Modulation of epithelial cell proliferation and luminal contents of the human large bowel. A link to carcinogenesis.
Cats, Annemieke

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Summary, conclusions and future perspectives

Summary

Colorectal cancer is the second most common non-skin malignancy in the Western world. The overall survival prospect for patients with this malignancy is 40-50%. Survival rates have not improved much over the last few decades. To achieve a reduction in mortality and morbidity rates primary and secondary prevention of colorectal cancer development will play an important role. Primary prevention is directed at inhibiting the onset of colorectal cancer. Secondary prevention is directed at inhibiting colorectal carcinogenesis through endoscopic surveillance of high-risk populations and removal of preneoplastic lesions. Before primary prevention can take place, it is essential to identify endogenous and exogenous factors involved in the process of colorectal carcinogenesis. Given the likely involvement of dietary factors in colorectal cancer (as outlined below), it is reasonable to study the luminal contents of the large bowel as well as the options to reduce its carcinogenic potential. The cytotoxic potential of the luminal contents can be determined in faecal water, the fraction of faeces containing the soluble, and thus cytotoxic, compounds. Animal and in vitro studies have shown that the extent of haemolysis of erythrocytes that have been incubated with faecal water, represents the amount of cytolyis of colonocytes induced by luminal compounds. In view of primary prevention, it is of interest to study the possibilities for modulation of the luminal contents in order to decrease its cytolytic activity. Using colorectal cancer as an endpoint for such studies would seem to be the most direct approach. However, such studies would require large numbers of subjects to be followed over considerable periods of time. Therefore, these studies are time-consuming and expensive. Studying small groups of subjects at increased risk for colorectal cancer development in combination with an intermediate endpoint for colorectal cancer can partly overcome these difficulties. Epithelial cell proliferation can be used as an intermediate biomarker of colorectal cancer, as colorectal epithelial cell proliferation accompanies the first stage of colorectal carcinogenesis, and since epithelial cell proliferation is increased in high risk subjects for colorectal cancer. Epithelial cell proliferation can be determined in vitro in endoscopically-obtained colorectal biopsy specimens after incubation with 5-bromo-2'-deoxyuridine (BrdU), a thymidine analogue. Following the incorporation of BrdU into the DNA of replicating cells, BrdU-labelled cells can be visualized by immunohistochemistry. Epithelial cell proliferation can subsequently be expressed as the percentage of BrdU-labelled epithelial cells in the colorectal crypts. Epidemiological studies have repeatedly associated a high intake of dietary fat and a low intake of dietary fibre with the development of neoplasia in the human large bowel. High intake of fat stimulates hepatic secretion of primary bile acids that are
required for fat solubilization. Primary bile acids not reabsorbed in the terminal ileum are bacterially degraded into secondary bile acids in the large bowel. Bile acids are capable of causing damage to colorectal cell membranes due to their physicochemical properties. From animal studies it is evident that especially the secondary bile acids, deoxycholic and lithocholic acids, have a cytotoxic potential and promote tumour development in the large bowel. In addition, increased concentrations of bile acids have been demonstrated in the intestinal contents of populations at high risk for colorectal cancer, who typically consume a diet high in fat, and in subjects with colorectal neoplasia. The consumption of dietary fiber probably reduces colorectal cancer risk. One of the physiological mechanisms that have been considered in this respect will be described here. The consumption of dietary fibre results in the generation of short-chain fatty acids by bacterial fermentation in the large bowel. It has been proposed that short-chain fatty acids, especially n-butyric acid, may have antineoplastic properties by inhibiting cell proliferation and inducing cell differentiation.

This thesis describes studies that evaluate modulation of colorectal epithelial and luminal factors associated with cancer development; specifically, epithelial cell proliferation, cytolytic activity of faecal water, short-chain fatty acids and bile acids. The development of colorectal cancer varies throughout the large intestine. This thesis also aims to clarify whether segmental heterogeneity of physiological processes could be involved in the regional variation of colorectal carcinogenesis. Also in this respect, colorectal epithelial and luminal factors that may be associated with cancer development are studied. Finally, this thesis focuses on endogenous factors and exogenous factors, other than nutritional ones, in relation to colorectal cancer development.

In general, the large bowel has been considered to be a uniform physiological and anatomical entity. Chapter 1 reviews recent indications for differences in function, metabolism and morphology between various segments of the human colorectum, and their consequences in terms of differences in susceptibility to neoplastic transformation.

Familial adenomatous polyposis is an autosomal dominantly inherited disorder, characterized by the presence of innumerable adenomatous polyps in the entire large bowel. Patients with familial adenomatous polyposis will inevitably develop cancer of the large bowel at a relatively young age, if left untreated. The treatment of choice is a prophylactic surgical resection of the colon to prevent the malignant degeneration of the adenomas. Of note, subtotal colectomy with ileorectal anastomosis in these patients can induce temporary regression of adenomas in the retained rectum. The mechanism for this phenomenon is unclear. Chapter 2 describes the short-term as well as long-term effects of subtotal colectomy on both rectal epithelial cell proliferation in familial adenomatous polyposis patients and on the proliferation and quiescent pool of the rectal mucosa large bowel. The acid pool size decreases shortly after surgery. In addition, the excretion of short-chain fatty acids after colonic resection was evaluated and compared with controls. After surgery, the concentrations of short-chain fatty acids were decreased in polyposis patients compared with controls. After subtotal colectomy, the rectal mucosa large bowel was studied in controls and in familial adenomatous polyposis patients. In sporadic neoplasia developing in the proximal part of the large bowel, the short-chain fatty acid pool is increased at the time of diagnosis. Consequently, the short-chain fatty acid pool is increased at the time of diagnosis. Consequently, the short-chain fatty acid pool is increased at the time of diagnosis. Consequently, the short-chain fatty acid pool is increased at the time of diagnosis. Consequently, the short-chain fatty acid pool is increased at the time of diagnosis. Consequently, the short-chain fatty acid pool is increased at the time of diagnosis. Consequently, the short-chain fatty acid pool is increased at the time of diagnosis. Consequently, the short-chain fatty acid pool is increased at the time of diagnosis.
cell proliferation as well as bile acid metabolism in 12 familial adenomatous polyposis patients. Shortly after surgery, a reduction of the initially increased size of the proliferative zone of rectal crypts was observed. This is consistent with a more quiescent proliferation state of the rectal mucosa. This beneficial effect on the rectal mucosa largely disappeared several years after surgery. The composition of the bile acid pool showed remarkable changes after subtotal colectomy, with similar patterns shortly as well as several years after surgery. Subtotal colectomy reduced the amount of cytotoxic secondary bile acids in duodenal bile and faeces. Total faecal bile acid excretion was increased after subtotal colectomy, due to elimination of bile acid absorption in the colon. Interactions between this changed bile acid composition and epithelial cells may be relevant for the regression of adenomas in familial adenomatous polyposis. Several years after surgery, the beneficial effect on epithelial cell proliferation diminishes, whereas the altered bile acid metabolism persists, suggesting that other factors, possibly genetic alterations, cause the redevelopment of neoplasia in this inherited disorder.

In view of the importance of short-chain fatty acids for the colonic epithelial function and the spontaneous regression and redevelopment of rectal polyps as mentioned above, chapter 3 describes the effects of subtotal colectomy with ileorectal anastomosis on bacterial flora and short-chain fatty acids in faeces. The same familial adenomatous polyposis patients were studied as those described in chapter 2. Results were compared with those in 10 healthy controls without large bowel resection. Preoperative cultural counts of aerobic and anaerobic bacteria and proportional short-chain fatty acid concentrations were not significantly different from those of the controls. After subtotal colectomy cultural counts of Bacteroides spp. and bifidobacteria were decreased compared to the controls and untreated familial adenomatous polyposis patients. Simultaneously, the ratio of acetic acid to total short-chain fatty acids increased at the expense of most other short-chain fatty acids, including n-butyric acid. Consequently, these alterations are primarily the result of the surgical resection and not due to the disease itself. No major differences were found between patients shortly and several years after subtotal colectomy. The lower anaerobe cultural counts and the change in short-chain fatty acid proportions after surgery in familial adenomatous polyposis patients do not explain the regression or late redevelopment of rectal polyps after subtotal colectomy in this genetically acquired disease.

In sporadic neoplasia, in which nutritional factors are aetiologically preponderant, neoplasia develop predominantly in the distal colorectum. This suggests that the proximal part of the large bowel has a less cytotoxic microenvironment than the distal part. Faecal compositions in 10 left and 19 right hemicolectomy patients were studied in chapter 4, in order to understand the function of the proximal and distal colons as well as their roles in processing contents. Faecal bacterial flora and short-chain fatty acids were compared with data from 10 healthy controls, whereas bile acids were compared with data from 17 subjects with an intact colorectum, in
which a polypectomy had previously been performed. In comparison with the intact colorectum, a decrease of total anaerobic counts was paralleled by a decrease of cultural counts of *Bacteroides* spp. only in left hemicolectomy patients. This suggests that alterations in bacterial flora after colonic resection are primarily a consequence of resection of the distal colon. In contrast to these changes, no differences between proportions of short-chain fatty acids were observed. Total faecal bile acid excretion was not affected by either a left or right hemicolectomy in comparison with the intact colorectum. This indicates that the remnant right and left colon are both capable of independently executing the colonic role in the enterohepatic circulation. Irrespective of this lack of effect on total bile acid excretion, remarkable differences in bile acid metabolism were observed between left and right hemicolectomy patients. Although bacterial degradation of primary bile acids into the more cytoxic secondary bile acids was reduced in both left and right hemicolectomy patients, conversion occurred to a larger extent in the proximal than distal colon. A higher proportion of deoxycholic acid in duodenal bile of right hemicolectomy patients indicates that a higher amount of protonated and hence cytoxic bile acids are present in the distal colon. This is thought to be due to the lower faecal pH in the distal colon than in the proximal colon. Hence, one expects the luminal content of the distal colon after right hemicolectomy to promote tumorigenesis to a greater extent than the luminal content of the proximal colon after left hemicolectomy. The main conclusions of this chapter are that the proximal and distal colons differ with respect to the processing of their luminal contents, and that differences between that proximal and distal colon may reflect regional variation in colorectal cancer risk.

In 1984 Newmark, Wargovich and Bruce drew attention to the interaction of bile and fatty acids, calcium and phosphate in relation to colorectal cancer. The authors postulated that calcium would have the potential to protect the colorectal epithelium against cytoxic bile and fatty acids by binding these surfactants. The authors further ascribed a competitive role to intestinal phosphate in this binding process, by causing the formation of insoluble calcium phosphate, which would then be incapable of binding the luminal surfactants. This hypothesis initiated a number of studies, both in vitro and in vivo, aimed at unravelling the intraluminal interactions between the above-mentioned compounds, their collective effects on the colorectal epithelium and their relation to colorectal cancer. These studies are reviewed in Chapter 5.

With reference to the part of the hypothesis of Newmark, Wargovich and Bruce, concerning the role of phosphate, in man most calcium intervention studies have been performed with calcium carbonate. A series of in vitro, animal and human studies have clarified that phosphate does not interfere with the effects of supplemental calcium on the luminal solubility and cytolytic activity of bile and fatty acids. On the contrary, an important role has actually been shown for phosphate in the binding of calcium supplements on intestinal calcium absorption, containing the assumption that calcium absorbs whereas that without phosphate supplements do not affect the luminal surfactants. With regard to luminal surfactants, the epithelial hyperproliferation and the formation of colorectal cancer in human mainly features. In 1989, the epithelial cell proliferation was suggested as a risk factor for hereditary colorectal cancer. A number of studies have demonstrated that the cell proliferation response to regional variations in the colorectum, the study of cell proliferation investigated the sigmoid colon, the healthy subjects, the large...
binding of calcium to bile and fatty acids. Therefore, in Chapter 6, the effects of supplementation of 1,500 mg of calcium as calcium phosphate $[\text{Ca}_3(\text{PO}_4)_2]$ tablets on intestinal bile acids and cytolytic activity of faecal water (the fraction of faeces containing the soluble and hence potentially harmful substances) were studied in 14 healthy volunteers. In duodenal bile, the proportion of cholic acid increased slightly, whereas that of chenodeoxycholic acid decreased slightly during calcium phosphate supplementation. Concurrently, proportional concentrations of bile acids in faeces were not affected during calcium phosphate supplementation. Consequently, supplemental calcium phosphate showed a tendency to alter intestinal bile acid metabolism, as was previously shown for calcium carbonate supplementation. In contrast to calcium carbonate supplementation in previous studies, however, calcium phosphate supplementation did not affect the cytolytic activity of faecal water. The results do not support a role for calcium phosphate, as used in this study, in the prevention of colorectal carcinogenesis.

With regard to colorectal cancer development, it is assumed that binding damaging luminal surfactants by calcium protects the colorectal epithelium by reducing the cytotoxic potential of its microenvironment, thereby preventing compensatory epithelial hyperproliferation. As a consequence, intervention studies with calcium in man mainly focused on the proliferation state of the epithelium in high risk populations. In 1985, Lipkin and Newmark were the first to report a decrease in rectal epithelial cell proliferation during calcium carbonate supplementation in subjects at risk for hereditary nonpolyposis colorectal cancer, an autosomally dominantly inherited syndrome with neoplasia predominantly developing in the proximal colon. A number of uncontrolled studies in other high risk populations also showed a reduction of rectal epithelial cell proliferation during calcium carbonate supplementation. Placebo-controlled studies, however, are not as uniform in their conclusions. Moreover, an uncontrolled study evaluating the effect of calcium in the sigmoid even reported an increase of epithelial cell proliferation. This suggests a differential response to calcium carbonate in various large bowel segments. In view of the regional variation of colorectal cancer development in hereditary nonpolyposis colorectal cancer and the indication of a site-specific response of the epithelium to calcium, the studies described in Chapter 7 & 8 were initiated. In chapter 7, epithelial cell proliferation in three different large bowel segments in 17 members of hereditary nonpolyposis colorectal cancer families was studied. Large bowel segments under investigation were the caecum, sigmoid and rectum. Epithelial cell proliferation in the sigmoid was markedly higher in this group of subjects than that in previously studied control subjects, and similar to that in previously studied patients with sporadic colorectal neoplasia. It was thus concluded that epithelial cell proliferation in hereditary nonpolyposis colorectal cancer family members is higher compared to healthy subjects and that this feature is found in both the proximal and distal parts of the large bowel. In chapter 8, the effects of 1,500 mg supplemental calcium as
calcium carbonate on epithelial cell proliferation in three different large bowel segments and on duodenal bile acids and cytolytic activity of faecal water, were studied in a randomized, double-blinded, placebo-controlled setting in 30 asymptomatic first degree family members of patients with hereditary nonpolyposis colorectal cancer. The population studied consisted partly of subjects described in chapter 7. Large bowel segments under investigation were the descending colon, sigmoid and rectum. In the descending colon and sigmoid no significant changes in epithelial cell proliferation were observed during calcium or placebo. Although rectal epithelial cell proliferation of whole crypts decreased markedly during both calcium and placebo supplementation, no significant difference in response was found between the calcium and placebo groups. A reduction of epithelial cell proliferation in this compartment was observed in the calcium group compared to the placebo group. This phenomenon has been connected with a quiescent proliferation state, and is consistent with earlier reported reductions of epithelial cell proliferation in the rectum during calcium. In duodenal bile a similar shift from chenodeoxycholic to cholic acid during calcium supplementation was observed as described in previous calcium supplementation studies. The cytolytic activity of faecal water decreased during calcium, but not during placebo supplementation. However, no significant difference between calcium and placebo could be demonstrated. The study casts considerable doubt on the value of calcium supplementation in the prevention of cancer at various sites of the large bowel in hereditary nonpolyposis colorectal cancer families.

The last part of this thesis is directed at obtaining additional information on other endogenous and exogenous factors in relation to colorectal cancer. Many growth-promoting properties of growth hormone are mediated by insulin-like growth factor-1, a hormone produced among others in the liver, pituitary gland and gastrointestinal tract. Insulin-like growth factor-1 receptors have been identified in normal colonocytes. In vitro, malignant epithelial cells respond to insulin-like growth factor-1 with hyperproliferation. Acromegalic patients have an intrinsic elevated production of insulin-like growth factor-1 due to growth hormone hypersecretion, as well as an increased risk for colorectal cancer development. Interestingly, patients with growth hormone deficiency have an altered bile acid pool. These observations resulted in the study, described in chapter 9, evaluating the relation between the levels of circulating growth hormone and insulin-like growth factor-1 on the one hand, and sigmoidal epithelial cell proliferation and intestinal bile acids on the other hand, in 30 acromegalic patients. A significant positive association existed between both growth hormone and insulin-like growth factor-1 on the one hand and epithelial cell proliferation on the other hand, suggesting that direct stimulation by one or both of these hormones leads to epithelial hyperproliferation. Although there was also a positive
The large bowel segments, were studied asymptomatic colorectal in chapter 7, sigmoid and rectal epithelial expression, were observed, was observed in epithelial colorectal epithelial proliferation state, proliferation in nodeoxycholic acid in pre-treatment, no differences analyzed. The association between both hormones and secondary bile acid proportions in faeces, faecal bile acid excretion was not dissimilar from normal. This study provides a possible mechanism for the reported increased incidence of neoplasia in patients with acromegaly. The study also demonstrates that some prudence with the administration of recombinant growth hormone to adults, as currently under investigation, is warranted.

Chronic use of anthranoid containing laxatives has recently been associated with an increased risk for colorectal cancer in man. Chapter 10 contains the results of a study evaluating the effect of a single high dose of a laxative containing sennosides A and B one day before endoscopy on sigmoidal epithelial cell proliferation in nine patients with macroscopically normal-appearing colorectal epithelium and a negative family history for colorectal cancer. Comparisons were made with three groups of subjects prepared without anthranoid laxatives; subjects without colorectal abnormalities, patients with sporadic neoplasia and members of hereditary colorectal cancer families. Following anthranoid administration, epithelial cell proliferation was impressively higher and epithelial crypt lengths were considerably shorter than in the three groups prepared without anthranoid laxatives. These data provide a possible mechanism through which anthranoid-containing laxatives may induce colorectal carcinogenesis.

Detoxifying enzymes in the enterocytes protect the gastrointestinal tract, and consequently also the portal and systemic circulation, from damage caused by lipophilic, potentially toxic compounds, by altering their nature, water-solubility and distribution. Up to now, studies involving the biotransformation system in the small intestine in man with reference to colorectal carcinogenesis are limited. In chapter 11 it is hypothesized that a defective biotransformation system, distinguished by low levels of enzyme activity, would allow an increasing load of carcinogens to reach the large bowel, thus stimulating colorectal carcinogenesis. On the other hand, chronic over-exposure to xenobiotics could be detected by abnormally high activity levels of these biotransformation enzymes. Therefore, the activity of one of these detoxifying enzymes, 7-ethoxycoumarin-O-deethylase, was quantitated in duodenal biopsies of 17 patients with metastasized colorectal cancer, and compared to values of 16 patients with metastasized breast cancer and 23 patients without malignant disease serving as a control group. No correlation between the presence of colorectal or breast cancer and the level of 7-ethoxycoumarin-O-deethylase activity could be demonstrated. This does not support that these malignant diseases are the consequence of initially decreased biotransformation activity. However, the possibility can not be rejected that normal 7-ethoxycoumarin-O-deethylase activity in colorectal and breast cancer is the result of changes in diet since the tumours developed, or otherwise that failure to increase biotransformation activity has played a role.
Conclusions and future perspectives

This thesis shows that it is possible to modulate factors associated with colorectal cancer development, specifically the composition of the luminal contents of the large bowel, the cytotoxic potential of these luminal contents, and the degree of proliferation of the colorectal epithelium. The factors associated with colorectal cancer can be modulated through dietary and surgical intervention. Dietary intervention can assist in the primary prevention of colorectal cancer. Of course, surgery is only an option for primary prevention in exceptional circumstances. The search for dietary compounds that can be used for primary prevention can be facilitated by intervention studies in small populations at increased risk for colorectal cancer that use a biomarker for colorectal cancer as an intermediate phenotypic endpoint. However, the outcomes of such intervention trials may be influenced by a number of elements. This thesis identified three of these elements: multifactorial aetiology, segmental heterogeneity, and interactions between dietary compounds. They may change the approach to future dietary intervention studies. These three elements and the impact they may have on primary prevention of colorectal cancer will be discussed below.

MULTIFACTORIAL AETIOLOGY

On the basis of knowledge obtained from epidemiological studies, it is generally accepted that the aetiology of large bowel cancer is multifactorial. Both genetic and environmental factors are known to substantially contribute to the risk of colorectal cancer. Colorectal malignancies arise from colorectal epithelial cells, in which alterations of the cellular genome have occurred. These genetic mutations can be inherited as well as induced by genotoxic substances. If these mutations give rise to activation of oncogenes or deletion of tumour suppressor genes, this may finally lead to neoplastic transformation. Cellular hyperproliferation plays an important role in the process of colorectal carcinogenesis because a large population of mutated cells can originate from one mutated cell. Additionally, non-genotoxic substances may induce hyperproliferation of normal colorectal epithelial cells (1). Hyperproliferation itself probably also increases colorectal cancer risk, because the process of cell replication makes a cell more susceptible to DNA alterations. Such a carcinogenic mechanism has been reported for various tumour-types, among others for endometrial and breast cancer. Thus, hyperproliferation may be an indicator for the risk of colorectal cancer.

In this thesis, hyperproliferation of the colorectal epithelium was observed in various populations at high risk for colorectal cancer development. In subjects at risk for hereditary nonpolyposis colorectal cancer epithelial cell proliferation throughout the colorectum is higher compared to controls. An abnormal proliferation pattern, with an extension of the proliferative zone, is found in the rectum of patients with familial adenomatous polyposis. In a group of patients with acromegaly, increased colorectal epithelial cell proliferation was also observed.
nic epithelial cell proliferation correlates with both circulating growth hormone and insulin-like growth factor-I levels. Short-term oral administration of a single high dose of anthranoid-containing laxatives also results in an increase of epithelial cell proliferation in the colon. The findings in these high risk populations demonstrate that the proliferation state of the colorectal epithelium is determined by multiple factors, such as inherited, hormonal and environmental factors. Although most of the studies in these patients described the effect of genetic, hormonal and environmental factors separately, it should be recognized that the effects of these factors overlap. This is supported by the study in familial adenomatous polyposis patients. This study shows that an altered luminal environment caused by subtotal colectomy is associated with a more quiescent proliferation pattern in the retained rectum of these patients. Several years after the surgical resection, the subsequently obtained beneficial effect on epithelial cell proliferation proves to be only temporary, whereas the changed luminal environment persists. Evidently, environmental factors can temporarily alter the expression of this inherited disorder, while at a later point in time the genotype prevails again. The ultimate decisive role of the genotype for colorectal carcinogenesis consequently restricts the possibilities for primary prevention. In view of the need for primary prevention, this underscores the importance of studying populations with a similar genetic background in future intervention studies. Additionally, as results from one population can not automatically be extrapolated to another population, it is mandatory to study the effect of a certain proposed protective agent in several populations at high risk for colorectal cancer.

SEGMENTAL HETEROGENEITY
The large bowel has long been considered a uniform physiological and anatomical entity. Several studies presented in this thesis support recent studies that suggest that this is not the case. In this thesis, differences between the luminal compositions of the proximal and distal colons have been described in patients after left or right hemicolectomy. Indications for differences in physiological functions between these large bowel segments specifically are differences in absorption of water and electrolytes, and differences in bacterial degradation and absorption of bile acids. Differences in epithelial cell proliferation in various segments of the colorectum are found in subjects from hereditary nonpolyposis colorectal cancer families. Epithelial cell proliferation in the rectum of these subjects is higher than in the sigmoid and descending colon. This is in agreement with the observation that the incidence of cancer in hereditary nonpolyposis colorectal cancer families is twice as high in the relatively short rectal segment as in the sigmoid and descending colon. It should be realized that regional differences in the composition of the luminal contents create a changing microenvironment for substances traversing the colorectum. As a consequence, interactions between various compounds will differ from one large bowel segment to another. Additionally, regional differences between the epithelium of various large
bowel segments may give rise to area-dependent responses of the epithelium to a certain luminal compound. In this thesis, evidence for site-specific responses of the epithelium to luminal compounds was revealed by the calcium supplementation study in hereditary nonpolyposis colorectal cancer families. In rectal crypts, epithelial cell proliferation was initially higher than in colonic crypts of the sigmoid and descending colon. Whereas calcium supplementation seemed to have no effect on epithelial cell proliferation in the sigmoid and descending colon, it induced a decrease of proliferation in the luminal compartment of rectal crypts. Obviously, effects of dietary intervention on one location of the colorectum cannot automatically be extrapolated to other parts of the colorectum. Regarding the issue of primary prevention, segmental heterogeneity of the luminal contents and epithelium of the large bowel underscores the importance of evaluating the effect of dietary intervention in various parts of the large bowel.

**INTERACTIONS BETWEEN DIETARY COMPOUNDS**

A prudent diet for the prevention of colorectal cancer grossly seems to be a diet low in animal fat and high in fibre (3). In order to achieve such a prudent diet, it would be necessary to markedly change habitual, culturally determined, diets of different populations at risk for colorectal cancer. As this will be difficult to accomplish, a more realistic approach would be supplementation of the regular diet with compounds that exert a protective effect directly or via counteracting the damaging potential of luminal substances. With respect to the latter, supplemental calcium has been proposed as an agent to bind cytotoxic surfactants. In this thesis, two different calcium formulas, calcium carbonate and tricalcium phosphate, are studied in relation to intestinal bile acids and cytolytic activity of faecal water. Whereas both calcium compounds tend to reduce the concentrations of hydrophobic cytolytic bile acids, only calcium carbonate supplementation tends to decrease the cytolytic activity. This indicates that the composition of the calcium formula is important for effective interactions with luminal surfactants. The finding raises serious doubts about the appropriateness of the used calcium phosphate formula for the primary prevention of colorectal cancer. However, in the meantime, similar results as with calcium carbonate have been reported with calcium phosphate as provided by milk products in healthy volunteers (4). Evidently, calcium phosphate within a complex liquid may not have the same effect on luminal surfactants as a solid calcium phosphate supplement. The human diet consists of an extensive amount and a large variety of nutritional substances. Co-administration of other nutrients may be relevant for the intraluminal binding of calcium compounds to cytotoxic surfactants, and should therefore be elucidated. Of interest may be luminal interactions between calcium, cytotoxic surfactants, and various types of fibre. It has been previously reported that fibres bind bile acids in vitro, the extent of binding being a function of both the type of fibre and the bile acid involved (5). In a more recent study, an increased produc-
tion of short-chain fatty acids was found in the caecum of rats fed fermentable fibres during calcium supplementation (6). Hence, interactions between various dietary substances may affect the protective potential of a compound under investigation for the prevention of colorectal cancer. As far as possible, such interactions should be taken into account in human intervention trials. Although in man, in contrast to animals, control of dietary intake is extremely difficult, the above-findings suggest that a baseline should be set for habitual diets in human intervention trials. Furthermore, if possible, input-output analyses of dietary supplements tested should be performed in such trials.

In general, it is assumed that primary prevention of colorectal cancer through dietary intervention is a promising avenue. However, it can be concluded from this thesis that it is essential to recognize the limitations of dietary intervention. Firstly, the genetic background of populations with an increased colorectal cancer risk due to an inherited disorder sets a limit to the extent to which these subjects can benefit from dietary intervention. Secondly, segmental heterogeneity of physiological processes and epithelial cell proliferation throughout the colorectum underlines the importance of studying the effect of dietary intervention in various large bowel segments. And thirdly, interactions between agents with a proposed beneficial potential and co-administered nutritional substances have to be considered to influence the outcome of dietary intervention. For the moment, it seems to be too early to advocate calcium supplementation for primary prevention of colorectal cancer in the general population. Large-scale primary intervention procedures may someday offer the potential of reducing the risk of colorectal cancer development in the general population. In the meantime, carefully performed small randomized phase III studies in selected groups of subjects at increased risk, combined with biochemical analyses, should precede large intervention trials.