same letter are not significantly different ($p < 0.05$).
Organ weights obtained on week 8.
l=lactulose
RCS=raw cornstarch
TCS=thermally-treated cornstarch

TABLE 6.5: THE EFFECT OF LACTULOSE, RAW, AND THERMALLY-TREATED STARCH ON THE NUMBER, CRYPT MULTIPLICITY, AND SIZE OF ACF IN THE COLON OF SPRAGUE-DAWLEY RATS

TREATMENT GROUP	MEAN ACF	MEAN AC/FOCUS	MEAN SIZE OF FOCUS $x10^{-2}mm^2$
	NUMBER OF ACF	CRYPT MULTIPLICITY	SIZE OF ACF
15% RCS + 1% l	311.3 ± 23.0	2.5 ± 0.1	2.9 ± 0.2
15% RCS	333.4 ± 34.0	2.3 ± 0.1	2.7 ± 0.1
45% RCS	313.8 ± 40.0	2.3 ± 0.1	2.5 ± 0.2
15% TCS	316.3 ± 31.0	2.4 ± 0.1	2.9 ± 0.1
45% TCS	281.0 ± 27.0	2.6 ± 0.1	3.0 ± 0.1

All values shown are mean ± SEM.
l=lactulose
RCS=raw cornstarch
TCS=thermally-treated cornstarch
15% RCS + 1% l, n=9
15% RCS, n=9
45% RCS, n=9
15% TCS, n=10
45% TCS, n=10
None of the values for within each column for each ACF parameter were found to be significantly different ($p<0.05$).

15% or 45% RCS diets (Table 6.5). Alternatively, according to the output from the orthogonal statements cooking had a significant impact on the number of AC per focus (p=0.04) and size (p=0.02) (Appendix K).

In all the dietary groups, trends for the distribution pattern of ACF with different crypt multiplicity revealed that a greater proportion of ACF consisted of 1-2 AC/focus (Appendix L). It was also evident that in the 45% TCS group, higher numbers of ACF with 3 or more AC/focus were present, than the 45% RCS diet group (39.8% versus 31.1%) (p=0.03) (Figure 6.2; Appendix M).

6.4.4 FAECAL PARAMETERS

Faecal pH for the 15% RCS group was significantly higher than for the 15% or 45% TCS groups (p=0.04) (Table 6.6). Faecal weight was not significantly affected by diet, but trends indicated that faecal output were higher in rats fed 45% RCS or 45% TCS diets (Table 6.7). The amount of faecal water was unaffected by dietary treatment (Table 6.7).

6.4.5 The Effects Of Dietary Treatment On Crypt Height, Mitotic Activity, And DNA-synthetic

6.4.5.1 Crypt Height (CH)

In general, there was no significant effect of dietary treatment on CH measurements. Trends were observed in which rats fed 45% TCS had elevated CH in the rectal and mid-region of their colons compared to other dietary groups (Table 6.8).

FIGURE LEGEND

FIGURE 6.2 **Percentage of ACF according to varying crypt multiplicity in the colons of rats fed 45% RCS (w/w) or 45% TCS (w/w). Bars with different superscript are significantly different ($p<0.05$). Bars represent % mean ACF in the colons of Sprague-Dawley rats. RCS=raw cornstarch; TCS=thermally-treated cornstarch. 45% RCS, n=9; 45% TCS, n=10.**

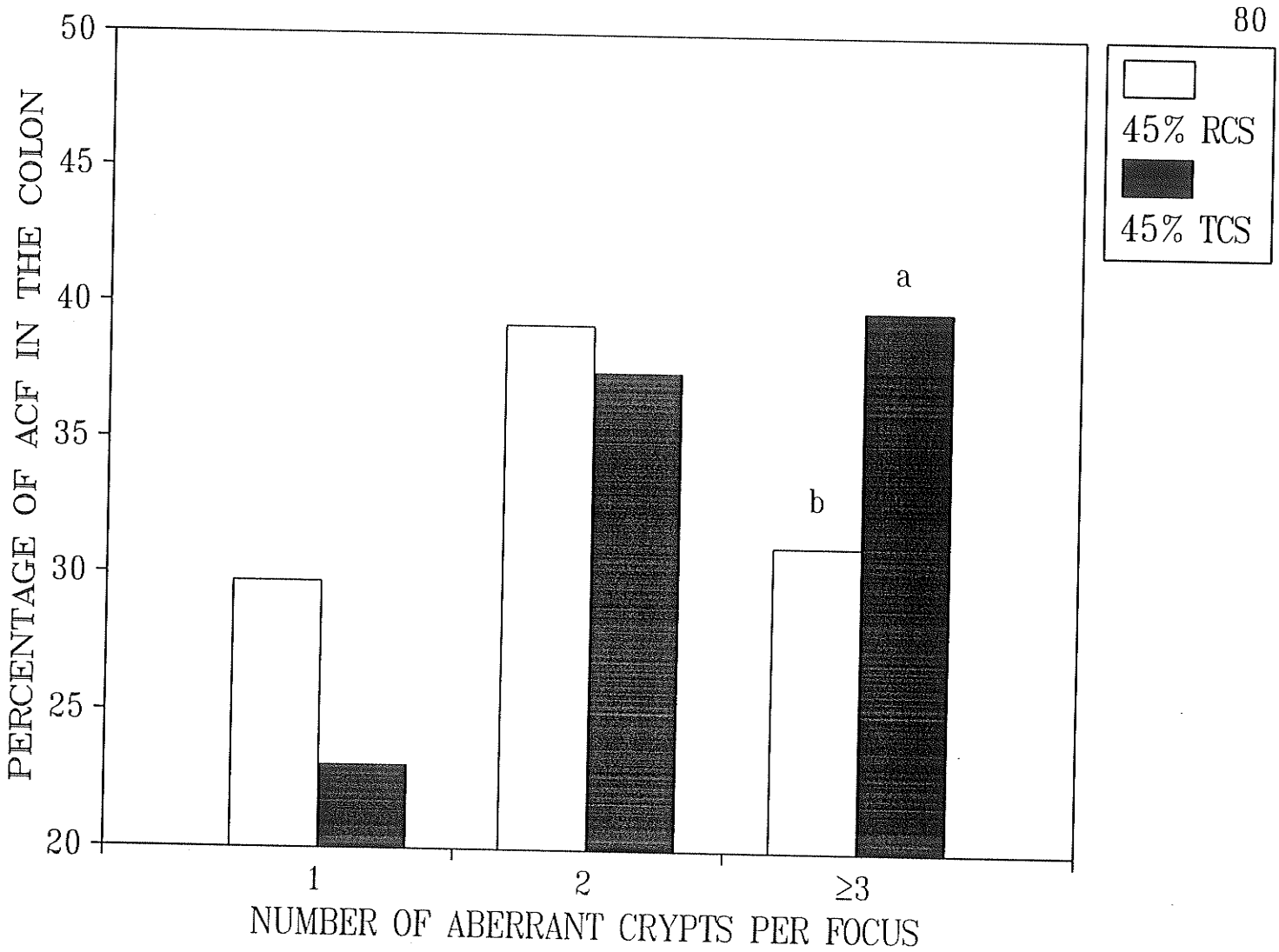

80
50
45
40
35
30
25
20
45% RCS
45% TCS
a
b
PERCENTAGE OF ACF IN THE COLON
1
2
≥3
NUMBER OF ABERRANT CRYPTS PER FOCUS

TABLE 6.6: THE EFFECT OF DIETARY TREATMENT ON FAECAL pH

TREATMENT GROUPS	N	FAECAL pH
15% RCS + 1% l	6	$7.1 \pm 0.2^{a,b,c}$
15% RCS	9	7.5 ± 0.1^{a}
45% RCS	9	$7.4 \pm 0.1^{a,b}$
15% TCS	10	$6.9 \pm 0.1^{b,c}$
45% TCS	10	6.8 ± 0.1^{c}

All values shown are mean ± SEM. Means in the column with the same letter are not significantly different ($p < 0.05$).
Faecal pH taken at week 8.
l=lactulose
RCS= raw cornstarch
TCS=thermally-treated cornstarch

TABLE 6.7: THE EFFECT OF DIETARY TREATMENT ON FAECAL WEIGHT

TREATMENT GROUP	FAECAL WET WEIGHT (g)	FAECAL DRY WEIGHT/WET WEIGHT
15% RCS + 1% l	1.5 ± 0.6	0.68 ± 0.02
15% RCS	1.4 ± 0.3	0.62 ± 0.02
45% RCS	1.8 ± 0.3	0.63 ± 0.10
15% TCS	1.2 ± 0.3	0.61 ± 0.01
45% TCS	2.5 ± 0.3	0.59 ± 0.01

All values shown are mean ± SEM.
24-hour wet faecal collection for week 8.
l=lactulose
RCS=raw cornstarch
TCS=thermally-treated cornstarch
15% RCS + 1% l, n=8
15% RCS, n=9
45% RCS, n=8
15% TCS, n=10
45% TCS, n=10
None of the values within each column for each parameter were found to be significantly different ($p<0.05$).

TABLE 6.8: EFFECT OF DIETARY TREATMENTS ON CRYPT HEIGHT IN THE COLON CRYPTS OF SPRAGUE-DAWLEY RATS

TREATMENT	N	MEAN CRYPT HEIGHT/CRYPT SECTION[1]	
		RECTAL REGION	MID REGION
15% RCS + 1% l	9	30.7 ± 1.4	31.3 ± 1.6
15% RCS	9	30.5 ± 0.7	31.2 ± 1.2
45% RCS	9	28.7 ± 1.0	32.4 ± 1.2
15% TCS	10	30.7 ± 0.9	30.4 ± 1.3
45% TCS	10	32.7 ± 1.0	33.0 ± 1.6

All values shown are mean ± SEM.
l=lactulose
RCS=raw cornstarch
TCS=thermally-treated cornstarch
[1]Number of cells per mid-axial crypt.
None of the values within each column for individual colonic sections were found to be significantly different ($p<0.05$).

6.4.5.2 **Mitotic Activity (MF)**

In the rectal region, MF in the colonic crypts were unaffected by dietary treatments (Figure 6.3; Appendix N). Interestingly, a trend was shown in which increased mitotic activity was noted in the mid-region of the colons of rats fed 45% RCS or 15% TCS diets (Figure 6.3; Appendix N).

6.4.5.3 **DNA-synthetic Activity**

Generally, the number of labelled cells (LC) in the rectal end of the colon were significantly unaffected by dietary treatments (Figure 6.4; Appendix O). In the mid-region, rats fed 45% RCS diet had significantly higher number of LC in the crypts compared to the 15% RCS + 1% l or 15% TCS groups ($p=0.03$) (Figure 6.4; Appendix O).

Trends suggested that LI was noticeably higher in the mid-region compared to the rectal end in the colons of rats fed 45% RCS diet (Figure 6.5; Appendix O).

Further analysis using the Least Squares t-test indicated that LI was higher in the mid-region of the colonic crypts of rats fed 45% RCS diet than in the rectal end ($p=0.002$) (Table 6.9).

A positive relationship between MF and LI was found in the 15% RCS + 1% l ($r=0.59$; $p=0.01$) and 45% RCS ($r=0.49$; $p=0.04$) groups, while a negative correlation was found in the 15% RCS or 15% or 45% TCS groups (Table 6.10).

FIGURE LEGEND

FIGURE 6.3 Mitotic activity in 2 regions of the colons of Sprague-Dawley rats. Bars represent mean number of mitotic figures/crypt sections/ group. L=lactulose; RCS=raw cornstarch; TCS=thermally-treated cornstarch. 1% L=15% RCS + 1% l, n=9; 15% RCS, n=9; 45% RCS, n=9; 15% TCS, n=10 45% TCS, n=10.

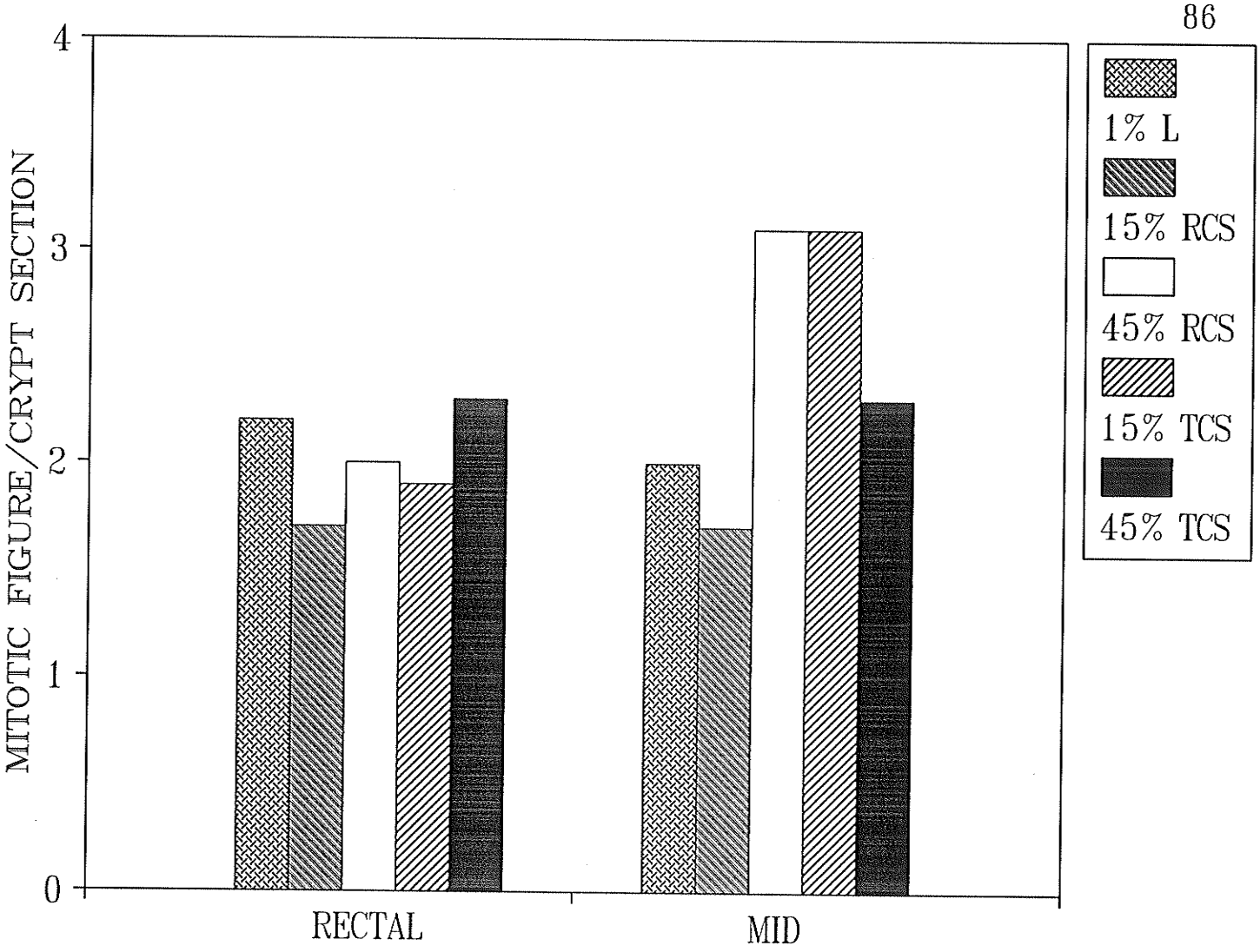

MITOTIC FIGURE/CRYPT SECTION
0
1
2
3
4
RECTAL
MID
1% L
15% RCS
45% RCS
15% TCS
45% TCS

FIGURE LEGEND

FIGURE 6.4 The effect of dietary treatment on the number of labelled cells per crypt in the colons of rats. Bars with the same letter are not significantly different ($p<0.05$). Bars represent mean number of labelled cells/crypt sections/ group. L=lactulose; RCS=raw cornstarch; TCS=thermally-treated cornstarch. 1% L=15% RCS + 1% l, n=9; 15% RCS, n=9; 45% RCS, n=9; 15% TCS, n=10; 45% TCS, n=10.

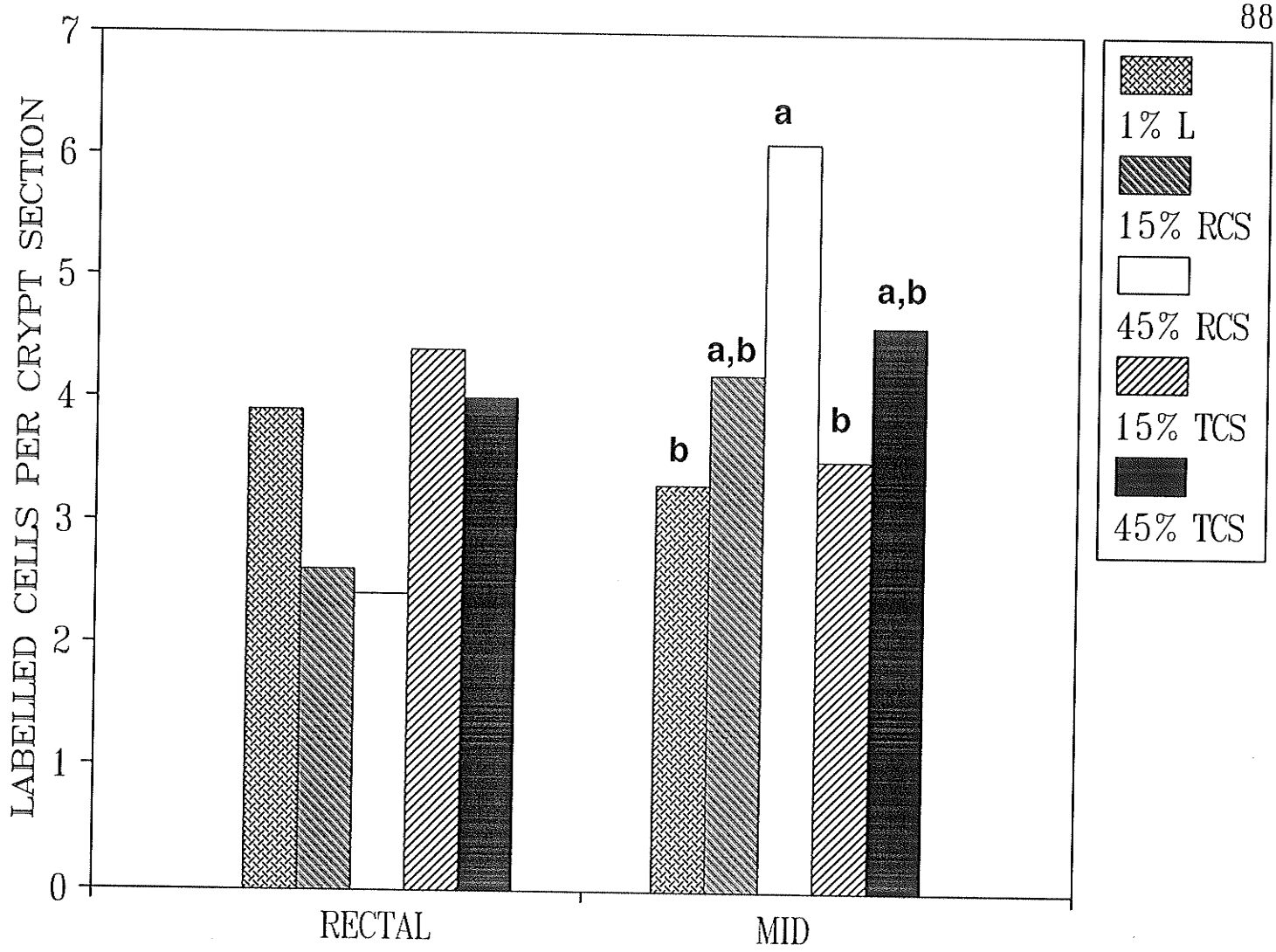

LABELLED CELLS PER CRYPT SECTION
0
1
2
3
4
5
6
7
RECTAL
MID
b
a,b
a
b
a,b
1% L
15% RCS
45% RCS
15% TCS
45% TCS

FIGURE LEGEND

FIGURE 6.5: The effects of diet on labelling index in 2 region of the colons in rats. L=lactulose; RCS=raw cornstarch; TCS=thermally-treated cornstarch. Bars represent mean labelling index/crypt sections/ group. 15% RCS + % L (1% L), n=9; 15% RCS, n=9; 45% RCS, n=9; 15% TCS, n=10; 45% TCS, n=10.

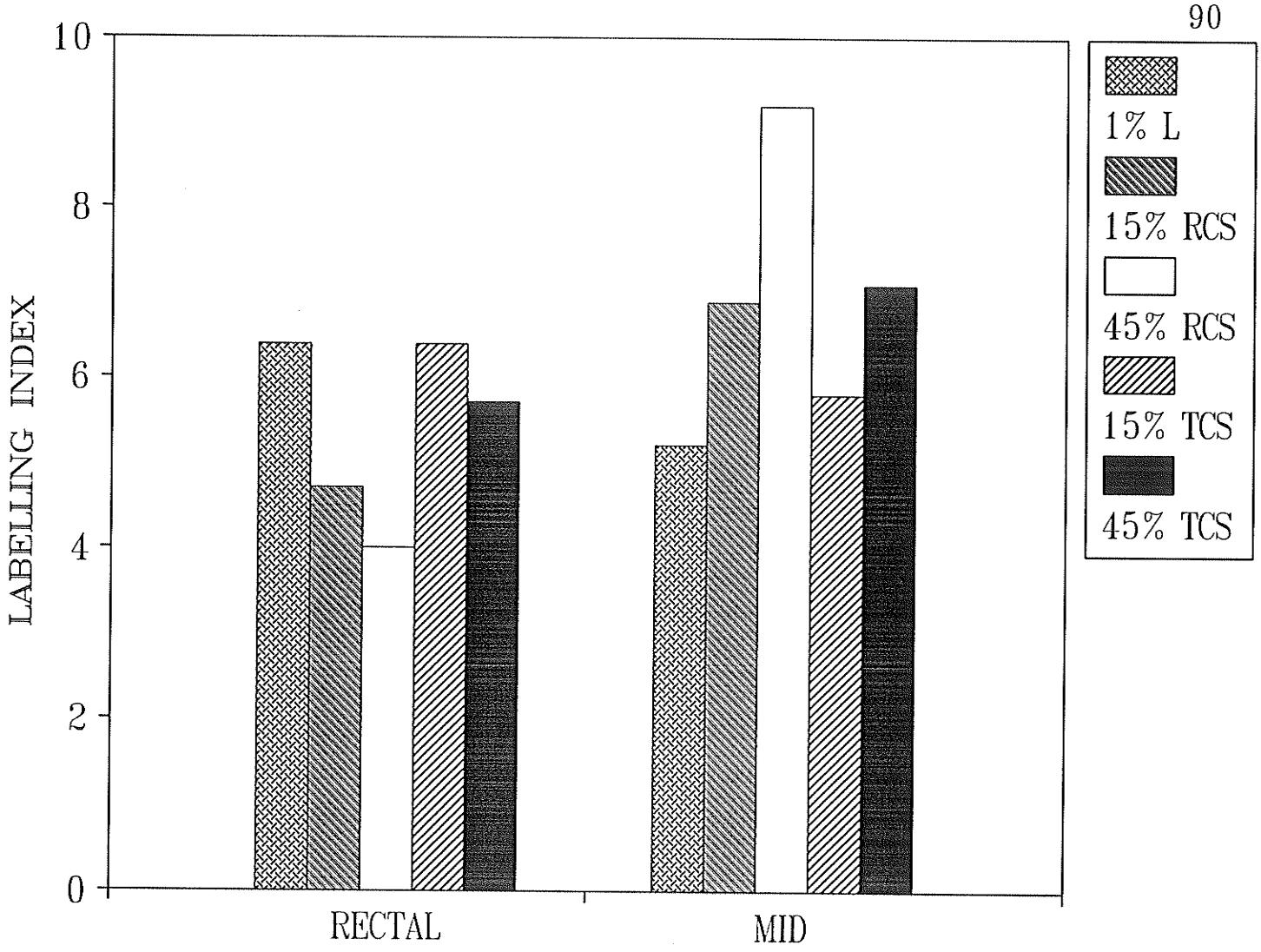

10
8
6
4
2
0
LABELLING INDEX
RECTAL
MID
1% L
15% RCS
45% RCS
15% TCS
45% TCS

TABLE 6.9: REGIONAL DIFFERENCES IN LABELLING INDEX IN THE COLONS OF SPRAGUE-DAWLEY RATS FED DIFFERENT DIETS

TREATMENT GROUPS	N	LIDIFF[1]	p-VALUE
15% RCS + 1% l	9	-1.09	0.54
15% RCS	9	2.19	0.22
45% RCS	9	6.03	0.002
15% TCS	10	-0.29	0.86
45% TCS	10	1.12	0.50

[1]LIDIFF=[mid region - rectal region]: regional differences in labelling index.
l=lactulose
RCS=raw cornstarch
TCS=thermally-treated cornstarch

TABLE 6.10: RELATIONSHIP BETWEEN MITOTIC FIGURE AND LABELLING INDEX DUE TO DIIFFERNT DIETS

TREATMENT GROUPS	N	r*	p-VALUE
15% RCS + 1% l	9	0.59	0.01
15% RCS	9	-0.59	0.01
45% RCS	9	0.50	0.04
15% TCS	10	-0.06	0.80
45% TCS	10	-0.14	0.57

*Pearson Product Moment correlation coefficient used to assess relationship mitotic figure and labelling index.
l=lactulose
RCS=raw cornstarch
TCS=thermally-treated cornstarch

6.5 DISCUSSION

The focus of Study III was to determine the effect of RCS and TCS on ACF and proliferative activity in the colons of Sprague-Dawley rats. The main question proposed was "would a diet containing starch which had undergone thermal-treatment and contained RS inhibit the induction and growth of preneoplastic lesions in the colons of rodents more than a diet containing untreated raw starch?" In spite of the positive conclusions obtained from the Differential Scanning Calorimetry and Enzymatic assays on the presence of RS in the TCS samples, this investigation did not support the hypothesis that a TCS diet which contained RS would reduce the development and growth of ACF in the colons of Sprague-Dawley rats.

Several findings were unexpected with respect to ACF. Statistical findings from the SAS orthogonal statements supported the contentions that diets containing fermentable compounds such as RS appear to be associated with a high risk for colon cancer development. Employing this type of statistical data analysis, it was apparent that ACF parameters such as crypt multiplicity and size were increase by feeding rats thermally-treated cornstarch (Appendix K). This observation appears to refute the proposed inhibitory role of NSP or RS in the development of colon cancer. It was further noted that the reduction in the mean number of ACF was accompanied by an increase in crypt multiplicity in the colons

of rats fed 45% TCS compared to those fed 45% RCS diet (Appendix M). This suggests that 45% TCS diet may lead to the induction and/or selection of lesions with higher growth rates.

Faecal pH is commonly used to measure fermentation in the large bowel (Kashtan et al, 1992). Using the statistical data provided, experimental diets containing 45% RCS, or 15% or 45% TCS led to a decrease in faecal pH. Some interesting trends in the present work indicated that the higher levels of RS in the 45% TCS diet resulted in a higher faecal output. Trends in this study appear to support studies which have reported that increased passage of unavailable CHO into the colon could increase faecal weight (Shetty and Kurpad, 1986; Ranhotra et al, 1991; Gee et al, 1991). In the literature, higher stool bulk has been implicated in reducing the risk for colon cancer. Therefore, as originally envisioned it is possible that increased faecal bulk may help in the rapid excretion of potential genotoxic or promotory agents from the colon (McPherson-Kay, 1986; Bartram et al, 1991). Further analysis accompanied with statistical significance between dietary groups would be warranted in order to confirm these proposals. Lactulose feeding did not increase faecal bulk, but trendwise it did decrease the faecal pH. This observation has been documented by others (Jenkins et al, 1986).

There were no major differences in mitotic activity, however this proliferative index was higher in the mid

compared to the rectal end of the colonic mucosa. It is believed that specific regions of the colon are sensitive to certain dietary components in a region-specific manner (Caderni et al, 1991b). Caderni and co-workers (1991b) demonstrated that starch-containing foods such as bread, pasta, rice and CS had a marked decrease on mitotic activity as measured by MI in the rectal rather than in the mid-region of the colon. Based on the previous studies, this investigation supports the contention that a selective effect of starch or other dietary components on specific sites of the colon may exist and that this phenomena could be vital in understanding the role of starch or other nutrients on colonic health and diseases.

In the present study, it was demonstrated that fermentation markers, such as faecal weight and pH were indeed affected by TCS diets. Therefore TCS in part served as a possible substrate for bacterial degradation in the colon. However, it is apparent that despite the high degree of fermentation in the 45% TCS group, an inhibitory effect on the growth characteristics of ACF was not demonstrated. Based on these findings, it seems appropriate to suggest that the proposed role of indigestible CHO in the prevention and treatment of large bowel disease must be re-evaluated.

CHAPTER VII

GENERAL DISCUSSION, CONCLUSIONS AND FUTURE RECOMMENDATIONS

7.1 GENERAL DISCUSSION

The main purpose of this thesis was to investigate the effect of a complex carbohydrate, dietary starch on the early stages of colon carcinogenesis. Three independent studies were carried out sequentially in order to examine the objective. Firstly, a preliminary study was conducted to determine which simple sugar (dextrose or sucrose) would have a positive growth effect on ACF. Following this verification, the sugar identified was chosen as the source of simple CHO in all the experimental diets to test the hypothesis that the effect observed with this sugar could be ameliorated by feeding rats diets containing high levels of starch. The information provided in Study I led to the design of Studies II and III but with the focus concentrated on starch. The specific purpose of Study II was to investigate the effects of varying levels of raw starch on the development and growth of ACF. Finally, Study III was designed to investigate and compare the effects of raw and thermally-treated starch on ACF. In all of these investigations, two proliferative indices, mitotic figure and labelling index, which have been

traditionally employed as reliable tools to predict the risk for colon cancer were also examined.

Firstly, it was evident in Study I, that dextrose had a distinct growth enhancing effect on ACF compared to sucrose. Based on this finding, it was reasoned that high levels of raw or thermally-treated starch might alter this effect in diets containing dextrose.

In Study II, high amounts of raw cornstarch, 45% (w/w), did not have an inhibitory effect on the induction and growth of ACF. In fact, the most noteworthy observation in Study II, was that the 30% starch diet suppressed the development and growth of ACF. The findings indicated that a 30% CS diet exerted an inhibitory effect on the development of ACF resulting in lower values for total number AC/focus than the 45% CS diets (Table 5.2). This observation was confirmed in an additional 4 week feeding study (Appendix F). Therefore, it can be proposed that a threshold level may exist at which starch is capable of exerting a protective effect against colon cancer. This is an area that must be pursued for future studies.

Study III did not support the research hypothesis that thermally-treated starch, because it is a source of indigestible CHO would prove more protective in the colon than raw starch. This reasoning was based on the literature pertaining to the protective role of fermentation against large bowel disease. Nevertheless, no beneficial effect was

noted with respect to the growth of ACF. Trends and orthogonal statements in this study indicated that diets with potentially fermentative substrates, such as RS in the thermally-treated starch samples or lactulose in the 15% RCS + 1% l diet supported the growth of selected ACF. This was demonstrated by increase in the number of ACF with higher crypt multiplicity (Table 6.5; Appendices K and L). Microscopically, the ACF in the colons of these rats were larger and dilated in appearance.

In countries where the population consumes high starch diet, there is not a high incidence of colon cancer. However, it is possible that in the prevention and treatment of colon cancer there are many contributing factors that must be taken into account, not just a single dietary factor such as starch. In less affluent societies, low fat, high starch diets are common. However, these diets contain high amounts NSP from lentils and grains, in addition to starch. The low incidence of large bowel disease that is observed in these populations may be attributed to the interaction of certain dietary components in the food, such as starch-and-NSP, to the low amount of fat and to the many other nutrients present in high starch and fibre containing foods. Therefore, we must question the role of starch and its influence in the colon with respect to development of preneoplastic lesions.

In addition it would appear that fermentation per se is more complicated than originally predicted. Jenkins and

colleagues (1991) proposed that the beneficial effects of fermentation may be dependent on the type of fermentable substrates and its' end-products that are present in the colon. Future studies must examine the effect of other starches, such as potato or amylomaize on colon cancer development.

A consistent effect on DNA-synthetic activity in the mid-colon compared to the rectal end was noted in diets containing high levels of raw starch (Study III) (Table 6.9) The mid-region exhibited approximately two-fold higher labelling index than the rectal region (Table 6.9). The findings in this thesis question the significance of proliferative indices, such as mitotic activity or LI as risk markers for colon tumorigenesis. These indices were not reliable in terms of disease development as measured by number, crypt multiplicity and size of ACF in the colons of rats. These indices yielded conflicting findings. For example, the group (Study II, 30% CS diet) with the least number of ACF had the highest mitotic activity (Appendix D). Furthermore in the same study, although the number of ACF in the 30% CS group was significantly lower than the 45% CS group, the labelling indices of their colon were similar. It has been proposed that increased cell proliferation is correlated with increased colon cancer incidence (Chang, 1980; Deschner, 1980; Jacobs, 1987). Several investigators have pointed out that individuals with ulcerative colitis or Crohn's disease

demonstrate increased LI in their colons which presumably predisposes them to colon cancer development (Deschner, 1987; Bleiberg et al, 1970; Eastwood et al, 1973; Gerdes et al, 1993). However, to the author's knowledge, there is no documented evidence that decreasing the colonic cell proliferative indices in these individuals, leads to reduced risk for cancer development. The findings on the proliferative indices of the colon lead one to suggest that cell kinetic measurements must be used with caution and can not be used as a substitute for the disease.

Colon carcinogenesis studies have focused on both animal and human model systems. The animal model allows investigators to work on a simple system compared to the more complex human model and to gain useful insight into the multistep process of cancer development. In addition, animal systems are useful in defining and refining the hypothesis linking diet in the aetiology and prevention of cancer. In terms of human health, the knowledge and information accumulated from animal model studies must be eventually be applied to human situations.

It is important to note that a possible protective role of high fibre or starch diets on colon cancer development may not be due to these components of the diets alone. Furthermore, it is interesting to note that the issue of fibre and starch-containing foods in the prevention and treatment of chronic diseases remains unresolved in the nutrition

community. Currently, health professionals appear to be advocating increased complex CHO per se in the diet without delegating responsibility of "protection" to any single nutrient. In keeping with this information, investigators must exercise caution in making statements which claim that a specific "dietary component" could be beneficial or harmful to ones health in terms of chronic diseases such as heart and colon cancer. Currently, the practice has been to "overclaim" that a high fibre/starch diet helps reduce risk for chronic diseases such as colon cancer and heart diseases, whereas a high fat diet is claimed to be responsible for these chronic illnesses. The development of different conceptions on nutrition-related diseases by different investigators creates confusion in the general public. This thesis contends that researchers must carefully scrutinized their findings by examining the advantages and disadvantages of specific diets or dietary components before making nutritional recommendations to the public. It must also be emphasized that no research to date has demonstrated a cancer modulating effect of dietary starch on colon cancer development.

7.2 CONCLUSIONS

The studies reported have systematically assessed the effect of starch on the early stages of colon carcinogenesis. Several important points were considered while conducting these studies. First, it was determined whether the source of simple sugar was important in the carcinogenesis process. Based on this, in subsequent studies, the level of simple sugar was modified in the experimental diets to synthesize diets containing various levels of starch and starch forms (raw, thermally-treated). Furthermore, several proliferative indices were assessed in the same dietary group to determine whether there was any concordance among these parameters, and whether anyone or more of these parameters consistently changed as the disease developed. The following conclusions can be drawn from this dissertation:

1) Dextrose affected the development of ACF by supporting the growth of ACF as seen by an increased number of AC/focus (crypt multiplicity) compared to numbers obtained with a sucrose diet.

2) Diets containing high amounts of raw (Study II) or thermally-treated (Study III) starch did not suppress the induction and growth of AOM-induced ACF based on the generalized ANOVA (Study II) and analysis by orthogonal tests (Study III).

3) Fermentation in the colon had no impact on number, crypt multiplicity or size of ACF in the thermally-treated starch groups.

4) Many of the proliferative indices quantified in the three studies failed to predict the state of the colon exhibiting different preneoplastic disease states.

7.3 RECOMMENDATIONS FOR FUTURE RESEARCH

Based on the findings from this thesis, the following areas can be explored in future research:

1. A longer term study must be conducted employing tumour incidence and growth parameters of ACF. This would help determine whether dietary starch, raw or thermally-treated, might have a disease modulating effect during later stages.
2. Further studies must be carried out employing different starches, in order to clarify the role of fermentation on the development and growth of preneoplastic lesions.
3. There should also be extensive examination of morphological features of ACF. McLellan and colleagues (1991b) reported in their study that a large population of ACF examined exhibited nuclear abnormalities, which is indicative of dysplasia, a feature of precancerous lesions.
4. Identification of consistent and reliable cellular and biochemical markers is needed. Currently, the expression of PCNA in colonic crypts is being used to measure proliferative activity. So far, PCNA activity in normal, pre-neoplastic and

neoplastic colonic tissues have shown favourable results. Yamada et al. (1992) reported that PCNA-labelling index identified more cells committed to DNA synthesis in the colonic crypts compared with BrdU-immunohistochemistry (S-phase cells). From this, they suggested that PCNA-expression could be a much more reliable and powerful tool.

APPENDIX A

EFFECT OF SIMPLE CARBOHYDRATE ON BODY WEIGHT

WEEKS	TREATMENT	BODY WEIGHT (GRAMS)
0	DEXTROSE	119.8 ± 3.7[a]
	SUCROSE	132.O ± 3.1[b]
2	DEXTROSE	222.3 ± 6.2
	SUCROSE	242.8 ± 10.4
4	DEXTROSE	317.8 ± 10.0
	SUCROSE	335.5 ± 17.0

All values shown are the mean ± SEM.
Means in the same row with different letter are significantly different ($p < 0.05$). Dextrose, n=4; Sucrose, n=4.

APPENDIX B

EFFECT OF CORNSTARCH (CS) ON WEEKLY MEAN BODY WEIGHTS GAIN IN SPRAGUE-DAWLEY RATS

WEEKS ON DIET	TREATMENT (% CS)[1]	BODY WEIGHT (BW)
0	15	168.4 ± 11.2
	30	185.2 ± 5.3
	45	167.2 ± 8.6
2	15	269.2 ± 13.0
	30	275.4 ± 4.3
	45	261.4 ± 8.8
4	15	345.0 ± 16.2
	30	359.3 ± 8.6
	45	357.6 ± 11.0
6	15	405.4 ± 19.3
	30	418.6 ± 11.0
	45	383.0 ± 13.0
8	15	451.0 ± 24.0
	30	446.6 ± 18.0
	45	446.6 ± 13.0

[1]Percentage of CS used in the diet. All values shown are the mean ± SEM. 15% CS, n=5; 30% CS, n=8; 45% CS, n=9. None of the values in each row were found to be significantly different ($p<0.05$).

APPENDIX C

ABERRANT CRYPT FOCI DISTRIBUTION WITH RESPECT TO CRYPT MULTIPLICITY

TREATMENT GROUP	NUMBER OF CRYPTS/ACF			
	1-2	3-4	5-6	≥7
15% CS	249.0 ± 24.0[a,b]	50.6 ± 8.2	7.6 ± 2.0	3.0 ± 2.1
30% CS	194.6 ± 28.4[b]	39.8 ± 6.6	6.1 ± 1.6	0.9 ± 0.3
45% CS	302.2 ± 17.0[a]	57.7 ± 6.3	7.1 ± 1.3	2.3 ± 0.7

All values shown are the mean ± SEM. Means in the same column with the same letter are not significantly different. 15% CS, n=5; 30% CS, n=8; 45% CS, n=9.

APPENDIX D

THE EFFECT OF CORNSTARCH (CS) ON MITOTIC ACTIVITY IN THE COLONIC CRYPT

TREATMENT GROUP	COLONIC SECTION	
	RECTAL	MID
	CRYPT HEIGHT/CRYPT SECTION[1]	
15% CS	32.0 ± 0.1	33.2 ± 1.2[b]
30% CS	31.0 ± 1.4	37.1 ± 1.2[a]
45% CS	31.0 ± 0.9	32.0 ± 0.3[b]
	MITOTIC FIGURE/CRYPT SECTION[2]	
15% CS	2.1 ± 0.1	1.4 ± 0.3
30% CS	2.7 ± 0.4	2.2 ± 0.4
45% CS	2.0 ± 0.3	2.3 ± 0.3

All values shown are the mean ± SEM. Means in the same column with the same letter are not significantly different ($p < 0.05$). 15% CS, n=3; 30% CS, n=5; 45% CS, n=4.
[1]Number of cells per mid-axial crypt.
[2]Number of mitotic figures/crypt section/group.

APPENDIX E

THE EFFECT OF VARYING LEVELS OF CORNSTARCH (CS) ON DNA-SYNTHETIC ACTIVITY IN THE COLONIC CRYPTS OF SPRAGUE-DAWLEY RATS

TREATMENT[1] GROUP	R^2	M^2
	LABELLED CELLS/ CRYPT SECTION	
15% CS	7.8 ± 0.2	4.0 ± 0.2
30% CS	6.6 ± 1.5	5.1 ± 1.3
45% CS	6.3 ± 0.9	5.4 ± 1.3
	LABELLING INDEX[3]	
15% CS	10.5 ± 0.5	6.8 ± 1.4
30% CS	11.3 ± 1.8	7.9 ± 1.8
45% CS	10.2 ± 1.2	9.8 ± 1.7

[1]Refers to %CS used in the diet.
[2]Colonic sections: R=rectal; M=midsection.
[3]Labelling index=(number of labelled cells divided by total number of epithelial cells)multiply by 100.
15% CS, n=2; 30% CS, n=3; 45%CS, n=5.
All values shown are the mean ± SEM.
None of the values within column for each parameter were found to be significantly different ($p<0.05$).

APPENDIX F

EFFECT OF DEXTROSE AND CORNSTARCH (CS) ON CRYPT MULTIPLICITY

TREATMENT GROUPS	NUMBER OF AC/ACF			
	1	2	3	≥4
DEXTROSE	58.0 ± 5.0	84.0 ± 12.0	34.0 ± 4.1	21.0 ± 2.0
30% CS	68.4 ± 9.2	78.0 ± 4.0	30.0 ± 3.1	16.3 ± 2.1

All values shown are the mean ± SEM.

[1]Study was conducted for 4 weeks. Animals were killed by CO_2 asphyxiation. Dextrose, n=4; 30% CS, n=4.
None of the values for each level of crypt multiplicity were found to be significantly different from each other ($p<0.05$).

APPENDIX G

MEAN BODY WEIGHTS IN SPRAGUE-DAWLEY RATS FED DIFFERENT DIETS

TREATMENT GROUP	N	BODY WEIGHTS (g) OF RATS AT WEEKS[1]				
		0	2	4	6	8
15% RCS + 1% l	9	128.0 ± 3.1	233.0 ± 4.4	315.5 ± 4.7	386.0 ± 1.3	414.4 ± 11.2[b,c]
15% RCS	9	131.6 ± 3.9	243.0 ± 5.8	324.7 ± 4.0	381.9 ± 5.2	395.6 ± 6.7[c]
45% RCS	9	129.6 ± 4.7	239.0 ± 5.8	334.8 ± 5.7	408.7 ± 7.5	452.9 ± 10.4[a]
15% TCS	10	134.0 ± 3.9	247.5 ± 5.3	339.6 ± 9.1	410.9 ± 4.0	436.0 ± 17.4[a,b]
45% TCS	10	127.8 ± 3.7	237.5 ± 4.9	326.5 ± 4.6	392.9 ± 4.6	433.6 ± 12.0[a,b]

All values shown are mean ± SEM. Means in the same column with the same letter are not significantly different ($p<0.05$).
[1]Weeks on dietary treatments.
L=lactulose
RCS=raw cornstarch
TCS=thermally-treated cornstarch

APPENDIX H
GENERAL LINEAR MODELS PROCEDURE FOR BODY WEIGHT AT WEEK 8

SOURCE	DF[1]	SUM OF SQUARES	p-VALUE
TREATMENT (MODEL)	4	17594.2	0.03
ERROR	42	59895.7	
CORRECTED TOTAL	46	77489.9	
CONTRAST2:		CONTRAST SUM OF SQUARES	
CONT VS. TREATMENT	1	1650.9	0.30
COOKING	1	1060.0	0.40
DOSE	1	7147.1	0.03
INTERACTION	1	8450.7	0.02

[1]DF=degree of freedom
[2]CONTRAST=SAS orthogonal statements used to analyze data for;
cont vs treatment=lactulose versus starch treatment (RCS and TCS)
cooking=raw cornstarch versus thermally-treated cornstarch
dose=15% versus 45%, (w/w) cornstarch
interaction=dose(15% or 45%, w/w) x treated/raw cornstarch

APPENDIX I

GENERAL LINEAR MODELS PROCEDURE FOR ORGAN WEIGHTS

SOURCE	DF[1]	p-VALUE		
		LIVER	KIDNEY	HEART
TREATMENT (MODEL)	4	0.16	0.28	0.09
ERROR	42			
CORRECTED TOTAL	46			
CONTRAST[2]:				
CONT VS TREATMENT	1	0.22	0.66	0.65
COOKING	1	0.88	0.56	0.22
DOSE	1	0.19	0.94	0.63
INTERACTION	1	0.05	0.04	0.02

[1]DF=degree of freedom
[2]CONTRAST=SAS orthogonal statements used to analyze data for:
cont vs treatment=lactulose versus starch treatment (RCS and TCS).
cooking=raw cornstarch versus thermally-treated cornstarch
dose=15% (w/w) versus 45% (w,w) cornstarch
interaction=dose(15% or 45%, w/w) x treated/untreated cornstarch

APPENDIX J
GENERAL LINEAR MODELS PROCEDURS FOR COLON LENGTH AND WEIGHT IN SPRAGUE-DAWLEY RATS

SOURCE	DF[1]	SUM OF SQUARES	p-VALUE
		COLON LENGTH	
TREATMENT (MODEL)	4	32.37	0.02
ERROR	42	105.31	
CORRECTED TOTAL	46	137.68	
CONTRAST[2]:		CONTRAST SUM OF SQUARES	
CONT VS TREATMENT	1	5.36	0.15
COOKING	1	22.76	0.004
DOSE	1	3.70	0.23
INTERACTION	1	0.00	0.98
		COLON WEIGHT	
TREATMENT (MODEL)	4	0.42	0.16
ERROR	42	2.56	
CORRECTED TOTAL	46		
CONTRAST2:			
CONT VS TREATMENT	1	0.03	0.49
COOKING	1	0.29	0.04
DOSE	1	0.00	0.78
INTERACTION	1	0.10	0.19

[1]DF=degree of freedom
[2]CONTRAST=SAS orthogonal statements used to analyze data for;
cont vs treatment=lactulos versus starch treatment (RCS and TCS)
cooking=raw cornstarch versus thermally-treated cornstarch
dose=15% versus 45%, (w/w) cornstarch
interaction=dose(15% or 45%, w/w) x treated/raw cornstarch

APPENDIX K

GENERAL LINEAR MODELS PROCEDURE FOR ACF

SOURCE	DF[1]	p-VALUE		
		NUMBER OF ACF	CRYPT MULTIPLICITY	SIZE OF ACF
TREATMENT (MODEL)	4	0.82	0.20	0.12
ERROR	42			
CORRECTED TOTAL	46			
CONTRAST[2]:				
CONT VS TREATMENT	1	0.99	0.55	0.55
COOKING	1	0.42	0.04	0.02
DOSE	1	0.38	0.54	0.59
INTERACTION	1	0.79	0.30	0.18

[1]DF=degree of freedom
[2]CONTRAST=SAS orthogonal statements used to analyze data for:
cont vs treatment=lactulose versus starch treatment (RCS and TCS)
cooking=raw cornstarch versus thermally-treated cornstarch
dose=15% versus 45% (w/w) cornstarch
interaction=dose(15% or 45%, w/w) x treated/untreated cornstarch

APPENDIX L

PERCENTAGE[1] OF ACF CONSISTING OF VARYING CRYPT MULTIPLICITY

TREATMENT GROUP	NUMBER OF AC/FOCUS						
	1	2	3	4	5	6	≥7
15% RCS + 1% l	27.5 ± 2.4	36.4 ± 1.4	17.8 ± 0.1	9.8 ± 1.2	3.6 ± 0.4	2.2 ± 0.4	2.9 ± 0.8
15% RCS	27.4 ± 2.2	40.5 ± 1.2	17.0 ± 1.0	8.9 ± 0.9	3.1 ± 0.8	1.3 ± 0.5	1.8 ± 0.5
45% RCS	29.7 ± 2.6	39.2 ± 1.9	17.1 ± 1.0	7.8 ± 1.1	3.2 ± 0.6	1.3 ± 0.4	1.7 ± 0.3
15% TCS	28.6 ± 3.4	37.0 ± 1.0	16.0 ± 1.2	10.4 ± 0.9	4.3 ± 0.8	1.7 ± 0.3	2.1 ± 0.5
45% TCS	23.0 ± 1.6	37.5 ± 1.4	19.5 ± 0.8	9.9 ± 1.1	5.1 ± 0.4	2.2 ± 0.4	3.1 ± 0.5

All values shown are mean ± SEM.
[1]Number of ACF consisting of varying numbers of crypts per colon divided by the total number of ACF per colon and multiplied by 100.
l=lactulose
RCS=raw cornstarch
TCS=thermally-treated cornstarch
15% RCS + 1% l, n=9
15% RCS, n=9
45% RCS, n=9
15% TCS, n=10
45% TCS, n=10
None of the values for each level of crypt multiplicity were found to be significantly different ($p<0.05$).

APPENDIX M

PERCENTAGE[1] OF ACF WITH VARYING CRYPT MULTIPLICITY IN RATS FED 45% (w/w) RAW OR 45% (w/w) THERMALLY-TREATED CORNSTARCH DIETS

TREATMENT GROUP	NUMBER OF AC/FOCUS		
	1 CRYPT	2 CRYPTS	≥3 CRYPTS
45% RCS	29.7 ± 2.6	39.2 ± 1.9	31.1 ± 2.8[b]
45% TCS	23.0 ± 1.6	37.5 ± 1.4	39.8 ± 2.3[a]

All values shown are mean ± SEM. Percentages of ACF in the same column with different letter are significantly different ($p < 0.05$).

[1]Number of ACF consisting of varying numbers of crypts per colon divided by the total number of ACF per colon and multiplied by 100.

45% RCS, n=9

45% TCS, n=10

APPENDIX N

THE EFFECT OF DIETARY TREATMENT ON MITOTIC ACTIVITY[1] IN THE COLONIC MUCOSA OF SPRAGUE-DAWLEY RATS

TREATMENT GROUP	N	COLONIC SECTIONS[2]	
		R	M
15% RCS + 1% l	9	2.2 ± 0.3	2.0 ± 0.4
15% RCS	9	1.7 ± 0.3	1.7 ± 0.6
45% RCS	9	2.0 ± 0.4	3.1 ± 0.6
15% TCS	10	1.9 ± 0.3	3.1 ± 0.4
45% TCS	10	2.3 ± 0.3	2.3 ± 0.5

All values shown are mean ± SEM.
[1]Mitotic figures per crypt sections=number of mitotic figures/crypt section/group.
[2]R=rectal, M=mid-region.
l=lactulose
RCS=raw cornstarch
TCS=thermally-treated cornstarch
None of the values for within each column for mitotic activity were found to be significantly different ($p<0.05$).

APPENDIX O

THE EFFECT OF DIETARY TREATMENTS ON DNA-SYNTHETIC ACTIVITY[1] IN THE COLONS OF RATS FED DIFFERENT DIETS

TREATMENT GROUP	N	LC/CRYPT SECTION		LI	
		R*	M	R	M
15% RCS + 1% l	9	3.9 ± 0.6	3.3 ± 0.7[b]	6.4 ± 0.8	5.2 ± 1.0
15% RCS	9	2.6 ± 0.8	4.2 ± 0.6[a,b]	4.7 ± 1.5	6.9 ± 1.0
45% RCS	9	2.4 ± 0.6	6.1 ± 0.4[a]	4.0 ± 0.9	9.2 ± 0.5
15% TCS	10	4.4 ± 0.7	3.5 ± 0.7[b]	6.4 ± 1.0	5.8 ± 1.1
45% TCS	10	4.0 ± 0.8	4.6 ± 0.9[a,b]	5.7 ± 1.0	7.1 ± 1.2

All values shown are mean ± SEM. Means in the same column with the same letter are not significantly different ($p < 0.05$).
l=lactulose
RCS=raw cornstarch
TCS=thermally-treated cornstarch
*R=RECTAL, M=MIDREGION
1DNA-SYNTHETIC ACTIVITY:
LC/CRYPT SECTIONS=labelled cells per crypt sections
LI=labelling index: number of labelled cells in the crypt divided by the total number of epithelial cells in the crypt multiplied by 100.

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