National Library of Canada
Canadian Theses Service
Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Services des thèses canadiennes

CANADIAN THESES

THÈSES CANADIENNES

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ MICROFILMÉE TELLE QUE NOUS L'AVONS REÇUE

NL-339 (r.86/06)

Canada

THE UNIVERSITY OF ALBERTA

VITAMIN A STATUS IN BENIGN AND MALIGNANT GASTROINTESTINAL DISEASE

(C)

BY

ANNE MARY HODGSON

A THESIS

SUBMITTED TO FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE.

IN NUTRITION

FACULTY OF HOME ECONOMICS

EDMONTON, ALBERTA

SPRING 1987

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-37755-0

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: ANNE MARY HODGSON

TITLE OF THESIS: VITAMIN A STATUS IN BENIGN AND MALIGNANT GASTROINTESTINAL DISEASE

DEGREE: MASTER OF SCIENCE

YEAR THIS DEGREE WAS GRANTED: 1987

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

Anne Hodgson

R.R. #1 Millarville
Alberta, T0L 1K0

Date: Apr. 17/87

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Vitamin A Status in Benign and Malignant ~~Gastro~~intestinal Disease." submitted by Anne Hodgson in partial fulfilment of the requirements for the degree of Master of Science in Nutrition.

.....
Supervisor

.....

.....

.....

Date 7/4/87

ABSTRACT

Several studies have demonstrated subnormal circulating retinol values in patients with cancer of epithelial origin. Conditions such as stress and diseases other than cancer are known to depress serum retinol levels. For this reason, vitamin A status in colorectal cancer patients was assessed using subjects with benign GI disease as controls. Serum samples from 31 healthy subjects, 40 benign GI disease patients and 41 colorectal cancer patients were obtained from the sera bank of the Mayo Clinic, Rochester. Serum concentrations of retinol and its carrier proteins, RBP and prealbumin, were higher in healthy subjects ($p<0.05$) than in benign or malignant GI patients. Circulating levels of zinc, which is required for the synthesis of the carrier proteins, were significantly higher in healthy subjects as compared to benign but not malignant patients. No significant differences were detected between benign GI and colorectal cancer patients for retinol or the factors involved in retinol metabolism. With the exception of zinc, the serum parameters were depressed to the same extent by both benign and malignant GI disease.

When discriminant function analysis was used to evaluate these serum parameters as diagnostic indicators, only 56.6% of subjects were correctly categorized as being healthy or diseased. Because of the considerable overlap in serum values between healthy and patient groups, these

biochemical indices lack the specificity required of diagnostic tumour markers.

Postoperative serum samples from 134 patients with curative resections of Duke's B2 or Duke's C colorectal cancer were analyzed in order to evaluate the prognostic significance of vitamin A and its related factors. These patients were involved in the adjuvant therapy trial for colorectal cancer at the Cross Cancer Institute, Edmonton. Serum levels of vitamin A, RBP, prealbumin and zinc did not significantly differ between patients who subsequently developed a recurrence of cancer and patients who remained disease-free. Circulating levels of these parameters do not appear to be of value in predicting the long-term survival status of postoperative colorectal cancer patients.

ACKNOWLEDGEMENTS

I wish to express my gratitude to my advisor, Dr. T.K. Basu, for providing advice and support throughout this project.

A special thanks is due to Joyce Ng for being a great source of information and help in the laboratory. Appreciation is also extended to Dr. G.B. Hill, Director of the Department of Epidemiology, Alberta Cancer Board, for his advice in analysis of data. I also wish to thank Mr. Ray Weingardt, Department of Animal Science, for his help and time spent in computer programming and statistical analysis.

I am indebted to my parents and last, but not least, my husband, Kevin, for their endless support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

BCG	= Bacille Calmette Guerin
C	= degrees Celsius
CCI	= Cross Cancer Institute
cm	= centimeter
CRABP	= cellular retinoic acid-binding protein
CRBP	= cellular retinol-binding protein
dl	= deciliter
DMH	= dimethyl hydrazine
DNA	= deoxyribonucleic acid
5-FU	= 5-fluorouracil
GI	= gastrointestinal
g	= gram
IU	= International Unit
kD	= kiloDalton
methyl-CCNU	= 1-(2-choloroethyl)-3-(4-methylcyclohexyl)-1-nitrosurea
mA	= milliamp
mg	= milligram
ml	= milliliter
mm	= millimeter
nm	= nanometer
ODC	= ornithine decarboxylase
RBP	= retinol binding protein
RE	= Retinol Equivalent
SD	= standard deviation
ug	= microgram

1. INTRODUCTION

Vitamin A has a considerable controlling influence on epithelial differentiation. During vitamin A deprivation, normal epithelium undergoes metaplastic change similar to that seen in early neoplasia (Wolbach & Howe, 1925). Extensive laboratory and epidemiological investigations have led to the suggestion that vitamin A is a natural inhibitor of the development and progression of preneoplastic and neoplastic lesions. Subnormal concentrations of circulating retinol have been demonstrated in patients with various epithelial cancers and subjects who subsequently developed cancer. The majority of such studies have used healthy subjects as controls. However, as stress, infections and diseases other than cancer are known to depress retinol levels, it is crucial that patients with appropriate benign disease be used as controls. The primary goal of this study was to evaluate the vitamin A status in colorectal cancer patients using subjects with benign gastrointestinal disease as controls.

Little information is available regarding either the prognostic significance of vitamin A status in patients with established cancer or the diagnostic significance of this vitamin in populations at risk for cancer. Therefore, the use of serum levels of vitamin A and its related factors as diagnostic and prognostic indicators for colorectal was investigated.

1.1 VITAMIN A ACTIVITY

Vitamin A was first described in 1915 by McCollum and Davis as a growth-promoting factor isolated from animal fat and fish oil. In the strictest sense, vitamin A refers to all-trans retinol, however, the term vitamin A is used to describe the naturally occurring analogues of retinol. Recently, the word "retinoids" has been used to encompass any natural or synthetic moiety of vitamin A.

Vitamin A is required for several important physiological functions; particularily vision, growth, reproduction and maintenance of epthelial tissue. These functions involve different forms of vitamin A. The biologically active form in mammalian tissues is all-trans retinol. Retinol can be oxidized to the aldehyde retinal, which is a visual pigment precursor (Wald, 1968). Further oxidation irreversibly forms retinoic acid. While retinoic acid is able to support normal body growth and differentiation of epithelial tissues, it cannot replace retinal's visual role (Dowling and Wald, 1960), nor can it support reproduction (Thompson et al., 1964). With exception of its role in vision, the molecular mechanisms of vitamin A activity are not fully understood.

1.2 EFFECT OF VITAMIN A ON EPITHELIUM

The retinoids are well known for their importance in the growth, differentiation and function of epithelial cells. While the exact mechanism by which vitamin A influences these cells remains unclear, deficiency studies have demonstrated the effect of vitamin A on epithelium. Squamous epithelium lines the external part of the body and its orifices while the gastrointestinal, lower respiratory and urogenital tracts are lined with glandular epithelium. During vitamin A deprivation, normal mucus-secreting epithelium undergoes metaplastic change into stratified, keratinizing squamous epithelium. Growth activity of this altered epithelial tissue is increased as well (Wolbach & Howe, 1925). Upon refeeding with vitamin A, this metaplastic process is reversed (Wolbach & Howe, 1933). An exception is the intestinal mucosa, where degeneration of mucus-secreting cells occurs without keratinization of the epithelium (DeLuca et al., 1969). Because the appearance of metaplasia is common to both vitamin A deficiency and early neoplasia, a deficiency of this vitamin might enhance the neoplastic response to chemical carcinogens or produce a preneoplastic condition.

Brevard et al., (1985) demonstrated that vitamin A supplementation increased the number of goblet cells in colorectal tissue of rats, concomitant with the normalization of mucin type and an increase in mucus

secretion. Since the mucus coat acts as a protective barrier against bacteria, viruses and foreign substances in normal intestinal epithelium, an increase in sulfated mucins could provide for a more protective mucus coat and prevent disease-causing agents from coming in contact with the epithelium. It has been indicated that vitamin A is required for the biosynthesis of glycoproteins; the principle constituent of goblet cells (DeLuca et al., 1972; DeLuca, 1977). As glycoproteins are common constituents of membranes necessary for a variety of cellular functions, the effects of retinoids on glycoprotein biosynthesis may explain many of the anomalies in cellular metabolism associated with vitamin A deficiency.

Isolation of cellular binding proteins for retinol (Bashor et al., 1973) and retinoic acid (Ong and Chytil, 1975), led to the hypothesis that retinoid action is comparable to that of steroid hormones. It has been suggested that intracellular binding proteins may play a direct role in biological expression of vitamin A activity, analogous to steroid hormone receptors. These proteins deliver retinol and retinoic acid specifically into the nucleus (Takase et al., 1979) where the retinoids seem to interact with nuclear components and influence gene regulation. In fact, evidence shows that protein synthesis and RNA metabolism are affected in several tissues of vitamin A deficient animals (Johnson et al., 1969; Zile and DeLuca, 1970). Zachman (1967) obtained a 2 to 3 fold

increase in the rate of RNA synthesis in the colon and intestinal mucosa by reintroduction of physiological levels of retinol into vitamin A-deficient rats. Cellular binding proteins are present in many epithelial tissues and may be present in elevated levels in some tumours as compared to normal adjacent tissue. It is possible that these proteins exist in any tissue where vitamin A is known to effect epithelial differentiation.

1.3 METABOLISM OF VITAMIN A

Dietary sources of vitamin A consist of long-chain retinyl esters in certain foods of animal origin (largely milk, cheese, butter, egg yolk and liver) and specific carotenoids, such as beta-carotene, which are precursor forms of the vitamin. Principal sources of carotene are dark green, leafy vegetables and certain yellow-red fruits and vegetables. Beta-carotene is largely converted to retinol in the intestinal mucosa, although humans can absorb small amounts of the intact provitamin (Goodman et al., 1966). Dietary retinyl esters are hydrolyzed in the intestine forming retinol, which is then absorbed into mucosal cells. In the mucosal cells, retinol from either the ester or provitamin source is reesterified primarily with palmitic acid (Ganguly, 1969). In association with chylomicrons, these retinyl esters are transported first via the lymphatic system and then via the systemic blood and are

taken up by the liver for metabolism and storage (Goodman et al., 1965). Most of the vitamin A in the body is stored in hepatic cells, largely as long-chain, saturated retinyl esters. Other tissues, including the kidneys, lungs, adrenals and intraperitoneal fat, contain about 9 percent of the total and the serum contains approximately 1 percent of the total body reserve (Moore, 1931). Total body reserves of vitamin A can be best approximated by the total liver concentration, which increases with the dietary intake of retinol. As dietary intake of vitamin A increases, the efficiency with which it is stored decreases. The liver responds to higher levels of vitamin A intake and storage by inducing a metabolic process for the increased excretion of vitamin A (Hicks, 1984; Olson, 1984). Retinol and its oxidized product, retinoic acid, are conjugated in the liver with glucuronic acid. The newly formed glucuronides are excreted in the bile and may be partially reabsorbed in the intestinal tract and re-excreted in the bile, thus establishing an enterohepatic circulation of vitamin A metabolites (Lippel and Olson, 1968). The majority of these biliary glucuronides, however, are hydrolyzed in the intestine and excreted in the feces as a mixture of free retinoic acid, possibly free retinol and the intact glucuronides. (DeLuca and Roberts,1969).

When a metabolic requirement for vitamin A exists, stored retinyl esters are hydrolyzed to form all-trans retinol. The retinol is then mobilized from the liver and

delivered to peripheral tissues bound to a specific transport protein, retinol-binding protein (RBP). RBP is a single polypeptide chain with a molecular weight of approximately 20,000 kD and a single binding site for retinol (Kanai et al., 1968) that is synthesized and secreted by hepatic parenchymal cells (Glover et al., 1974). In serum, RBP normally circulates bound to retinol, forming holo-RBP, and complexes with prealbumin. Prealbumin is a tetrameric protein of 54,900 kD molecular weight which is also synthesized in the liver (Kanada et al., 1974). Although prealbumin is thought to have four binding sites for RBP, the two proteins form a 1:1 complex.

The interaction of retinol with RBP serves to solubilize the vitamin in serum and protect the unstable retinol molecule against oxidative damage. Formation of the protein-protein complex appears to stabilize the binding of retinol to RBP and prevent glomerular filtration of the relatively small RBP molecule (Kanai et al., 1968).

Circulating retinol that is bound to RBP is taken up by non-hepatic cells without a concomitant uptake of RBP. The uptake process appears to be mediated by a specific membrane receptor that recognizes RBP but not retinol (Peterson et al., 1974; Heller, 1975; Rask and Peterson, 1976). Neither free retinol nor a retinol-albumin complex can be taken up by cells (Chan and Heller, 1977), indicating a specific need for the recognition of RBP by target cells. During the uptake process an altered form of RBP is produced which

lacks the terminal arginine residue (Rask et al., 1971). This apo-RBP cannot bind retinol or prealbumin and is selectively filtered by the renal glomeruli for catabolism.

Serum RBP levels appear to be regulated by the nutritional status of vitamin A. In rats, retinol deficiency results in low serum RBP concentrations together with elevated liver RBP levels. Administration of oral or injected vitamin A stimulates the rapid secretion of RBP from the expanded liver pool into the serum (Muto et al., 1972). These findings suggest that vitamin A deficiency specifically interferes with the secretion, rather than the synthesis of RBP by the liver. This effect appears to be specific for RBP as prealbumin levels are not affected by a vitamin A deficiency (Smith and Goodman, 1979).

Because of its role in the plasma transport and cellular uptake of retinol, RBP is necessary not only for cellular utilization of this vitamin, but also for the prevention of vitamin A toxicity. Vitamin A toxicity occurs when body stores of vitamin A are so great that retinol begins to circulate and interact with membranes in a complex bound to lipoproteins rather than RBP (Smith and Goodman, 1979). As retinol is known to be a highly surface-active compound which is potentially membranolytic, binding of the vitamin to RBP may serve to prevent retinol from damaging biological membranes.

1.4 REGULATION OF SERUM AND TISSUE VITAMIN A LEVELS

Serum concentrations of retinol are highly characteristic of the individual and less characteristic of vitamin A intake; with the exception of severe dietary deficiency and excess. The homeostatic level of circulating retinol is known to vary considerably among well-nourished individuals living in industrialized countries (Wald et al., 1980). Most of this diversity can be attributed to the influence of physiological, nutritional, clinical and genetic factors which affect serum retinol levels by a variety of mechanisms.

Only in cases of extreme hypo- and hypervitaminosis A do serum levels of retinol correlate with liver stores (Rietz et al., 1974). Under conditions of inadequate dietary intake, liver stores are drawn upon to maintain a relatively constant serum retinol value until the stores are nearly exhausted. When the liver is depleted of its reserve, serum levels fall rapidly. Serum levels rise to a plateau when liver storage concentrations reach 10 - 30 ug retinol/g liver. Homeostatic control maintains this plateau until a storage level of over 300 ug retinol/g liver is reached (Olson, 1984). At this point the liver storage mechanism is largely saturated and despite an increase in retinol metabolism, serum vitamin A rises to toxic levels. Therefore, within a wide range of vitamin A concentrations (30 - 300 ug/g liver) serum retinol values are not good

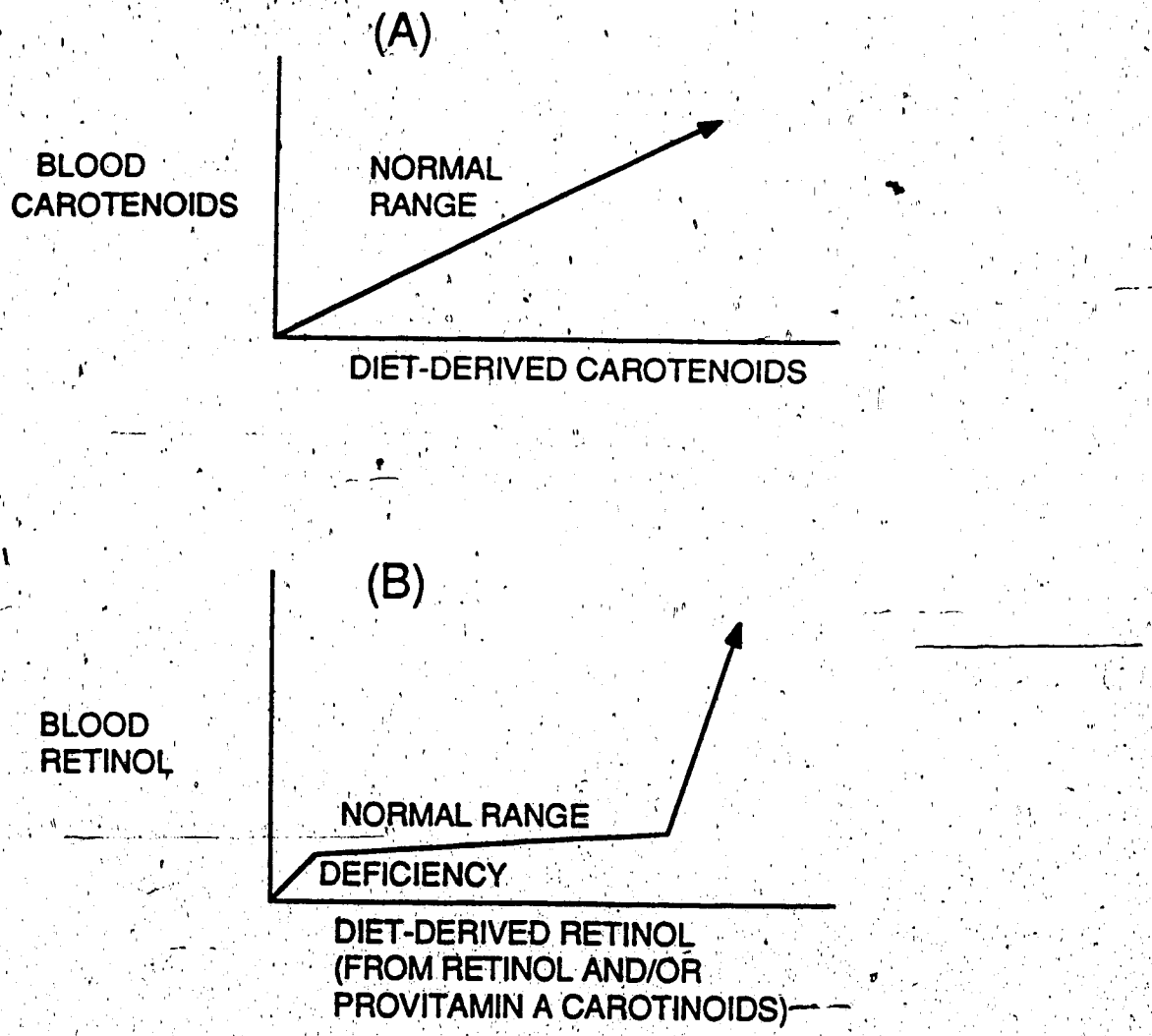
indicators of total body reserves. Serum carotene levels, however, are much more variable than those of vitamin A in that they directly reflect dietary intake of carotenoids. (See figure 1.1)

1.4.1 EFFECT OF DIETARY VITAMIN A

The majority of individuals in developed countries consume adequate amounts of vitamin A so that additional vitamin A supplementation produces little, if any, increase in RBP-bound retinol. In studies of Willett et al. (1983), supplementation with 25,000 IU retinyl palmitate failed to significantly elevate plasma retinol levels in healthy subjects. In the same subjects, supplementation with 30 mg (5,000 RE) of beta-carotene tripled plasma carotene levels but again did not influence retinol concentrations. However, in a subgroup of women with relatively low plasma retinol levels, a daily 10,000 IU supplement of vitamin A produced a small increase in plasma retinol (Willett et al., 1984). This increase was greatest amoung individuals with low prior vitamin A intake. Retinol supplementation in cancer patients failed to show significant changes in five patients with normal pretreatment plasma retinol concentrations while three patients with low pretreatment retinol levels all had a rise in plasma vitamin A (Goodman et al., 1984). These increases in plasma retinol levels may simply represent the normalization of a vitamin A deficiency.

FIGURE 1.1

SCHEMATIC RELATIONSHIP BETWEEN BLOOD AND DIET-DERIVED AMOUNTS OF (A) CAROTENOIDS AND (B) RETINOL



SOURCE: PETO,1983

1.4.2 INFLUENCE OF OTHER NUTRIENTS

In addition to the intake of the vitamin itself, the dietary intake and nutritional status of several other nutrients effects body vitamin A concentrations. Low fat diets hamper the absorption of both vitamin A and carotenoids, thus resulting in low serum retinol levels. It follows then, that diseases involving defective fat absorption either due to reduced absorptive surface area or pancreatic and/or biliary insufficiency are also associated with depressed serum vitamin A concentrations (Underwood, 1974).

It has been observed that blood levels of vitamin A and prealbumin correlate linearly to levels of RBP in both healthy subjects and in patients with a variety of diseases (Smith et al., 1971; Underwood, 1974). Because vitamin A is transported in association with two proteins, protein-deficiency conditions accentuate retinol deficiency. Patients with protein-energy malnutrition typically have low serum retinol concentrations which reflect an impairment in hepatic release of vitamin A due to defective synthesis of plasma proteins (McLaren et al., 1969; Smith et al., 1973). Depressed synthesis of RBP and prealbumin due to liver disease results in low serum levels of these proteins accompanied by decreased serum vitamin A concentrations (Smith et al., 1971). In contrast to depressed levels associated with liver disease, elevated concentrations of

serum vitamin A are seen in renal disease. As the kidney is normally the main catabolic site for RBP, in renal disease the levels of both RBP and vitamin A are elevated. Prealbumin levels remain normal in this condition.

The trace element zinc is also essential for maintenance of normal blood vitamin A levels. Zinc deficient rats were shown to have very low plasma retinol concentrations with an accumulation of vitamin A in their livers (Smith et al., 1973). After dietary or intraperitoneal zinc repletion, the plasma vitamin A returned to normal. (Brown et al., 1976). Normal weanling rats demonstrated a decrease in hepatic and an increase in plasma levels of vitamin A two hours after the intraperitoneal administration of zinc (Ette et al., 1979). These results reflect the involvement of zinc in the mobilization of vitamin A from the liver to the circulation within a short time period. Smith further demonstrated that a severe zinc deficiency in rats reduced plasma RBP to one quarter the normal values accompanied by liver RBP of approximately one half the normal (Smith et al., 1974). Recently, a 75 day metabolic study of low zinc intake resulted in a significant reduction of serum RBP, albumin and prealbumin levels in six men (Wada and King, 1986). The low plasma vitamin A levels seen in zinc deficiency were attributed to an impaired mobilization of vitamin A from the liver in the form of the retinol-RBP complex. Low RBP and total plasma protein concentrations in these rats suggests

that zinc deficiency results in depressed synthesis of hepatic proteins. This is supported by the fact that zinc is essential for protein and nucleic acid synthesis in microorganisms (Wegener and Romano, 1973) and rats (Swenerton et al., 1969). Several enzymes involved in protein synthesis, such as thymidine kinase and DNA-dependent RNA polymerase are known to be zinc dependent.

Zinc deficiency also affects the utilization of vitamin A. Alcohol (retinol) reductase, a zinc metalloenzyme, is necessary for the interconversion of retinol to retinal, a process essential for normal vision. Zinc deficiency results in a significant reduction in liver and retina levels and activity of this enzyme (Huber and Gershorf, 1975). Improvement of dark adaptation in alcoholic cirrhotics upon zinc supplementation may be due to enhanced activity of previously depressed retinol reductase (Morrison et al., 1978).

1.4.3 HORMONAL REGULATION

Some regulation of serum vitamin A is likely mediated hormonally as males have higher levels than do females (Pearson, 1967) and administration of estrogens as oral contraceptives causes a marked increase in serum levels of RBP-bound retinol (Gall et al., 1971; Banji and Ahmed, 1978).

1.4.4 EFFECT OF STRESS

Stress, from physical or emotional sources is also known to influence circulating retinol, causing a transient reduction in blood concentrations. This effect has been demonstrated in postoperative patients (Kasper et al., 1975) and subjects with severe burns (Cynober et al., 1985) and infections (Sivakumar and Reddy, 1972). The decreases in body vitamin A levels observed under conditions of stress may be mediated by alterations in the absorption or metabolism of the vitamin.

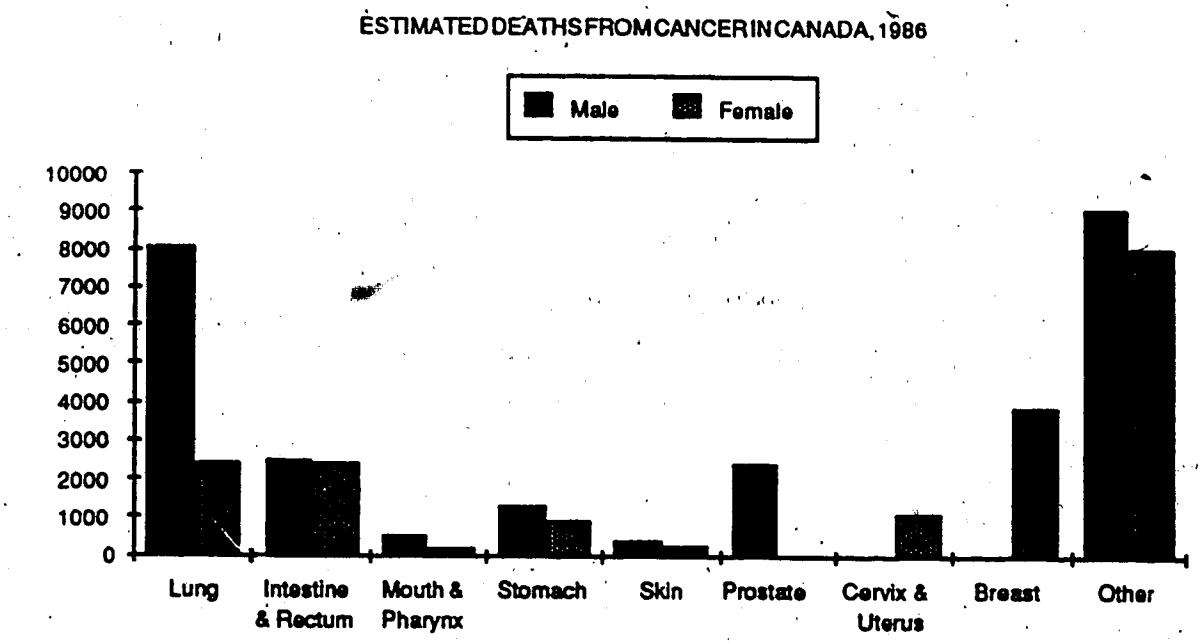
1.5 COLORECTAL CANCER

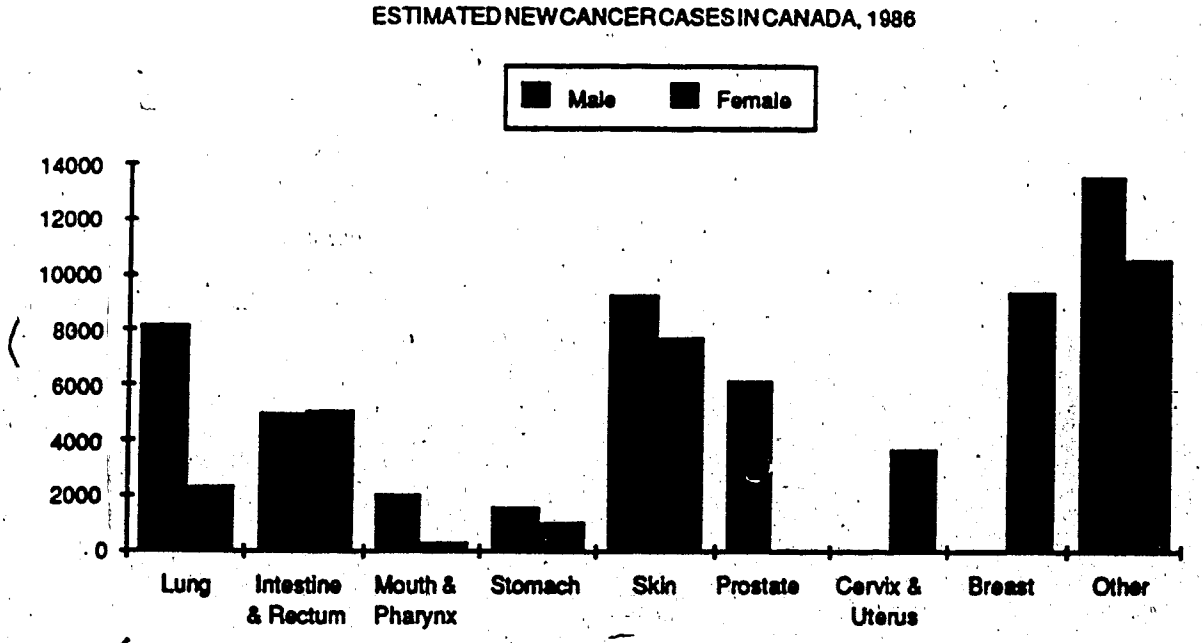
Colorectal cancer is one of the most important cancers in developed countries in terms of both incidence and mortality. (See figure 1.2) The incidence rates for colorectal cancer for 1985 in Alberta were 36.6 and 35.9 per 100,000 for men and women, respectively.

It is expected that every person born in the western world today has a five percent chance of developing cancer of the large intestine (Van de Velde et al., 1986). As neither the incidence rate nor the survival rate of patients following surgical treatment have markedly improved in the past decades, efforts into the prevention of this disease are crucial.

Figure 1.2

CANCER INCIDENCE AND MORTALITY RATES FOR CANADIANS





Source: Canadian Cancer Society

1.5.1 PREDISPOSING FACTORS

Factors which predispose to colorectal cancer include rare genetic disorders as well as the presence of specific polyps, diverticular disease, chronic ulcerative colitis and regional enteritis or Crohn's disease.

It has recently been confirmed that the risk for development of colorectal cancer is 3 times greater for patients with benign polyps (particularily adenomatous polyps) in comparison to the general population (Lofti et al., 1986). While the majority of benign polyps never become malignant, it is generally accepted that adenomas should be regarded as precancerous lesions (Wegener et al., 1986). In the populations studied, the risk of large bowel cancer increases with the size and number of adenomas, thus suggesting a dose-response relationship. Clinical observations indicate that it usually takes many years for a polyp to evolve into cancer (Morson, 1974), corresponding to the long latent period before patients present with colorectal cancer symptoms.

There is a very close parallel in the epidemiology of large bowel cancer and adenomatous polyps. Cancer and benign polyps share similar shifts in frequency when comparing low and high risk populations (Stemmerman and Yatani, 1973). It has also been demonstrated that diverticular disease seldom occurs in populations which experience a low risk for colorectal carcinoma or polyps.

Patients with inflammatory bowel disease are known to be at an increased risk for developing large bowel cancer. The basis for this relationship, however, is unknown since the genesis of both the predisposing diseases and the carcinoma remain largely unclear (Watson, 1974). Rather than being a cause-effect relationship, associated etiologic factors for the diseases may be involved. Before cytotoxic agents, including carcinogens, can reach cells of the colonic epithelium, the protective mucosal layer must be damaged or removed. Bennet (1986) proposed that in genetically susceptible individuals, ulcerative colitis is caused by a bacterial metabolite of bile acids or cholesterol and that this substance is similar or identical to bacterial metabolites implicated in the development of colon cancer. Bile acids can, in fact, cause the removal of the mucus layer of colonic epithelium, accompanied by the loss of the upper-most layer of the epithelial cells.

Several types of gastrointestinal operations have also been reported to predispose to the development of colorectal cancer by affecting cell kinetics and modifying intestinal microflora (Trichopoulos, 1986). The increased incidence of large bowel cancer in women who had undergone cholecystectomy may have been the result of an altered bile acid composition (Weiss et al., 1982).

1.5.2 DIET AND COLORECTAL CANCER

Epidemiological studies indicate that exogenous factors are major determinants of many common cancers, particularily in epithelial-derived tumours of the lung, breast and gastrointestinal tract. Doll and Peto (1981) estimated that approximately 90 percent of colon cancer in men and women in the United States was potentially avoidable, as was 85 percent of rectal cancer in men and 75 percent of rectal cancer in women. Substantial international variation in the incidence of colorectal cancer is present with high values in western Europe and north America and low values in southeast Asia and Africa. Migrant studies indicate that the risk of colorectal cancer may be dramatically altered within a person's lifetime and that dietary differences may explain the increased frequency of these tumours in developed western countries compared with the underdeveloped countries.

Because the development of colorectal carcinoma is thought to be a multistep process involving initiation, promotion and progression, there may be potential for inhibition at each step. The food we eat may carry potentially mutagenic and carcinogenic substances as well as possible anticarcinogens. Anticarcinogenic nutrients may offer protection either by modifying the production or metabolism of potentially harmful substances or indirectly by maintaining the normal integrity of tissues and of

cellular processes (Underwood, 1984). Studies in man and in animal models suggest that the most significant dietary factors involved in colorectal cancer are the amounts of dietary fat and fibre. However, the relationship between dietary consumption of vitamin A and cancer risk is currently of considerable interest.

1.6 VITAMIN A AND CANCER

Extensive laboratory and epidemiological investigations have led to the conclusion that vitamin A is a natural inhibitor of the development and progression of preneoplastic conditions, such as hyperplasia and metaplasia, as well as neoplastic lesions. As epithelial tissues, dependent on vitamin A for normal cellular differentiation and growth, account for over half of the total primary cancer in humans, investigations have been undertaken to study the effects of retinoids on epithelial tissues after exposure to various carcinogens. The hypothesis that the etiology of epithelial cancers might be related to dietary deficiencies of vitamin A suggests the possibility that retinoids may be used, through dietary or pharmaceutical means, as a preventive measure to reduce the mortality and morbidity of cancer.

1.6.1 EXPERIMENTAL STUDIES

In experimental animals, two general lines of research have been pursued; the first related to the cancer-promoting effects of vitamin A deficiency and the second concerning beneficial effects of the administration of retinoids. The association between vitamin A and cancer was discovered in the 1920's when a vitamin A-deficient diet was interpreted to be the cause of gastric carcinoma in rats (Fujimaki, 1926). In the same decade Wolbach and Howe (1925) discovered that a deficiency of vitamin A leads to metaplastic changes in specific epithelial tissues which may be considered as the first step in the transformation from normal to preneoplastic and finally neoplastic tissue.

While it has been established that vitamin A deficiency alone does not cause cancer (Kummet and Meyskens, 1983), numerous animal studies have demonstrated that the feeding of a diet deficient in retinoids followed by exposure to carcinogens results in a larger than normal incidence of neoplastic and preneoplastic lesions. Newberne and Rogers (1973) showed that, when given the hepatic carcinogen aflatoxin , rats on a vitamin A-deficient diet were more susceptible to liver cancer and developed a 29 percent incidence of colon cancer. In additional work by Newberne, a marginal deficiency of dietary vitamin A increased susceptibility to dimethyl hydrazine (DMH), a carcinogen that requires metabolic activation. In rats exposed to DMH,

100 percent of those deficient in vitamin A developed colon carcinoma compared to 60 percent of those supplemented with vitamin A (Newberne and Suphakarn, 1977). Similarily, vitamin A deficiency has been shown to enhance susceptibility to chemical carcinogens in the trachea (Rowe and Gorlin, 1959), bladder (Cohen et al., 1976) and lung (Nettesheim et al., 1975).

Retinoid deficiency enhances the binding of the metabolite of the carcinogen benzo(a)pyrene to tracheal epithelial DNA. Approximately four times as much carcinogen was bound to tracheal DNA from hamsters fed a diet deficient in vitamin A as compared to normal animals (Genta et al., 1974).

Ornithine decarboxylase (ODC) activity correlates with cell proliferation and can be stimulated by tumour promotors such as bile acids. It was recently shown that vitamin A deficiency, without clinical manifestations, resulted in elevated ODC activity in the colon mucosa of rats (Daliam et al., 1986). Administration of a bile acid led to a significantly larger ODC response in a vitamin A deficient group compared to non-deficient rats. This suggests that a deficiency of this vitamin increases sensitivity to the promotor action of bile acids. Hence, retinol is physiologicallly necessary for natural resistance of intestinal cells against chemically induced tumour promotion.

Because vitamin A deficient animals lose weight and

show impaired immune function, reduced mucus secretion and other physiological defects, the enhanced growth of some neoplasms may result from general physiological debilitation rather than specifically from the absence of vitamin A.

Numerous studies have shown that retinoids can suppress and prevent both the process of carcinogenesis induced by various agents in experimental animals and the development of the malignant phenotype in vitro. Natural and synthetic vitamin A derivatives can inhibit proliferation and stimulate differentiation and/or maturation in normal and many transformed cells. This effect has been demonstrated in tissues that depend on vitamin A activity for maintenance of normal differentiation and integrity, such as that of the respiratory tract, skin, mammary gland, urinary bladder, intestine, stomach, esophagus, cervix, pancreas, lung and liver. Although a number of studies indicate that retinoids act primarily at the stage of tumour promotion, other studies show that retinoids, given prior to initiation, might also be effective in inhibiting tumour growth. (For reviews see Hill and Grubbs, 1982; Sporn and Roberts, 1984.)

Hyperplastic and anaplastic epithelial lesions induced by chemical carcinogens can also be reversed by supplementation with retinoids, even after the lesions have developed. The retinoids are thus able to cause cellular repair of the neoplastic process induced by chemical carcinogens (Chopra and Wilkoff, 1975). The effectiveness of a retinoid as a chemopreventive agent is dependent upon

the type of retinoid, the carcinogen and the target tissue.

In reviewing the role of vitamin A in cancer prevention, Peto et al. (1981) suggested that beta-carotene may be the major molecule providing antineoplastic activity. Beta-carotene may exert a beneficial effect, independent of its provitamin A role, by deactivating reactive singlet oxygen and free radicals, thereby protecting lipids and DNA from oxidative degradation.

Supplementation with large doses of beta-carotene, three to nine weeks after carcinogen administration, resulted in a dramatic inhibition of rat mammary tumorigenesis (Rettura et al., 1983). Muto and Mariwaki (1984), however, found that beta-carotene was less active than retinol or retinol esters in suppressing the development of either skin or liver tumours. In another study, vitamin A and beta-carotene were both able to reduce genotoxic damage in oral mucosa of betal nut and tobacco chewers, while canthoxanthin demonstrated no protective effect (Stich et al., 1984). As canthoxanthin is a good trapper of oxygen singlets but cannot be converted into vitamin A, it appears that vitamin A and beta-carotene exerted their inhibitory effect on precancerous lesion development by a mechanism not involving the scavenging of free radicals. A difference in the preventive activity of beta-carotene and canthazanthin was also observed when they were applied to hairless mice painted with dimenthyl benz(a) anthracene (Mathews-Roth, 1982).

While the exact mechanism by which retinoids exert antineoplastic activity is as yet unknown, proposed mechanisms include immunological effects, interaction with cell membranes, effects on carcinogen metabolism, interaction with the genetic machinery of cells and the quenching of reactive species by carotenoids (Kummet and Meyskens, 1983).

1.6.2 EPIDEMIOLOGICAL STUDIES

1.6.2.1 DIETARY INTAKE OF VITAMIN A

Epidemiologic studies carried out by several different investigators show a reduced cancer risk with increased consumption of dietary vitamin A. Such investigations can be divided into retrospective studies, where the diet histories of cancer patients are compared to those of non-cancer controls, and prospective studies, in which the cancer incidence is observed in large populations of known dietary habits. Dietary information obtained by history is usually translated into nutrient intakes using food composition tables. Although the reliability of dietary records is questionable, vitamin A tends to occur in predictable amounts in a limited number of food items thus enabling reasonable estimation. An example of a food items list used to determine vitamin A intake is listed in Table 1.1.

In the first prospective dietary study, 8,278 Norwegian

TABLE 1.1 VITAMIN A CONTAINING FOODS LISTED IN DIET HISTORY QUESTIONAIRES

I. VEGETABLES

Peas	Tomatoes	Squash
Carrots	Cabbage	Pumpkin
Frozen mixed vegetables	Lettuce	Sweet potatoes
Green beans	Spinach	Potatoes
Broccoli	Swiss Chard	Corn
Asperagus	Green pepper	Carrot juice
Zucchini	Vegetable soup	Tomato juice
	Tomato soup	

II. FRUITS

Papayas	Mangoes	Pineapple juice
Canteloupe	Watermelon	Apricot nectar
Apricots	Oranges	Fruit drinks
Pineapple	Peaches	
Apples	Bananas	
Avocado	Citrus juice	

III. DAIRY PRODUCTS

Milk	Margarine
Cream	Cheese
Ice cream	Cottage cheese
Butter	Yogurt

IV. MISCELLANEOUS

Liver	Vitamin-fortified breakfast cereals
Beef	
Eggs	Vitamin supplements

Adopted from Hankin et al., 1984

men were followed for 5 years during which time 36 developed lung cancer. A vitamin A index, which chiefly assessed beta-carotene intake, showed that low intake values were inversely associated with lung cancer incidence at all levels of cigarette smoking (Bjelke, 1975).

The 265,118 respondants to a dietary questionaire, given in Japan's 1965 census, were followed for 10 years. From the 7,377 people who subsequently developed cancer, a significant negative association was detected between cancer of the lung, colon, stomach and prostate and daily consumption of vegetables rich in beta-carotene. (Hirayama et al., 1979).

Shekelle et al. (1981) found that the intake of beta-carotene was inversely related to the 19 year incidence of lung cancer in a prospective study of 1,954 middle-aged men while the intake of retinol was not significantly related to the risk of lung or other cancers. The estimated carotene intake was lowest for colon and rectum cancers compared to all other carcinomas developed in the entire study group.

A fourth prospective study, using an elderly population, examined the association between consumption of carotene-containing vegetables and subsequent 5 year mortality (Colditz et al., 1985). Again, a significant trend of decreased cancer incidence associated with increased intake of carotene was demonstrated.

It is possible that the negative association between carotene-containing foods and cancer risk, shown in these

four studies , is not causal. Other protective agents within the vegetables, including vitamin C; indoles, plant phenols or trace elements such as selenium, may be responsible for the demonstrated effect.

Numerous retrospective dietary studies have been used to investigate the relationship between vitamin A and cancer. The majority of these studies have combined preformed vitamin A and its precursor, beta-carotene, as the total vitamin A intake so that the effects of preformed and pro-vitamin A cannot be distinguished. Because such a large proportion of dietary vitamin A is actually contributed by beta-carotene, only a few case-control studies have examined the specific effect of retinol intake in relation to cancer risk.

In the first retrospective dietary study, Bjelke (1974) showed that patients with stomach, colon and rectum cancers from Norway and the United States had significantly lower intakes of vitamin A as compared to control subjects. Two other independent studies failed to find any relationship between total vitamin A intake and gastrointestinal cancer, while they did demonstrate a protective effect of beta-carotene containing vegetables (Graham et al., 1978; Moden et al., 1981).

Intake of preformed vitamin A from the diet or supplements was found to be significantly lower in male lung cancer patients versus controls (Gregor et al., 1980). Similarily, Hankin et al. (1984) showed that relative to

those men with the highest intakes, the men who consumed less vitamin A or carotene had a higher risk for lung cancer in a dose-response relationship. These findings were not apparent for females in either study. Ziegler et al. (1986) also found that men with low carotenoid intake had greater risk for lung cancer while no increased risk was associated with low consumption of retinol or total vitamin A. In this study, the intake of dark green and yellow-orange vegetables showed a stronger association with lung cancer than did carotenoid intake.

The effect of retinol-containing preparations on cancer incidence was investigated by comparing 800 newly diagnosed cancer patients to 3,433 patients with non-malignant conditions (Smith and Jick, 1978). While there was a slight protective effect for men, no overall evidence was provided to suggest that regular consumption of vitamin A preparations protected against the development of cancer.

In reviewing case-control studies, Graham (1984) concluded that retinoids appear to have no relationship to risk of cancers of the colon, rectum, esophagus, stomach and corpus uteri. However, they do appear to be associated with a lower risk of cancers of the lung, bladder, mouth, larynx, cervix, breast and ovary. Despite inconsistent findings and instances in which an association has not been observed, the weight of evidence suggests that the intake of vitamin A may inhibit the onset of cancer. It remains to be seen, however, whether intervention based on this knowledge will

reduce cancer incidence in humans. In one intervention study, 50,000 IU of vitamin A, given on a daily basis for over a year along with riboflavin and zinc, had no effect on the prevalence or severity of precancerous esophageal lesions in a high risk group (Munoz et al., 1985).

1.6.2.2 SERUM VITAMIN A LEVELS IN CANCER PATIENTS

Prospective studies have been conducted in which blood samples were collected from a large group of subjects who were then followed for the development of cancer. Biochemical parameters were compared between those subjects who developed cancer and those who did not. The purpose of these studies was to determine if the presence of low blood vitamin A levels preceded the development of cancer. A summarization of these prospective studies is given in Table 1.2.

In the first prospective investigation, conducted in England by Wald et al. (1980), 16,000 men were followed for 3 to 5 years by which time 86 men had developed cancers of various sites. As compared to 172 control subjects, the cancer patients had significantly lower serum retinol levels; the greatest difference being for lung and gastrointestinal cancers. The authors suggested that serum retinol levels in man have predictive value for subsequent cancer development.

In a study from the United States over 3,000

TABLE 1.2 PROSPECTIVE STUDIES RELATING SERUM VITAMIN A AND CANCER.

AUTHOR	YEAR PUBLISHED	COUNTRY	CONTROLS	CANCER SITE	SERUM LEVELS OF PARAMETERS CANCER PATIENTS VS. CONTROLS
Wald	1980	Britain	healthy (172)	lung (18) GI (21) other (51)	decreased retinol
Kark	1981	USA	healthy (174)	various (85)	decreased retinol
Haines	1982	Britain	healthy	lung	decreased retinol
Stahelin	1982	Switzerland	healthy (357)	lung (36) stomach (16) colon (12) other (44)	decreased retinol only in stomach cancer patients
Wald	1984	Britain	healthy (78)	breast (39)	no difference in retinol decreased beta-carotene
Willett	1984	USA	healthy	various	decreased retinol & RBP in GI cancer patients
Nomura	1985	USA	healthy (302)	various	no difference in retinol decreased beta-carotene in lung cancer patients
Salonen	1985	Finland	healthy (51)	GI (18) respiratory (15) other (18)	decreased retinol in GI & respiratory cancer patients

individuals were followed for 12 to 14 years (Kark et al., 1981). Again, serum retinol levels were significantly lower in subjects who developed cancer versus those who did not. The effect was greater for men than for women.

Further prospective studies suggest that the relationship between serum vitamin A levels and cancer development may be site specific. Stahlein et al. (1982) found significantly low serum vitamin A levels only in subjects who subsequently developed stomach cancer. Similarily, no significant association was detected between retinol, RBP or total carotenoid levels in prediagnostic serum and the incidence of cancer of any site other than that of the gastrointestinal system (Willett et al., 1984). In addition, the association between low serum retinol concentrations and an increased cancer risk was found to be significant for respiratory cancer and for all cancers among smokers (Salonen et al., 1985). A weak relationship was also seen for gastrointestinal cancers in this study.

In conflicting reports, Wald et al. (1984) failed to confirm a relationship between plasma retinol and the development of breast cancer. Beta-carotene levels, however, showed a tendency to be lower in women who developed breast cancer than in controls, but the difference was not statistically significant. A ten-year follow-up study of 6,800 Japanese men in Hawaii showed no significant association of serum retinol with any epithelial cancers. However, there was a strong negative association between

lung cancer and beta-carotene (Nomura et al., 1985).

While findings of these prospective serum studies are not consistent, they do suggest that subnormal serum vitamin A, and perhaps beta-carotene, levels precede the development of specific cancers. This relationship appears to be particularily strong for gastrointestinal cancers. Because such prospective studies present many methodological problems, including the necessity of very large numbers of subjects and prolonged follow-up time, the relationship between human cancer development and serum vitamin A has been investigated more extensively through retrospective studies.

The possible protective effect of vitamin A in cancer development has been assessed by comparing levels of vitamin A and related parameters in the blood of cancer patients with those in healthy persons or subjects with non-malignant diseases. The majority of such retrospective studies have shown that serum retinol concentrations are lower in patients with cancer compared to control subjects. Findings of depressed serum beta-carotene levels in cancer patients have also been reported. Retrospective studies investigating the vitamin A-cancer relationship have been summarized in Table 1.3.

Because the relationship between blood vitamin A levels and cancer may be site-specific, many studies have investigated specific neoplastic diseases. In the first study of this type, Abels et al. (1941) measured plasma

TABLE 1.3 RETROSPECTIVE STUDIES RELATING SERUM VITAMIN A AND CANCER.

AUTHOR	YEAR PUBLISHED	COUNTRY	CONTROLS	CANCER SITE	SERUM LEVELS OF PARAMETERS CANCER PATIENTS VS. CONTROLS
Abels	1941	USA	healthy precancerous (47)	GI (51)	decreased retinol & beta-carotene
Wahi	1965	India	hospital patients (60)	oral (409) precancerous oral (127)	decreased retinol
Clifford	1972	Kenya	healthy (53)	nasopharyngeal (17)	decreased retinol
Cohen	1977	USA	standards	lung (67)	no difference in retinol
Ibrahim	1977	Pakistan	healthy (112)	oral (26)	decreased retinol & beta-carotene
Milano	1978	USA	non-cancer patients	colorectal (180)	decreased RBP & prealbumin
Atukorala	1979	Britain	non-malignant lung disease (10) non-lung disease	lung (26)	decreased retinol, beta-carotene, RBP & zinc

TABLE 1.3 CONTINUED

AUTHOR	YEAR PUBLISHED	COUNTRY	CONTROLS	CANCER SITE	SERUM LEVELS OF PARAMETERS CANCER PATIENTS VS. CONTROLS
Lambert	1981	USA	non-cancer patients (10)	precancerous cervix (14)	no difference in retinol or beta-carotene
Lopez	1981	USA	hospital patients (17)	lung (17)	decreased retinol, beta-carotene & RBP
Mellow	1981	USA	non-cancer patients (11)	esophageal (17)	decreased retinol & zinc
Basu	1982	Britain	healthy (30)	myeloma (53) epithelial (2)	decreased retinol in both cancers & decreased RBP in epithelial cancer
Mahmoud	1982	Egypt	healthy (10)	bladder (30)	decreased retinol & beta-carotene
Bichler	1983	Austria	healthy (20)	head & neck (20)	decreased retinol, RBP & prealbumin
Basu	1985	Canada	healthy	postoperative colorectal (103)	decreased retinol & RBP
Orr	1985	USA	standards	cervix (78)	decreased beta-carotene

TABLE 1.3 CONTINUED

AUTHOR	YEAR PUBLISHED	COUNTRY	CONTROLS	CANCER SITE	SERUM LEVELS OF PARAMETERS CANCER PATIENTS VS. CONTROLS
Sawicki	1985	Poland	healthy & non-cancer patients	colon & lung (55)	decreased retinol
Tyler	1985	Britain	healthy	sarcomas (16)	decreased retinol, RBP, prealbumin & carotenoids
				malignant melanomas (15)	decreased carotenoids & prealbumin no difference in retinol or RBP
Flaim	1986	USA	non-cancer patients	various	decreased retinol, RBP & prealbumin
Harris	1986	Britain	healthy (226)	cervix (32)	no difference in retinol or beta-carotene
				precancerous cervix (81)	no difference in retinol decreased beta-carotene

vitamin A levels in 51 patients with gastrointestinal cancer, including that of the esophagus, stomach and rectum. When compared to values from healthy subjects, 86 percent of the cancer patients had subnormal vitamin A concentrations. Patients with precancerous lesions (oral leukoplakia and atrophy of the gastric mucosa) or benign gastrointestinal lesions (ulcers, colitis or sprue), however, did not demonstrate low plasma vitamin A levels. After evaluating diet histories and plasma levels of other vitamins, the authors concluded that in the majority of the cancer patients, depressed plasma vitamin A concentrations could not be attributed to dietary deficiency or malabsorption of the vitamin.

Recent studies have also demonstrated low serum retinol concentrations in patients with colorectal cancer present (Sawicki et al., 1985) and with colorectal cancer surgically removed (Basu et al., 1985). Basu et al. (1985) found that patients with more advanced large bowel tumours, ie. with lymph-node metastases, had significantly lower serum RBP levels than patients with no nodal involvement. When all cancer patients in this study were followed, those patients with subsequent cancer recurrence had initial, post-operative serum retinol and RBP concentrations significantly less than those who remained disease free. It was postulated that the levels of circulating retinol and RBP may have prognostic and predictive value in colorectal carcinoma.

Milano et al. (1978) suggested that the depressed levels of serum RBP and prealbumin seen in patients with colorectal cancer were a consequence of malignancy due to altered protein biosynthesis in the liver. Low concentrations of these transport proteins are not unique to large bowel cancer; the same effect has been seen in patients with a variety of cancers (see Table 3).

An increased requirement for zinc, due to tumour load, may depress serum levels of this mineral in cancer patients. Subnormal zinc concentrations have been detected in the blood of esophageal (Mellow et al., 1981) and lung (Atukorala et al., 1979) cancer patients. If body zinc stores are reduced or depleted in cancer patients, the synthesis of RBP and prealbumin may be inhibited, resulting in the observed low circulating levels of vitamin A.

1.7 CONCLUSION AND PLAN OF PRESENT STUDY

The relationship between vitamin A deficiency and epithelial cancer is well documented. Numerous prospective and retrospective studies of both dietary intake and serum levels have shown an inverse relationship between vitamin A and the incidence of cancer. Whether the depressed serum levels of vitamin A demonstrated in cancer patients are a cause or result of malignant disease is presently unknown.

Several nutritional and physiological factors can influence vitamin A status, however, serum retinol

concentrations are not strongly influenced by dietary vitamin A intake. Because of this, measurement of serum retinol alone provides little information about the cause of depressed serum levels of the vitamin. As low serum retinol concentrations may be related not to vitamin A intake but to protein-energy balance, Tyler and Dickerson (1984) suggested that serum levels of vitamin A transport proteins (RBP and prealbumin) be measured in addition to serum retinol.

Diseases other than cancer are known to affect circulating retinol levels. It is not clear, therefore, whether the depression of vitamin A demonstrated in cancer patients is a result of malignancy or the result of stress and trauma associated with disease in general. In the majority of case-control studies evaluating vitamin A status in cancer patients, healthy subjects or non-specified hospital patients were used as controls. In order to adjust for the general influence of disease, patients with appropriate benign conditions should be used as controls.

Objectives of the present study were as follows:

1) To investigate the status of vitamin A, its transport proteins (RBP and prealbumin) as well as zinc, which is required for synthesis of the proteins, in patients with colorectal cancer.
2) To compare the levels of these parameters in cancer patients with healthy subjects and patients with benign

GI disease. Healthy subjects were used primarily to establish normal values of these parameters using specified methods and laboratory conditions.

3) To investigate the possible effect of surgery on vitamin A status.

4) To evaluate the possible relationship between vitamin A status and prognosis in apparently disease-free, post-operative colorectal cancer patients.

2 METHODOLOGY

2.1 STUDY POPULATIONS

2.1.1 MAYO CLINIC PATIENTS

Serum samples from 41 patients with metastatic colorectal cancer, 40 patients with benign colorectal disease and age and sex-matched healthy controls were obtained from the National Cancer Institute (U.S.) serum sample bank for use in this study. This serum bank was established at the Mayo Clinic in 1972 for provision of sera for the evaluation of potential tumour markers. The serum samples had been donated primarily by patients treated at the Mayo Clinic, after an informed consent was obtained. Serum from this bank was frozen in 1 ml vials and stored at -75 ° C. Samples were shipped, packed in dry ice, to Edmonton and subsequently stored at -70 ° C until analysis of biochemical indices was carried out.

Information concerning age, sex, previous treatment, site of primary tumour, sites of metastatic tumour, date of surgery, date of blood collection, and clinical status of patient at the time of blood collection was withheld until laboratory analysis was completed.

2.1.2 CROSS CANCER INSTITUTE

A total of 134 patients from the Cross Cancer Institute (CCI) in Edmonton, who had previously undergone curative resections of colon and rectal adenocarcinomas, were also included in this study. Of these patients, 75 had Duke's B2 tumours (involving the full thickness of the bowel wall but without lymph node involvement) and 59 had Duke's C tumours (with regional lymph node metastases). These patients were all involved in a Phase III adjuvant trial of chemo-immunotherapy (using methyl-CCNU, plus 5-FU and BCG) versus immunotherapy (BCG alone) as compared to a concurrent control group receiving no adjuvant therapy. Patients were eligible for this trial only if they had not received preoperative radio-, chemo- or immunotherapy within the previous year.

Serum samples were collected from patients within 1 to 6 months postoperatively, before the initiation of the first course of chemo- or immunotherapy in those patients receiving adjuvant treatment. Serial serum samples were collected only from patients who were not receiving any form of adjuvant therapy.

Vials containing 2 ml of serum were frozen at -20 C for a period of 4 to 10 years. These samples were transported from the CCI in February 1986 and subsequently stored at -40 C until laboratory analysis was carried out. The effect of long-term storage of vitamin A is of

particular concern, as undocumented mechanical freezer problems and power failures took place, resulting in thawing of the serum samples. However, investigators have found that in serum samples stored for a period of 14 to 16 years, repeated thawing and refreezing had no detectable effect of vitamin A levels (Kark et al., 1981).

Because the serum samples used in this study were initially collected for determination of carcinoembryonic antigen (CEA), no attempt was made to protect the samples from exposure to light. As vitamin A is extremely sensitive to ultraviolet light, it is likely that levels of the vitamin were reduced during the collection and storage of these serum samples. The aim of this study, though, was not to determine absolute values of vitamin A and its related factors but rather to compare the values from one subgroup of patients within the total sample to values from another subgroup. As all serum samples from the CCI patients were exposed to the same conditions, they were considered to be adequate for the purposes of this study.

2.2 ANALYTICAL METHODS

All biochemical measurements were carried out in blind manner in that the identity and classification of patients was not revealed until laboratory analysis was completed.

As low levels of serum retinol are not strongly influenced by dietary intake of vitamin A, it has been

suggested that the measurement of serum retinol alone provides little information regarding vitamin A status (Tyler & Dickerson, 1984). Thus, in order to assess vitamin A status in healthy and diseased subjects, serum levels of retinol, its transport proteins (RBP and prealbumin) as well as zinc, which is required for synthesis of the proteins, were determined.

2.2.1 SERUM RETINOL DETERMINATION

Because of the sensitivity of vitamin A to light, retinol determination was carried out in a dark room. Serum concentrations of retinol were measured by a modification of the fluorometric method of Hansen & Warwick (1968). These investigators measured the fluorescence of retinol at an excitation wavelength of 340 nm and an emission wavelength of 480 nm. As there appears to be considerable interference from carotenoids at this wavelength (Thompson et al., 1973), it was suggested that fluorescence should be measured at an emission wavelengtn of 550 nm, where interference from carotenoids is absent (Van Stevenick & De Goeij, 1973). Thus, the fluorescence of retinol in this study was measured accordingly at an emission wavelenght of 550 nm.

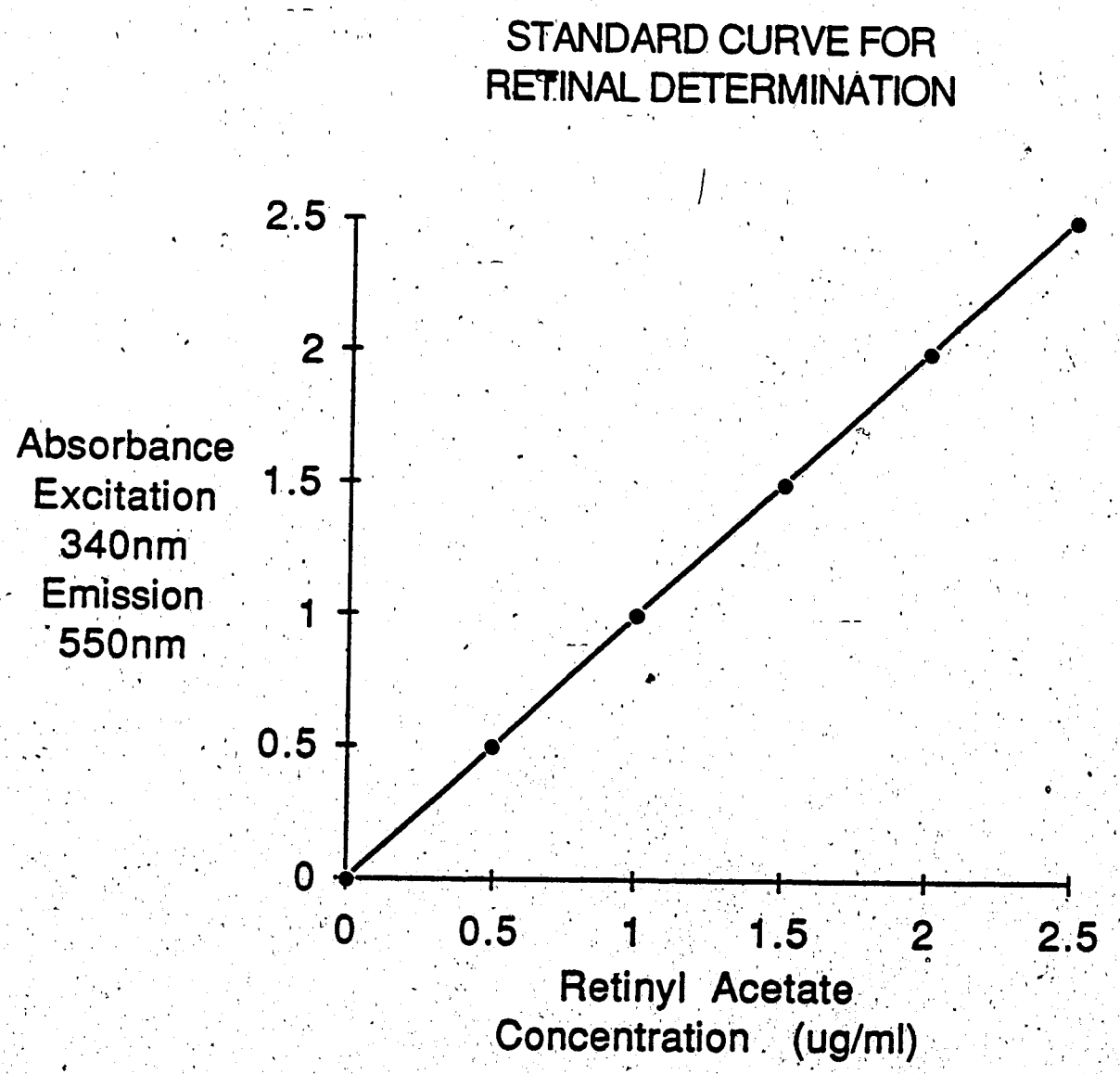
Thompson (1983) has recently argued that the shift in emission wavelength from 480 to 550 nm does not eliminate interference from the fluoresecence of the carotenoid, phytofluene. Phytofluene is a pigment which is commonly

present in fruits and vegetables. Nonetheless, serum retinol levels determined by this modified fluorometric method are significantly correlated with serum RBP concentrations and therefore do reflect vitamin A status (Tyler & Dickerson, 1984). As the intent of this study was not to measure precise retinol levels in individual patients, but to compare the status of vitamin A in different categories of healthy and diseased subjects, this analytical method was thought to be satisfactory.

All trans retinyl acetate (Sigma), diluted in absolute ethanol at a range of 0.5 to 2.5 ug/ml, was used as the standard for vitamin A analysis. As shown in figure 2.1, the intensity of fluorescence increased linearly with the concentration of these standards.

Aliquots (0.2 ml) of serum, distilled water and standard were pipetted into 15 ml Sovril tubes for preparation of patient samples, blank and standards, respectively. All samples and standards were measured in triplicate. After the addition of 1.0 ml of distilled water to each tube, 2.0 ml of absolute ethanol were added slowly, with mixing, to precipitate all serum proteins. Five milliliters of high pressure liquid chromatography (HPLC) grade hexane were added and the tubes were capped with teflon-lined screw caps. The tubes were mixed for 1 minute on a vortex mixer to ensure complete extraction of vitamin A from the alcohol phase to the hexane layer and then centrifuged at 2000 rpm for 5 minutes in a Sorvall RC2-

Figure 2.1


STANDARD CURVE FOR
RETINAL DETERMINATION
Absorbance
Excitation
340nm
Emission
550nm
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
Retinyl Acetate
Concentration (ug/ml)

B centrifuge. The upper hexane layer was removed and pipetted into a fluorometric cuvette for measurement of vitamin A in a Perkin Elmer 650-10S Fluorescence Spectrophotometer. Because retinyl acetate was used as the standard in this analysis, the average value of retinol for each sample was calculated using a conversion factor of 0.3/0.344.

2.2.2 DETERMINATION OF SERUM RBP AND PREALBUMIN

Single radial immunodiffusion, as described by Mancini et al. (1965), was used to measure serum levels of RBP and prealbumin. Standardized human serum for NOR- Partigen (Behring Diagnostics), diluted with physiological saline (0.9%), was used for the preparation of standards for RBP and prealbumin. Behring Diagnostics also supplied human plasma containing 5.5 mg/dl of RBP and 24.5 mg/dl of prealbumin for use as a control.

LC-Partigen and M-Partigen immunodiffusion plates (Behring Diagnostics) were used to determine serum concentrations of RBP and prealbumin, respectively. Each immunodiffusion plate contained 12 wells. Aliquots of 20.0 ml of standard, control or serum sample were pipetted into each well for RBP determination, while 5.0 ml aliquots were pipetted in wells in the prealbumin plates. All samples were measured in duplicate. After filling the wells, the plates were left uncovered for 20 minutes and then covered and left

Figure 2.2

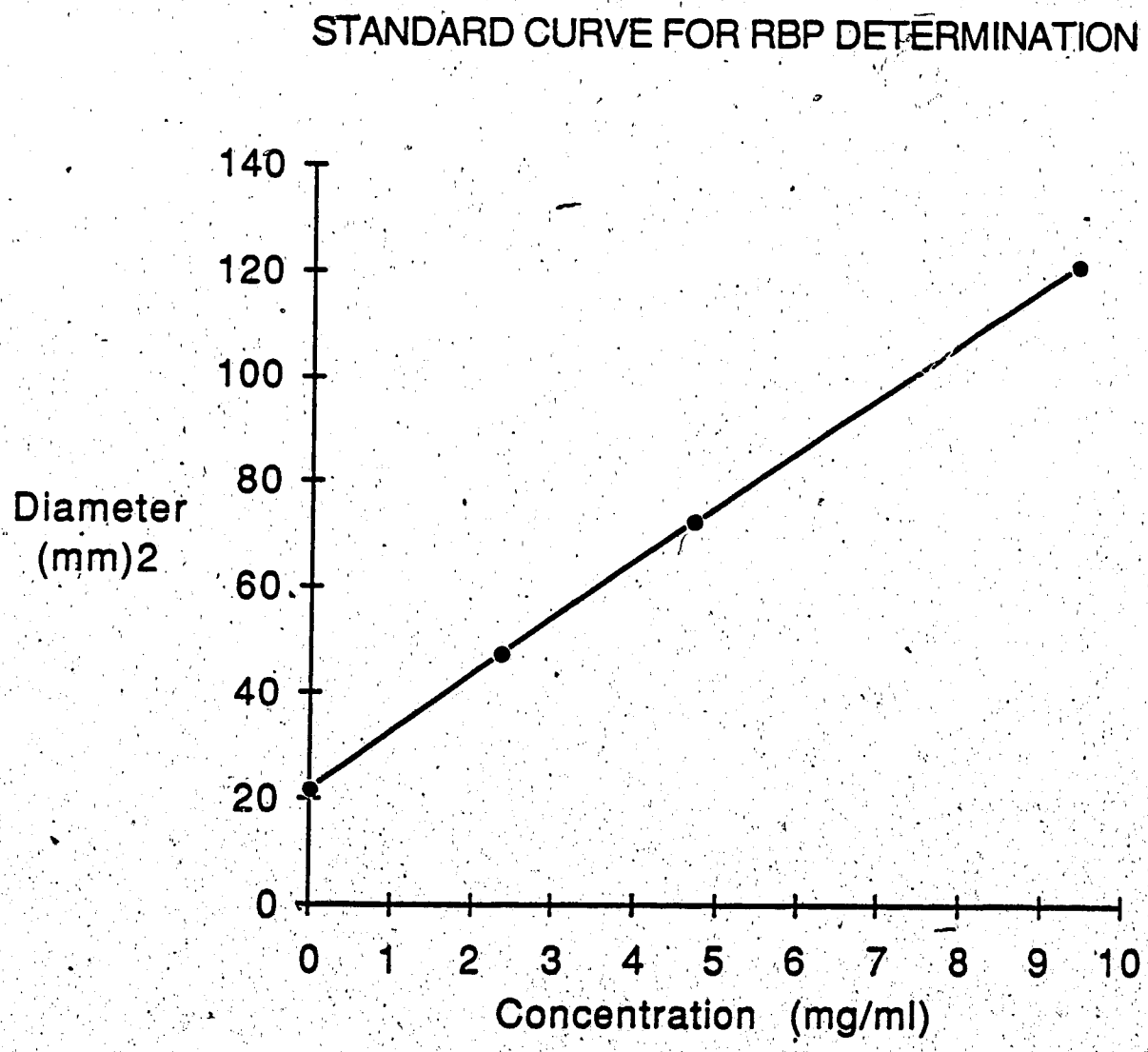
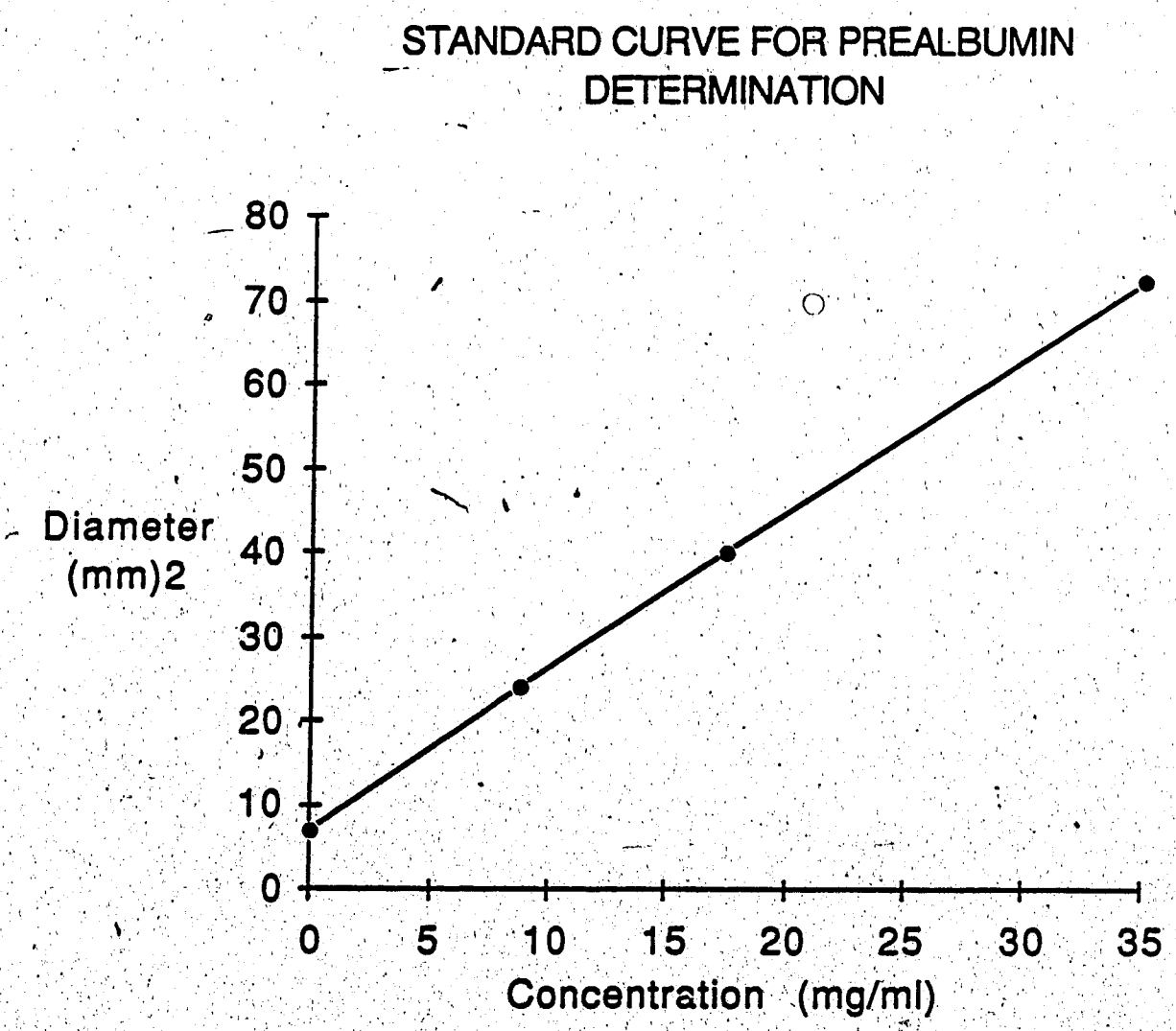

STANDARD CURVE FOR RBP DETERMINATION
Diameter (mm)2
140
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10
Concentration (mg/ml)

Figure 2.3


STANDARD CURVE FOR PREALBUMIN DETERMINATION
Diameter (mm)2
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
Concentration (mg/ml)

to incubate for 72 hours. The diameters of the precipitation rings were measured in a magnifying viewer, made by Behring Company, to an accuracy of 0.1 mm. The sample concentrations of these 2 proteins were obtained directly from the plot of the square of the diameter on the standard curves.

2.2.3 SERUM ZINC DETERMINATION

Zinc concentrations in serum samples were measured by atomic absorption sprectrophotometry (AAS). In order to avoid the interference of the atomic absorption signal by the serum matrix, samples were diluted 1 in 10 with distilled water. Because this dilution procedure reduces the sensitivity of AAS, a slotted quartz tube was used in the AAS. This tube slows the thermal decomposition of zinc in the presence of an air-acetylene flame and thereby increases the residence time of zinc atoms in the light path of the spectrophotometer.

A 1000 ug/ml zinc atomic absorption standard (Aldrich) was used as a stock solution from which standard solutions of 50, 100, 150, 200, and 250 ug/dl were made. Zinc concentrations in serum samples were read directly from the spectrophotometer's SP9 computer which was calibrated using the 5 zinc standards. The following settings were used on the Philip's SP9 AAS:

wavelength 213.9 nm

band pass	0.2 nm
lamp current	10 mA
burner type	10 cm air/acetylene

2.3 STATISTICAL ANALYSIS

All statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSSX, 1986). In order to assess the relationship between vitamin A status and colorectal cancer, means and standard deviations of vitamin A, RBP, prealbumin and zinc were determined for each grouping of patients from the Mayo Clinic and the CCI. The patients were grouped according to age, sex, disease status, treatment received, surgery, and followup status. Conventional statistical analyses (one-way analysis of variance and Student's t-test) were used to detect statistical differences within groups for each of the serum parameters. To rule out the effect of age and sex on levels of the parameters in the CCI patients, multiple regression was used to determine whether or not a significant difference existed in parameters according to groupings of disease status, time lapse between surgery and blood collection, and followup status. A pairwise t-test was used to identify any significant change in serum parameters between serial samplings. The Pearson Correlation Coefficient was used to quantify the strength of the

association between the 4 biochemical indices. In order to assess the ability of these parameters to distinguish between patient groups and survival status of patients, discriminant function analysis was carried out.

3 RESULTS

3.1 MAYO CLINIC SUBJECTS

This study was undertaken to investigate the biochemical status of vitamin A in patients with benign and malignant gastrointestinal disease. The essence of this investigation was to determine the metabolic availability of the vitamin. Since RBP and prealbumin are involved as carriers of vitamin A and zinc is required to synthesize these proteins, the metabolic availabiltiy of vitamin A was assessed by measuring the circulating levels of the carrier proteins and the trace element along with serum retinol.

Subjects included 31 healthy controls, 40 patients with benign GI disease and 41 patients with malignant colorectal disease. Before comparisons of the serum parameters were made between the groups, the subjects within each group were categorized according to their age and sex to determine the influence of these factors on the parameters. Such measures were taken in order to validate the interpretations of the results.

3.1.1 EFFECT OF AGE, SEX AND DIAGNOSTIC GROUP ON VITAMIN A AND ITS RELATED FACTORS

All subjects were divided into age groups as shown in Table 3.1. Statistical analysis using ANOVA failed to detect any significant difference in the serum parameters

TABLE 3.1 EFFECT OF AGE ON SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
HEALTHY SUBJECTS				
1-39 YEARS (3)	110.7 ± 39.3	5.3 ± 1.3	40.2 ± 5.1	122.7 ± 18.9
40-49 YEARS (3)	120.7 ± 46.1	4.4 ± 0.4	33.2 ± 5.8	184.0 ± 31.1
50-59 YEARS (10)	128.2 ± 60.6	4.8 ± 1.1	35.2 ± 6.5	159.9 ± 42.5
60-69 YEARS (13)	132.8 ± 52.4	4.9 ± 0.9	31.5 ± 4.5	149.2 ± 30.2
70-99 YEARS (2)	147.0 ± 9.9	6.6 ± 2.6	36.0 ± 15.4	118.5 ± 20.5
ANOVA SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.
BENIGN GI DISEASE				
1-39 YEARS (6)	76.2 ± 17.9	3.5 ± 1.0	28.0 ± 8.3	116.0 ± 9.2
40-49 YEARS (7)	87.3 ± 39.4	4.6 ± 1.6	34.2 ± 11.6	131.8 ± 31.7
50-59 YEARS (11)	72.5 ± 22.0	3.3 ± 1.1	26.0 ± 7.5	132.4 ± 38.8
60-69 YEARS (10)	96.1 ± 33.0	4.5 ± 0.9	29.9 ± 5.8	141.8 ± 23.8
70-99 YEARS (6)	68.2 ± 30.4	3.9 ± 1.5	25.6 ± 10.9	138.0 ± 11.6
ANOVA SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.
COLORECTAL CANCER				
1-39 YEARS (3)	78.3 ± 24.4	3.5 ± 0.7	26.3 ± 2.4	128.7 ± 30.5
40-49 YEARS (3)	100.7 ± 8.1	5.2 ± 1.6	32.3 ± 8.9	155.7 ± 30.9
50-59 YEARS (10)	94.1 ± 52.6	4.4 ± 1.6	28.5 ± 7.7	146.2 ± 28.1
60-69 YEARS (18)	85.4 ± 37.5	4.5 ± 2.0	27.7 ± 10.4	154.8 ± 33.2
70-99 YEARS (7)	57.4 ± 21.8	3.6 ± 1.3	19.2 ± 7.2	155.2 ± 52.2
ANOVA SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.

ANOVA = analysis of variance.
N.S. = not significant.

between any age group for either healthy or diseased subjects. It should be noted that the number of subjects involved in each group was small. Similarily, the differences in serum parameters between males and females in healthy and patient groups were found to be nonsignificant (Table 3.2). As serum levels of vitamin A and related indices were not found to be influenced by age or sex, subjects within each group were combined together irrespective of age and sex for further statistical analyses.

Table 3.4 shows the comparison of all measured biochemical indices for 21 colon and 20 rectal cancer patients. As no significant differences were detected (vitamin A, $p=0.93$; RBP, $p=0.62$; prealbumin, $p=0.73$; zinc, $p=0.60$), the two diagnostic categories were combined into a single group; colorectal cancer patients.

When comparing serum parameters in subjects with various benign disorders, no statistically significant differences were observed using analysis of variance (Table 3.3). As a result, these subjects were also combined together for further analyses as benign GI disease patients. The small numbers of subjects within each disease category may preclude the detection of any true differences in vitamin A, protein or zinc levels between different benign GI disorders.

TABLE 3.2 EFFECT OF SEX ON SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
HEALTHY SUBJECTS				
MALES (n=13)	126.3 ± 54.2	4.8 ± 1.0	35.1 ± 4.8	160.9 ± 37.4
FEMALES (n=18)	130.8 ± 48.6	5.1 ± 1.2	33.3 ± 7.3	141.2 ± 32.1
SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.
BENIGN GI DISEASE				
MALES (n=11)	82.0 ± 24.6	4.3 ± 1.3	31.1 ± 10.3	134.7 ± 26.0
FEMALES (n=29)	80.5 ± 32.0	3.8 ± 1.3	27.7 ± 8.1	133.6 ± 28.6
SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.
COLORECTAL CANCER				
MALES (n=19)	96.2 ± 43.7	4.6 ± 1.9	29.3 ± 9.7	150.5 ± 40.3
FEMALES (n=22)	72.2 ± 30.6	4.0 ± 1.5	24.4 ± 8.3	150.7 ± 27.6
SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.
TOTAL				
MALES (n=43)	101.7 ± 45.9	4.6 ± 1.5	31.5 ± 8.9	150.3 ± 36.9
FEMALES (n=69)	91.0 ± 43.4	4.2 ± 1.4	28.1 ± 8.5	141.0 ± 29.7
SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

N.S. = not significant.

TABLE 3.3 SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS IN PATIENTS WITH VARIOUS BENIGN GI DISEASES

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
COLON POLYPS (n=9)	84.0 ± 29.0	4.1 ± 1.2	28.8 ± 7.3	136.2 ± 22.1
RECTAL POLYPS (n=1)	102.0	4.9	32.2	141.0
GASTRIC ULCER (n=6)	80.0 ± 17.7	4.2 ± 1.4	34.2 ± 11.2	104.7 ± 17.7
REGIONAL ENTERITIS (n=1)	70.0	4.3	30.5	136.0
ULCERATIVE COLITIS (n=9)	73.9 ± 26.7	3.9 ± 1.1	28.1 ± 7.9	138.2 ± 35.0
DIVERTICULA (n=5)	72.6 ± 30.2	4.0 ± 1.5	28.5 ± 8.4	135.2 ± 28.1
CHRONIC LIVER DISEASE (n=4)	87.0 ± 36.8	2.6 ± 0.8	20.2 ± 4.2	121.7 ± 26.4
CHOLITHIASIS (n=5)	90.4 ± 52.3	4.1 ± 1.7	28.3 ± 12.1	153.8 ± 21.7
TOTAL (n=41)	80.9 ± 29.8	3.9 ± 1.3	28.6 ± 8.7	133.8 ± 27.5
ANOVA SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

ANOVA = analysis of variance.
N.S. = not significant.

TABLE 3.4 EFFECT OF TUMOUR SITE ON SERUM LEVELS OF VITAMIN A, RBP, PREALBUMIN AND ZINC

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
COLON (n=21)	82.9 ± 43.1	4.1 ± 2.0	26.2 ± 10.5	147.5 ± 31.8
RECTUM (n=20)	83.8 ± 35.6	4.4 ± 1.3	27.2 ± 7.8	153.4 ± 35.8
SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

N.S. = not significant.

3.1.2 SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS IN HEALTHY CONTROL SUBJECTS AND PATIENTS WITH EITHER BENIGN GI DISEASE OR COLORECTAL CANCER

Serum concentrations of vitamin A, RBP and prealbumin in healthy control subjects were significantly higher ($p<0.05$) than those of both benign GI and colorectal cancer patients, while zinc levels in healthy controls were significantly higher ($p<0.05$) than those of benign GI patients only. However, no significant differences were detected between benign GI and colorectal cancer patients for any of the biochemical indices (Table 3.5). Of the four parameters, the difference in average values between healthy subjects and patients was greatest for vitamin A. The healthy control subjects' average vitamin A level was 157% that of the patients'.

Frequency charts, given in Figures 3.1 to 3.4, display individual values for the serum parameters from healthy subjects and patient groups. The serum retinol levels for benign and malignant GI patients averaged approximately 80 ug/dl with a range of 12 to 154 ug/dl and 32 to 195 ug/dl, respectively (Figure 3.1). In the healthy subjects the average value was 130 ug/dl with a range of 55 to 259 ug/dl. Although the serum values for the vitamin in the three groups overlapped each other to a considerable extent, 17.5% of benign GI and 19.5% of colorectal cancer patients were found to have values which were less than the lowest value observed in the healthy patients.

TABLE 3.5 SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS IN HEALTHY CONTROL SUBJECTS AND PATIENTS WITH EITHER BENIGN GI DISEASE OR COLORECTAL CANCER

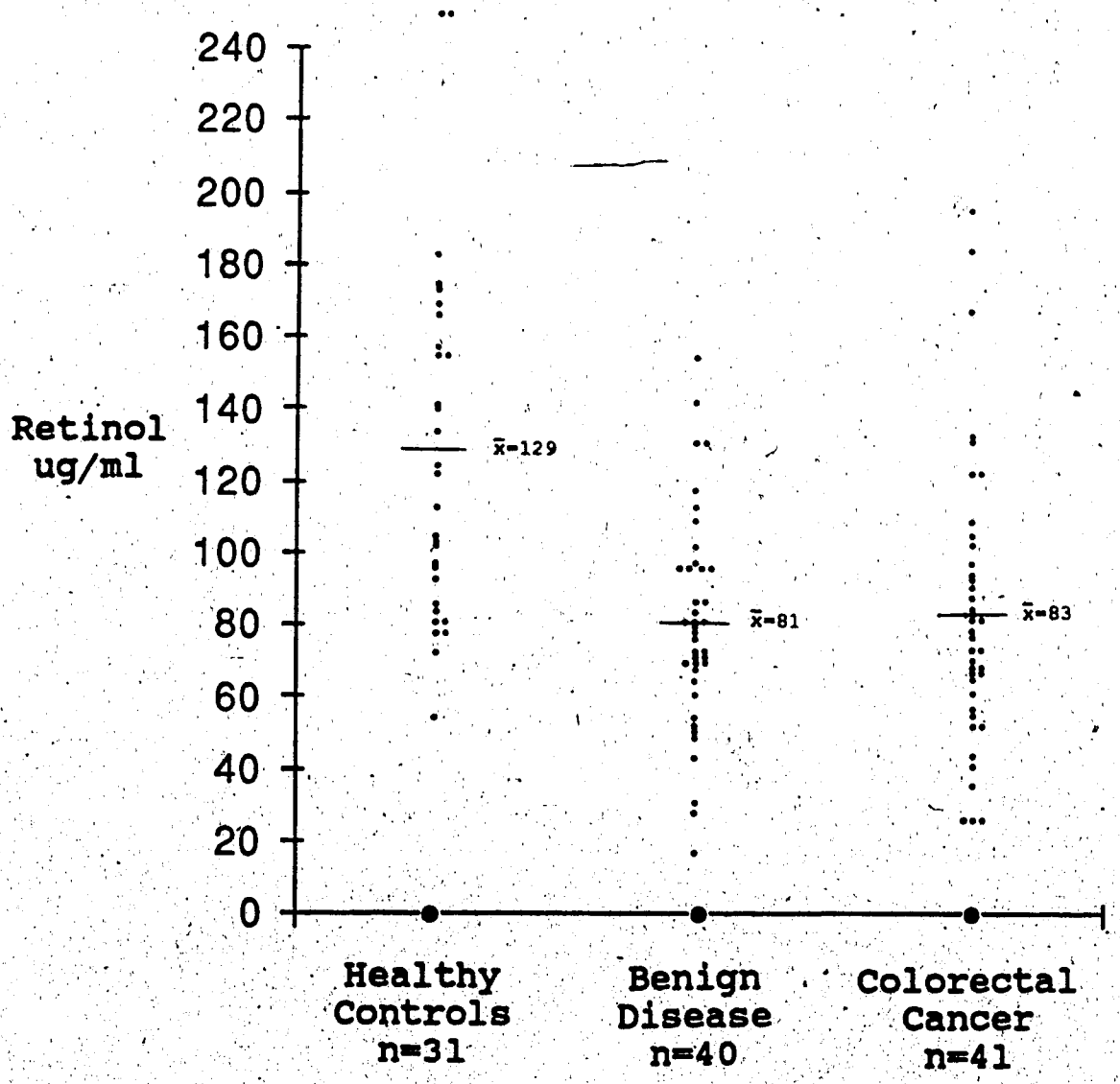
GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
HEALTHY SUBJECTS (n=31)	128.9 ± 50.2	5.0 ± 1.1	34.1 ± 6.4	150.1 ± 35.4
BENIGN GI DISEASE (n=40)	80.9 ± 29.8*	3.9 ± 1.3*	28.6 ± 8.7*	133.8 ± 27.5*
COLORECTAL CANCER (n=41)	83.3 ± 38.7*	4.3 ± 1.7*	26.7 ± 9.2*	150.6 ± 33.7

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

*Significantly different from the controls, $p < 0.05$.

Figure 3.1

DISTRIBUTION OF SERUM VITAMIN A VALUES IN HEALTHY SUBJECTS AND PATIENTS WITH EITHER BENIGN OR MALIGNANT GI DISEASE



The average circulating RBP level in the healthy group was 5.0 mg/dl with a range of 3.1 to 10.45 mg/dl (Figure 3.2). Benign GI patients had a mean RBP value of 3.9 mg/dl with a range of 1.95 to 6.80 mg/dl, while the colorectal cancer patients had a mean value of 4.3 mg/dl with a range of 1.85 to 9.70 mg/dl. The lowest serum RBP level from the healthy subjects (3.1 mg/dl) was greater than 30.0% and 19.5% percent of values from the benign and malignant patients, respectively. Similarily, figure 3.3 shows that prealbumin concentrations from 17.5% of benign subjects and 24.4% of colorectal cancer patients were below the lowest value detected in the healthy subjects (20.8 mg/dl).

A virtually identical mean zinc level was detected between healthy subjects and colorectal cancer patients (150.1 versus 150.6), although the overall range of values was somewhat greater for the colorectal cancer patients. Benign GI patients had the narrowest range of serum zinc levels (90.1 to 210.0 mg/dl) with an average value of 133.8 mg/dl. Interestingly, little difference was seen in the low values for zinc between the healthy and patient groups.

3.1.3 EFFECT OF ADJUVANT TREATMENT ON SERUM LEVELS OF VITAMIN A, RBP, PREALBUMIN AND ZINC

Of the 41 colorectal cancer patients being investigated, 22 were undergoing chemo-, immuno- and/or radiotherapy during the time period when their blood was

Figure 3.2

DISTRIBUTION OF SERUM RBP VALUES IN HEALTHY SUBJECTS AND PATIENTS WITH EITHER BENIGN OR MALIGNANT GI DISEASE

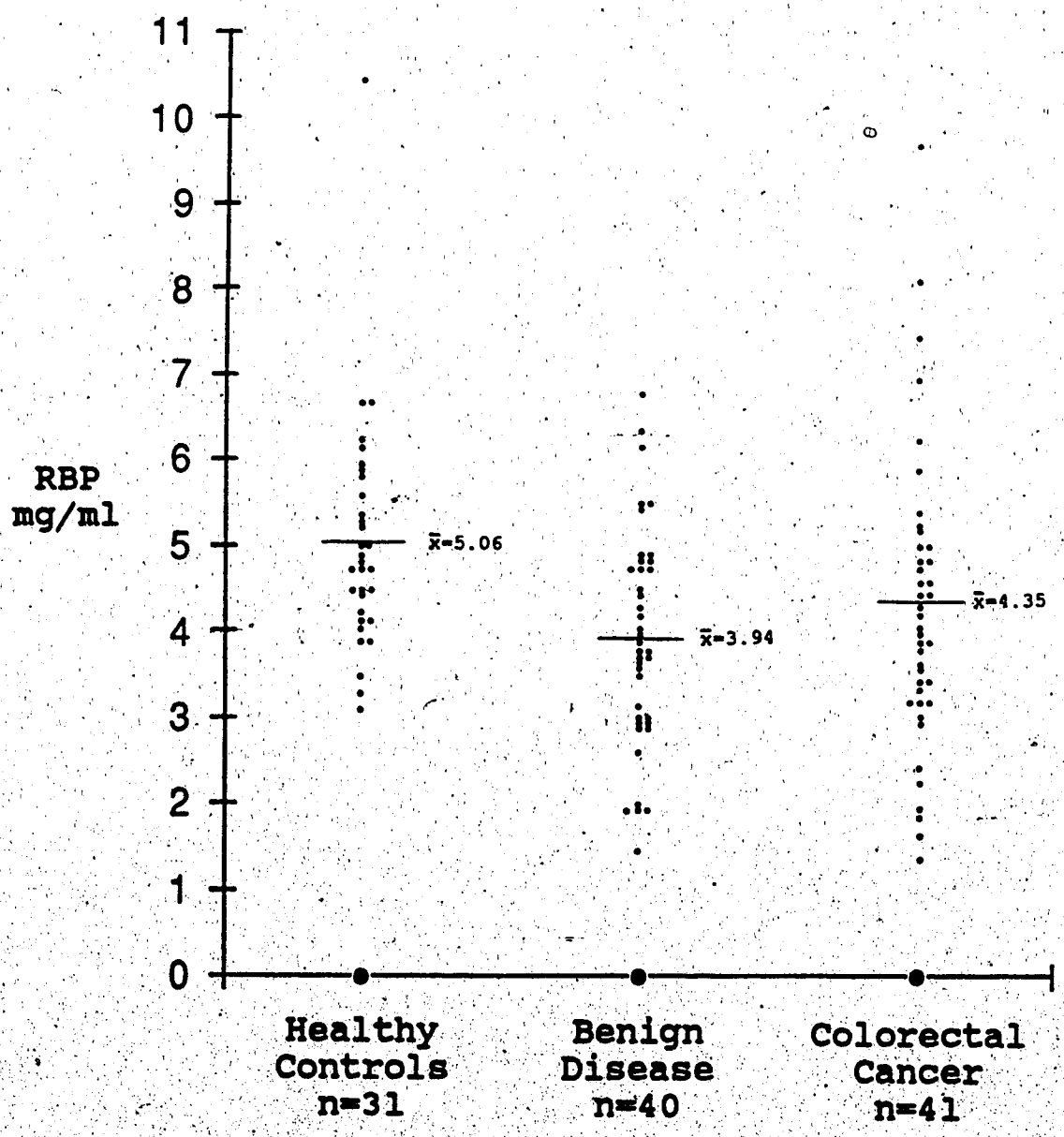


Figure 3.3

DISTRIBUTION OF SERUM PREALBUMIN VALUES IN HEALTHY SUBJECTS AND PATIENTS WITH EITHER BENIGN OR MALIGNANT GI DISEASE

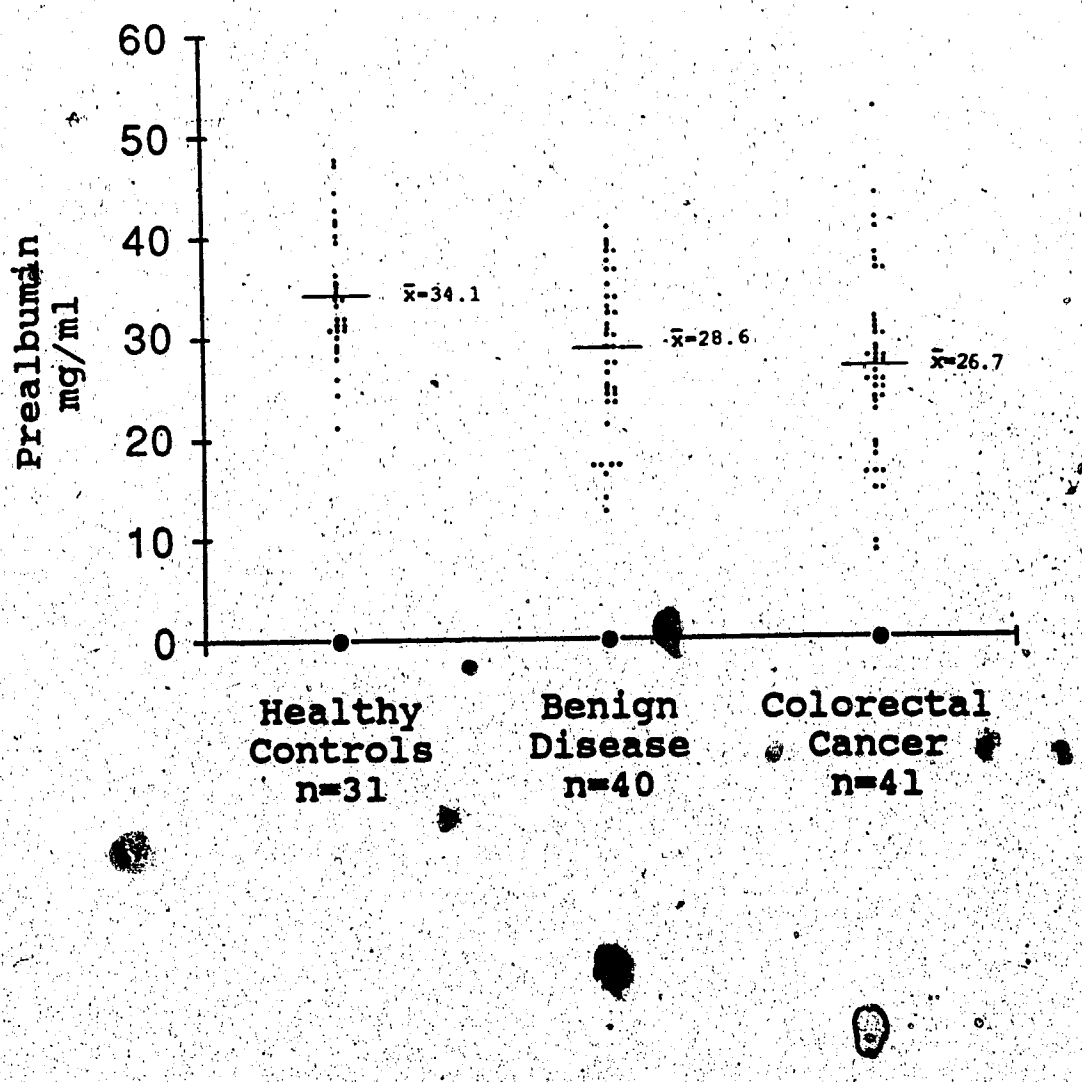
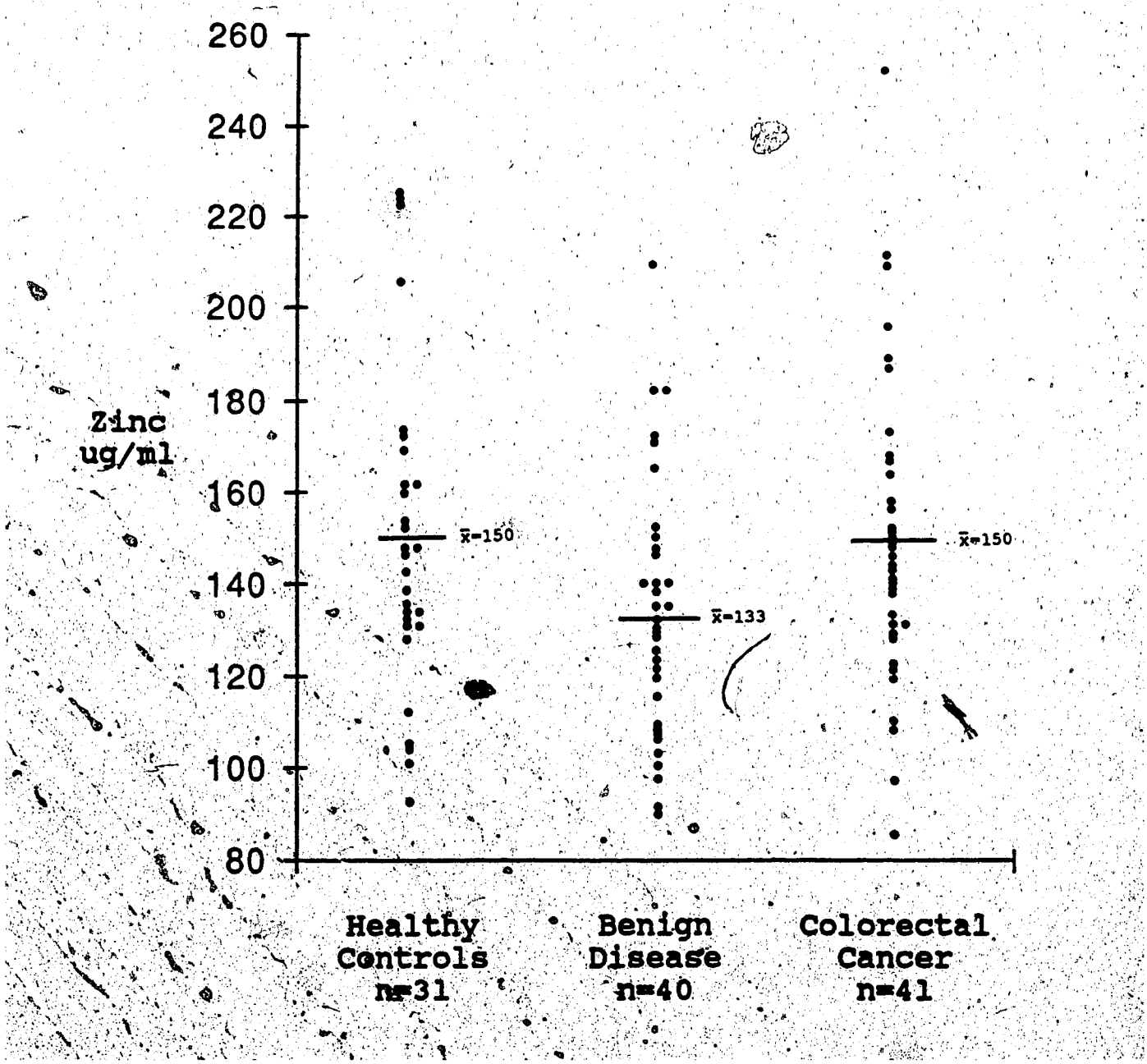


Figure 3.4

DISTRIBUTION OF SERUM ZINC VALUES IN HEALTHY SUBJECTS AND PATIENTS WITH EITHER BENIGN OR MALIGNANT GI DISEASE



collected for biochemical analysis. In order to determine if these adjuvant treatments had any effect on serum vitamin A status, patients were grouped according to the type of cancer therapy that they were receiving, as shown in table 3.6. Analysis of variance showed that no statistically significant differences existed between these groups for vitamin A ($p=0.54$), RBP ($p=0.11$), prealbumin ($p=0.23$) or zinc ($p=0.98$).

3.1.4 EFFECT OF SURGERY ON VITAMIN A AND ITS RELATED FACTORS

Approximately 46% of colorectal cancer and 65% of benign GI patients underwent gastrointestinal surgery. In order to determine if surgery had any effect on the serum biochemical indices, these were compared between the patients who did not have surgery and the patients who had surgery (Table 3.7). There appeared to be a trend towards decreased serum levels of all parameters in the post-operative patients, the difference being most marked for vitamin A and zinc. Statistical analysis did not show any significant differences in these biochemical indices (vitamin A, $P=0.15$; RBP, $p=0.47$; prealbumin, $p=0.72$; zinc, $p=0.06$). But, when the benign and malignant groups were analyzed separately, serum zinc levels were significantly lower in post-operative colorectal cancer patients versus colorectal cancer patients who did not undergo surgery

TABLE 3.6 EFFECTS OF ADJUVANT TREATMENT ON SERUM LEVELS OF VITAMIN A, RBP, PREALBUMIN AND ZINC

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
NO TREATMENT (19)	78.2 ± 30.2	3.8 ± 1.1	24.3 ± 7.1	149.9 ± 40.9
CHEMOTHERAPY (7)	107.6 ± 57.2	5.5 ± 2.5	30.8 ± 13.0	153.8 ± 29.2
IMMUNOTHERAPY (1)	87.0	3.6	23.7	144.0
CHEMO-IMMUNOTHERAPY (1)	44.0	1.4	9.2	165.0
RADIOTHERAPY (5)	79.6 ± 52.9	4.1 ± 1.3	28.1 ± 8.1	143.6 ± 40.7
RADIO-IMMUNOTHERAPY (3)	69.7 ± 39.2	5.1 ± 2.5	32.7 ± 14.3	135.5 ± 7.8
RADIO-CHEMOTHERAPY (2)	115.0 ± 9.9	6.1 ± 1.8	34.4 ± 3.9	149.0 ± 2.8
RADIO-IMMUNO-CHEMOTHERAPY (3)	70.3 ± 8.3	3.9 ± 0.7	25.8 ± 1.3	169.3 ± 18.0
ANOVA SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

ANOVA = analysis of variance.
N.S. = not significant.

TABLE 3.7 EFFECTS OF SURGERY ON VITAMIN A, RBP, PREALBUMIN AND ZINC

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
COLORECTAL CANCER				
NO SURGERY (n=22)	89.7 ± 37.9	4.4 ± 1.6	27.4 ± 8.4	163.1 ± 32.3
SURGERY (n=19)	75.9 ± 39.3	4.1 ± 1.9	25.9 ± 10.2	136.7 ± 30.2*
BENIGN GI DISEASE				
NO SURGERY (n=14)	85.9 ± 20.2	3.9 ± 1.0	29.3 ± 7.3	129.1 ± 33.5
SURGERY (n=26)	78.2 ± 33.9	3.9 ± 1.4	28.3 ± 9.5	136.8 ± 23.5
COLORECTAL CANCER AND BENIGN GI DISEASE				
NO SURGERY (n=36)	88.2 ± 31.9	4.2 ± 1.4	28.1 ± 7.9	149.3 ± 36.4
SURGERY (n=43)	77.1 ± 36.7	4.0 ± 1.6	27.4 ± 9.9	134.7 ± 24.9

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

*Significantly different from non-surgery patients, p = 0.02.

($p<0.02$).

3.1.5 EFFECT OF TIME LAPSE IN BETWEEN SURGERY AND BLOOD SAMPLE COLLECTION ON SERUM PARAMETERS

All patients who underwent gastrointestinal surgery for either a benign or malignant condition were divided into two groups according to the time interval between the date of surgery and the date of blood sample collection (< 1 month versus < 2 months). While no statistically significant differences were observed for any of the biochemical indices between the two periods of time (Table 3.8), samples collected less than one month after surgery had lower mean serum vitamin A, RBP and zinc levels than those collected approximately one month later (mean difference = 17.1 ug/dl, 0.37 mg/dl and 29.8 ug/dl respectively). The average serum prealbumin concentration appeared to be higher in the patient group whose blood was collected within one month after surgery. It should be pointed out that the validity of this comparison is questionable as only 3 subjects had blood collected more than one month after surgery.

Because more patients with benign disease had surgery than did cancer patients and because surgery appeared to have an effect on the serum parameters, benign and malignant GI disease patients were compared after adjusting for the effect of surgery using two-way analysis of variance. The differences in serum levels of vitamin A, RBP and prealbumin between these two patient groups still were not significant.

TABLE 3.8 EFFECT OF TIME LAPSE IN BETWEEN SURGERY AND BLOOD SAMPLE COLLECTION ON SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
< 1 MONTH (n=40)	75.6 ± 36.9	3.9 ± 1.6	27.3 ± 10.2	133.9 ± 24.9
< 2 MONTHS (n=3)	92.7 ± 35.0	4.3 ± 0.8	22.9 ± 4.1	163.7 ± 39.5
SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.

Each value represents the mean ± S.D. for the number of subjects in parenthesis.

N.S. = not significant.

However, the mean circulating level of zinc was significantly lower in the subjects with benign disease versus colorectal cancer ($p<0.02$), after adjusting for the effect of surgery

3.1.6 EFFECT OF CANCER RECURRENCE ON VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS

All colorectal cancer patients from the Mayo Clinic had distant metastases at the time of blood sample collection (See Table 3.10 for the metastatic sites). In order to determine whether the extent of cancer recurrence effected the biochemical parameters, cancer patients were grouped by their number of distant metastases. Those patients with only one metastasis had higher serum vitamin A, RBP, and prealbumin values than did patients with two or more metastatic sites, although these differences did not reach a significant level (Table 3.9). Patient groups with one or more than one metastatic metastases had identical mean serum zinc levels.

One-way analysis of variance showed no significant differences in the levels of vitamin A and its related factors in patients with different metastatic sites (Table 3.10). Of the 41 colorectal cancer patients, 45% developed liver and 20% developed lung metastases. The remaining patients developed recurrences in the peritoneum (10%), connective tissue (10%), ovary (5%), kidney (5%), and

TABLE 3.9 EFFECT OF RECURRENCE ON VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
ONE METASTASIS (n=26)	88.3 ± 38.1	4.6 ± 1.7	28.1 ± 8.7	150.6 ± 26.9
MORE THAN ONE METASTASES (n=15)	74.8 ± 39.6	3.6 ± 1.7	24.3 ± 9.9	150.5 ± 44.5
SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

N.S. = not significant.

TABLE 3.10 EFFECT OF SITE OF RECURRENCE ON VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
LIVER (n=18)	92.5 ± 44.4	4.1 ± 1.7	26.4 ± 8.7	154.4 ± 27.1
PERITONEUM (n=4)	64.7 ± 30.1	3.4 ± 1.0	22.0 ± 6.7	130.0 ± 21.1
LUNG (n=8)	90.1 ± 40.0	4.9 ± 2.1	29.9 ± 10.9	154.4 ± 45.6
CONNECTIVE TISSUE (n=4)	90.3 ± 25.4	5.2 ± 1.6	29.6 ± 6.0	174.2 ± 32.1
OVARY (n=2)	31.0 ± 7.1	2.0 ± 0.5	12.4 ± 5.4	86.0 ± 20.9
KIDNEY (n=2)	75.5 ± 7.8	4.9 ± 0.4	27.8 ± 12.4	159.3 ± 53.0
LYMPH (n=3)	66.0 ± 14.5	4.5 ± 1.5	31.0 ± 9.0	134.3 ± 12.4
ANOVA SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

ANOVA = analysis of variance.
N.S. = not significant.

lymphatic system (7.5%).

3.1.7 CORRELATIONS BETWEEN ASSAY MEASUREMENTS

The relationships between serum vitamin A, RBP, prealbumin and zinc are shown in table 3.11. Vitamin A was significantly correlated with RBP and prealbumin as was RBP with prealbumin for all healthy and patient groups. A statistically significant negative relationship existed between RBP and zinc in the healthy subjects while a positive correlation reaching statistical significance was seen between these two parameters in the benign GI disease patients. RBP was significantly correlated with zinc in the benign patient group only.

3.1.8 USE OF SERUM LEVELS OF VITAMIN A, RBP, PREALBUMIN AND ZINC AS PROGNOSTIC INDICATORS

Discriminant function analysis was carried out in order to determine if the values of the four serum parameters, used together, were able to distinguish between healthy subjects and patients with either benign or malignant GI disease. Standardized canonical discriminant function coefficients for the biochemical indices are shown in Table 3.12. Both functions 1 and 2 from this model were found to be statistically significant ($p < 0.0001$ and $p = 0.002$, respectively). Using these canonical discriminant

TABLE 3.11 CORRELATIONS BETWEEN ASSAY MEASUREMENTS

	PEARSON'S CORRELATION COEFFICIENT (r)	p-value
HEALTHY SUBJECTS (31)		
VIT-A WITH RBP	0.60	< 0.0001
VIT-A WITH PRE-A	0.35	0.027
VIT-A WITH ZINC	-0.29	0.061
RBP WITH PRE-A	0.78	< 0.0001
RBP WITH ZINC	-0.36	0.025
PRE-A WITH ZINC	-0.20	0.155
BENIGN GI DISEASE (40)		
VIT-A WITH RBP	0.75	< 0.0001
VIT-A WITH PRE-A	0.70	< 0.0001
VIT-A WITH ZINC	0.40	0.009
RBP WITH PRE-A	0.96	< 0.0001
RBP WITH ZINC	0.44	0.005
PRE-A WITH ZINC	0.20	0.129
COLORECTAL CANCER (40)		
VIT-A WITH RBP	0.78	< 0.0001
VIT-A WITH PRE-A	0.76	< 0.0001
VIT-A WITH ZINC	0.20	0.12
RBP WITH PRE-A	0.92	< 0.0001
RBP WITH ZINC	0.09	0.29
PRE-A WITH ZINC	0.09	0.31
TOTAL		
VIT-A WITH RBP	0.71	< 0.0001
VIT-A WITH PRE-A	0.64	< 0.0001
VIT-A WITH ZINC	0.10	0.167
RBP WITH PRE-A	0.88	< 0.0001
RBP WITH ZINC	0.11	0.133
PRE-A WITH ZINC	0.07	0.232

TABLE 3.12 STANDARDIZED CANONICAL DISCRIMINANT FUNCTION COEFFICIENTS FOR SERUM VALUES OF VITAMIN A, RBP, PREALBUMIN AND ZINC FROM MAYO CLINIC SUBJECTS.

	FUNCTION 1	FUNCTION 2
VITAMIN A	1.03553	0.24494
RBP	-1.53102	1.49083
PREALBUMIN	1.31485	-1.31665
ZINC	0.05019	0.56618

functions, 56.6% of cases were correctly classified into healthy, benign GI disease or colorectal cancer categories.

3.2 CROSS CANCER INSTITUTE SUBJECTS.

One hundred and thirty four subjects were followed for a time period of 4 to 107 months (average= 56.5 months) after undergoing curative surgery for cancer of the colon or rectum. Serum levels of vitamin A, RBP, prealbumin and zinc were measured in postoperative blood samples in order to determine whether these biochemical indices could predict the survival outcome of the colorectal patients.

When analyzing all postoperative cancer patients together, no statistically significant differences in serum levels of vitamin A and its related factors were seen between patients with colon tumours versus rectal tumours (Table 3.13). Consequently, colon and rectal cancer patients were combined together for further analysis.

3.2.1 EFFECT OF AGE AND SEX ON SERUM LEVELS OF VITAMIN A AND ITS RELATED FACTORS

In order to assess the effect of age on circulating levels of vitamin A and its related biochemical indices, patients were grouped by age as shown in table 3.14. Using analysis of variance, no significant differences were detected between the age categories for any of the measured

TABLE 3.13 EFFECTS OF TUMOUR SITE ON SERUM LEVELS OF VITAMIN Z, RBP, PREALBUMIN AND ZINC.

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
COLON CANCER (n=76)	85.2 ± 30.6	5.5 ± 1.8	36.4 ± 9.5	129.8 ± 34.3
RECTAL CANCER (n=58)	79.5 ± 22.0	5.3 ± 1.5	36.6 ± 9.2	122.3 ± 26.3
SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

N.S. = not significant.

TABLE 3.14 EFFECT OF AGE ON SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC.

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
DUKE'S B2				
1-39 YEARS (n=3)	111.3 ± 17.2	5.1 ± 0.7	36.7 ± 3.5	110.5 ± 17.7
40-49 YEARS (n=8)	85.1 ± 37.2	6.3 ± 1.7	44.1 ± 11.1	139.0 ± 43.2
50-59 YEARS (n=35)	83.7 ± 29.1	5.5 ± 1.5	37.4 ± 9.0	132.3 ± 31.0
60-69 YEARS (n=23)	85.4 ± 23.0	5.4 ± 2.0	35.8 ± 8.8	126.3 ± 19.8
70-99 YEARS (n=6)	97.5 ± 32.2	7.0 ± 1.3	45.1 ± 8.4	164.7 ± 60.3
SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.
DUKE'S C				
1-39 YEARS (n=5)	69.8 ± 29.2	4.1 ± 1.4	31.4 ± 8.2	102.8 ± 7.2
40-49 YEARS (n=12)	65.2 ± 23.8	4.5 ± 1.5	32.3 ± 9.4	137.3 ± 33.7
50-59 YEARS (n=18)	86.2 ± 22.9	5.8 ± 1.5	38.7 ± 9.5	113.1 ± 19.1*
60-69 YEARS (n=16)	75.9 ± 22.6	5.3 ± 2.1	32.0 ± 7.9	117.3 ± 24.1*
70-99 YEARS (n=8)	86.5 ± 32.5	5.1 ± 1.5	35.7 ± 5.9	106.9 ± 20.7*
SIGNIFICANCE	N.S.	N.S.	N.S.	p = 0.03
TOTAL				
1-39 YEARS (n=8)	85.4 ± 32.2	4.5 ± 1.3	33.4 ± 7.0	105.3 ± 10.5
40-49 YEARS (n=20)	73.2 ± 30.6	5.2 ± 1.8	37.0 ± 11.5	138.0 ± 36.9
50-59 YEARS (n=53)	84.5 ± 26.9	5.6 ± 1.5	37.8 ± 9.1	125.8 ± 28.9
60-69 YEARS (n=39)	81.5 ± 23.0	5.4 ± 2.0	34.3 ± 8.5	122.9 ± 21.7
70-79 YEARS (n=14)	91.2 ± 31.6	5.9 ± 1.7	38.7 ± 9.6	131.6 ± 50.1
SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

*Significantly different from the 40-49 year age group, $p < 0.05$.
N.S. = not significant.

parameters in patients with Duke's B2 colorectal cancer (vitamin A, p=0.46; RBP, p=0.19; prealbumin, p=0.07; zinc, p=0.11). While no statistically significant differences were detected for vitamin A (p=0.17), RBP (p=0.16) or prealbumin (p=0.16) between the different age groups in Duke's C patients, serum zinc levels were significantly lower in these patients over the age of 50 as compared to those in the 40 to 49 year age group.

It was of interest that in contrast to findings from the Mayo Clinic, there did appear to be a sex difference in the patients from the Cross Cancer Institute. Serum values of all four parameters were found to be lower in females as compared to males (Table 3.15). When evaluating all colorectal cancer patients together, sex differences reached a statistically significant level for RBP, prealbumin and zinc. The effect of sex on serum parameters was, however, more pronounced in the patients with Duke's C colorectal cancer.

Because of the differences in serum biochemical indices between age groups and sex groups detected in this patient population, the effects of these two factors were adjusted for in further statistical analysis.

TABLE 3.15 EFFECT OF SEX ON SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS.

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
DUKE'S B2				
MALES (n=43)	84.4 ± 27.7	5.9 ± 1.8	40.0 ± 9.5	137.0 ± 33.6
FEMALES (n=32)	89.6 ± 29.0	5.3 ± 1.4	35.8 ± 8.5	128.2 ± 32.9
SIGNIFICANCE	N.S.	N.S.	0.05	N.S.
DUKE'S C				
MALES (n=24)	83.7 ± 24.1	5.9 ± 1.3	39.3 ± 7.5	126.9 ± 23.1
FEMALES (n=35)	73.7 ± 26.0	4.7 ± 1.8	30.9 ± 8.3	111.3 ± 25.1
SIGNIFICANCE	N.S.	p = 0.003	< 0.0001	p = 0.02
TOTAL				
MALES (n=67)	84.1 ± 26.3	5.9 ± 1.7	39.8 ± 8.8	133.5 ± 30.6
FEMALES (n=67)	81.3 ± 28.4	5.0 ± 1.6	33.2 ± 8.7	119.5 ± 30.1
SIGNIFICANCE	N.S.	p = 0.001	p < 0.0001	p = 0.01

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

N.S. = not significant.

3.2.2 SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS IN DUKE'S B2 AND DUKE'S C POSTOPERATIVE COLORECTAL CANCER PATIENTS

Serum levels of all parameters measured were lower in Duke's C versus Duke's B2 colorectal cancer patients (Table 3.16). After adjusting for the effect of age and sex, however, these differences reached statistically significant levels only for zinc ($p=0.01$). Probability values determined by the Student's t-test almost reached statistically significant levels for vitamin A ($p=0.09$) and prealbumin ($p=0.07$).

3.2.3 EFFECT OF TIME LAPSE IN-BETWEEN SURGERY AND SUBSEQUENT BLOOD SAMPLE COLLECTION ON SERUM VITAMIN A AND ITS RELATED FACTORS

Patients were grouped by time interval from the date of surgery to the date of blood sample collection (Table 3.17). The majority (63.4%) of blood samples were collected between 1 and 2 months after surgery. After adjustments had been made for the influence of age and sex, a significant difference between the different time periods was detected for both vitamin A ($p=0.05$) and prealbumin ($p=0.05$). The lowest average values of these two parameters were found in samples collected less than one month after surgery while the highest average values were found in samples collected more than 6 months after surgery. A similar trend was demonstrated for RBP and zinc, with the samples collected

TABLE 3.16 SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS IN DUKE'S B2 AND DUKE'S C POSTOPERATIVE COLORECTAL CANCER PATIENTS

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (µg/dl)
DUKE'S B2 (n=75)	86.6 ± 28.1	5.6 ± 1.7	38.2 ± 9.3	133.2 ± 33.4
DUKE'S C (n=59)	77.8 ± 25.5	5.2 ± 1.7	34.3 ± 9.0	117.5 ± 25.3
AGE AND SEX ADJUSTED SIGNIFICANCE	N.S.	N.S.	N.S.	p = 0.01

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

N.S. = not significant.

TABLE 3.17 EFFECT OF TIME LAPSE IN BETWEEN SURGERY AND SUBSEQUENT BLOOD SAMPLE COLLECTION ON SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC.

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
< 1 MONTH (n=15)	63.5 ± 22.0	5.3 ± 2.9	33.0 ± 11.9	129.2 ± 33.4
< 2 MONTHS (n=58)	81.0 ± 23.5	5.4 ± 1.5	36.0 ± 8.5	127.9 ± 33.8
< 3 MONTHS (n=27)	88.0 ± 28.7	5.3 ± 1.4	35.9 ± 9.2	116.9 ± 21.2
< 4 MONTHS (n=19)	97.0 ± 28.5	5.7 ± 1.6	39.7 ± 8.6	127.8 ± 35.1
< 6 MONTHS (n=12)	75.8 ± 19.2	5.4 ± 1.7	37.0 ± 10.3	132.8 ± 28.2
> 6 MONTHS (n=3)	101.7 ± 63.5	6.9 ± 1.1	45.6 ± 7.9	139.3 ± 26.5
AGE AND SEX ADJUSTED SIGNIFICANCE	p = 0.05	N.S.	p = 0.05	N.S.

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

N.S. = not significant.

more than 6 months after surgery having the highest values. However, the differences in mean serum values between the time periods did not reach statistical significance for either RBP ($p=0.42$) or zinc ($p=0.93$).

3.2.4 SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS IN PATIENTS WITH SERIAL BLOOD SAMPLING

Of the 134 patients, 35 had serial samples taken at two different periods of time. The time interval between the first and second samples ranged from 15 to 137 days with an average time difference of 79.4 days. Table 3.18 shows that the average mean serum levels of all four biochemical indices were higher in the second sample, although the difference between average values in the first and second samples reached a level of significant difference only for vitamin A ($p=0.03$).

3.2.5 SERUM VITAMIN A STATUS IN PATIENTS REMAINING DISEASE-FREE VERSUS PATIENTS WITH SUBSEQUENT CANCER RECURRENCE OR CANCER-DEATH FOLLOWING SURGERY

During the postoperative period, which averaged 4.7 years, 32.0% of Duke's B2 and 65.9% of Duke's C colorectal cancer patients subsequently developed a recurrence of cancer (Table 3.19). Currently, 21.3% of Duke's B2 and 50.8% of Duke's C patients have died of cancer.

The survival rate for all apparently disease-free

TABLE 3.18 SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS IN PATIENTS WITH SERIAL BLOOD SAMPLING.

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
SAMPLE 1 (n=35)	77.9 ± 22.4	5.3 ± 1.6	36.0 ± 8.9	122.5 ± 25.9
SAMPLE 2 (n=35)	88.6 ± 25.4	5.6 ± 1.5	37.3 ± 7.6	126.3 ± 30.2
PAIRWISE T-TEST 2-TAIL PROBABILITY	0.03	N.S.	N.S.	N.S.

Each value represents the mean ± S.D. for the number of subjects in parenthesis.

N.S. = not significant.

TABLE 3.19 DISEASE FREE SURVIVAL OF POST-OPERATIVE COLORECTAL CANCER PATIENTS.

	DUKE'S B2	DUKE'S C	TOTAL
DISEASE-FREE	51 (68.0%)	26 (44.1%)	77
CANCER RECURRENCE	8 (10.7%)	3 (5.1%)	11
DEATH FROM RECURRENCE	16 (21.3%)	30 (50.8%)	46
TOTAL	75	59	134

patients included in this study is shown in figure 3.5. The survival rate was calculated from the date of surgery to either the date of cancer recurrence or death.

Table 3.20 shows that the mean levels of all biochemical indices measured in postoperative blood samples were lower in patients who subsequently died of cancer as compared to patients who survived. However, the differences in these values did not reach a significant level, after age and sex adjustments had been made. A relatively small number of patients developed a recurrence of epithelial cancer that was not fatal. The mean serum levels of vitamin A and its transport proteins from these 11 subjects were actually higher than mean levels of these parameters from post-operative colorectal cancer patients who remained disease-free after several years of follow up.

In Table 3.21, all 57 patients who developed a cancer recurrence were compared to the 77 patients who remained disease-free. Again, the mean value of every parameter was lower in the recurrence group, but, significant differences were not observed (vitamin A, $p=0.497$; RBP, $p=0.901$; prealbumin, $p=0.324$; zinc, $p=0.068$).

3.2.6 USE OF SERUM PARAMETERS AS PROGNOSTIC INDICATORS IN POSTOPERATIVE COLORECTAL CANCER PATIENTS

Post-operative serum levels of vitamin A, RBP, prealbumin and zinc were assessed together as prognostic indicators in apparently disease-free colorectal cancer

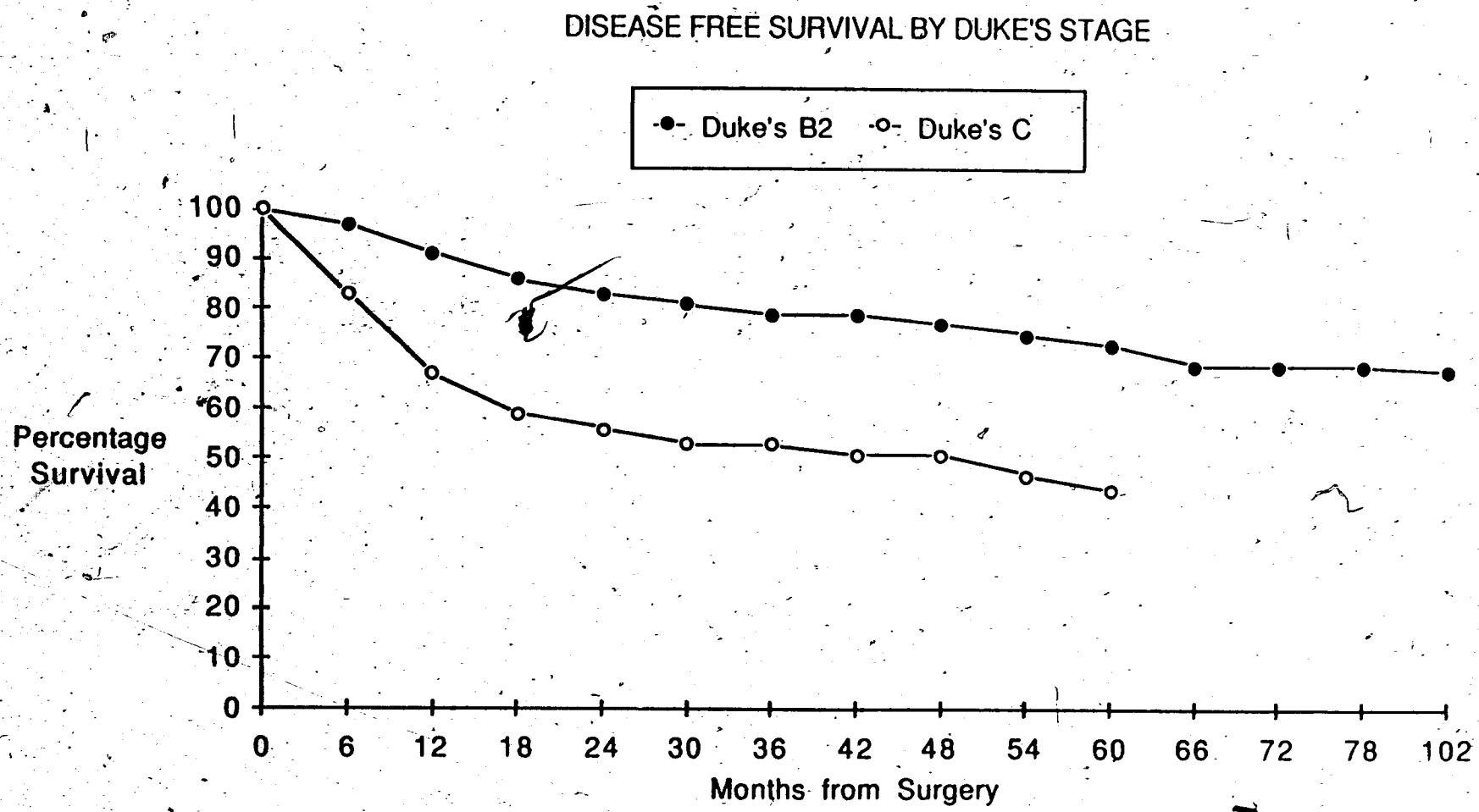

DISEASE FREE SURVIVAL BY DUKE'S STAGE
Duke's B2
Duke's C
Percentage Survival
100
90
80
70
60
50
40
30
20
10
0
0
6
12
18
24
30
36
42
48
54
60
66
72
78
102
Months from Surgery

Figure 3.5

TABLE 3.20 SERUM VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS IN PATIENTS REMAINING DISEASE-FREE VERSUS PATIENTS WITH SUBSEQUENT CANCER RECURRENCE OR CANCER-DEATH FOLLOWING SURGERY.

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
DISEASE-FREE (n=77)	84.1 ± 28.9	5.5 ± 1.9	37.1 ± 10.2	130.6 ± 35.0
RECURRENCE (n=11)	90.0 ± 24.9	5.6 ± 1.3	38.1 ± 7.4	127.0 ± 21.3
DEAD (n=46)	78.7 ± 24.9	5.4 ± 1.5	35.0 ± 8.1	119.5 ± 24.8
AGE AND SEX ADJUSTED SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

N.S. = not significant.

TABLE 3.21 EFFECT OF CANCER RECURRENCE ON VITAMIN A, RBP, PREALBUMIN AND ZINC LEVELS.

GROUPS	VITAMIN A (ug/dl)	RBP (mg/dl)	PREALBUMIN (mg/dl)	ZINC (ug/dl)
DISEASE-FREE (n=77)	84.1 ± 28.9	5.5 ± 1.9	37.1 ± 10.2	130.6 ± 35.0
RECURRENCE (n=57)	80.9 ± 25.1	5.4 ± 1.4	35.6 ± 8.0	121.0 ± 24.1
AGE AND SEX ADJUSTED SIGNIFICANCE	N.S.	N.S.	N.S.	N.S.

Each value represents the mean ± S.D. for the number of subjects shown in parenthesis.

N.S. = not significant.

patients. Table 3.22 shows the standardized canonical discriminant fuction coefficients for the biochemical parameters generated for categorizing the cancer patients by their survival status after a follow up of several years. Neither function in this model reached a statistically significant level (function 1, p=0.32; function 2, p=0.88). Only 43.1% of patients were correctly classified using serum levels of the four parameters in discriminant function analysis.

3.2.7 CORRELATION BETWEEN ASSAY MEASUREMENTS IN POST-OPERATIVE COLORECTAL CANCER PATIENTS

Figure 3.23 shows the correlations between serum vitamin A, RBP, prealbumin and zinc levels in the post-operative colorectal cancer patients. In both Duke's B2 and Duke's C patients all parameters were correlated with each other at a statistically significant level with the exception of vitamin A and zinc.

TABLE 3.22 STANDARD CANONICAL DISCRIMINANT FUNCTION COEFFICIENTS OF SERUM OF VITAMIN A, RBP, PREALBUMIN AND ZINC FROM POST-OPERATIVE COLORECTAL CANCER PATIENTS.

	FUNCTION 1	FUNCTION 2
VITAMIN A	0.37762	0.80752
RBP	-1.64327	-0.17354
PREALBUMIN	1.52689	0.25772
ZINC	0.53776	-0.62917

TABLE 3.23 CORRELATION BETWEEN ASSAY MEASUREMENTS IN POST-OPERATIVE COLORECTAL CANCER PATIENTS.

	PEARSON'S CORRELATION COEFFICIENT (r)	p-value
DUKE'S B2		
VIT-A WITH RBP	0.30	0.004
VIT-A WITH PRE-A	0.33	0.002
VIT-A WITH ZINC	0.08	0.257
RBP WITH PRE-A	0.89	< 0.0001
RBP WITH ZINC	0.42	< 0.0001
PRE-A WITH ZINC	0.47	< 0.0001
DUKE'S C		
VIT-A WITH RBP	0.59	< 0.0001
VIT-A WITH PRE-A	0.55	< 0.0001
VIT-A WITH ZINC	-0.04	0.393
RBP WITH PRE-A	0.90	< 0.0001
RBP WITH ZINC	0.28	0.017
PRE-A WITH ZINC	0.35	0.004
TOTAL		
VIT-A WITH RBP	0.47	< 0.0001
VIT-A WITH PRE-A	0.44	< 0.0001
VIT-A WITH ZINC	0.07	0.210
RBP WITH PRE-A	0.89	< 0.0001
RBP WITH ZINC	0.38	< 0.0001
PRE-A WITH ZINC	0.46	< 0.0001

4. DISCUSSION

Vitamin A has an important role in the growth, differentiation and maintenance of epithelial tissues. Retinoic acid is believed to be the principal form of vitamin A to influence differentiation of epithelial cells (De Luca, et al., 1971), whereas retinol acts to stabilize biological membranes (Roels et al., 1971). Through its role in glycoprotein synthesis, vitamin A is also involved in the maintenance of the mucus coat which protects epithelial cells (Brevard, et al., 1985). Current data from laboratory and epidemiological investigations suggest that vitamin A deficiency, reflected either by an inadequate dietary intake of vitamin A or subnormal serum retinol levels, results in an increased susceptibility to the development and progression of preneoplastic and neoplastic lesions. The effects of vitamin A deficiency at a cellular level are most readily seen in those differentiated tissues that have a rapid turnover rate, such as epithelia of the respiratory and gastrointestinal systems. Indeed, evidence for the relationship between vitamin A and cancer risk is greatest for malignancies of these tissues.

Numerous retrospective studies have generally shown that levels of circulating retinol are depressed in patients with cancer when compared to control subjects (see table 1.3). While the majority of such investigations conducted to date have detected subnormal serum vitamin A levels in

cancer patients, this was not the finding in a study of lung cancer patients conducted by Cohen et al., (1977). No controls were used in this study, rather the cases were compared to standard values (50 ± 20 ug/100ml). Serum retinol levels fell within this standard range in 66 out of 67 patients studied, but comparisons between predetermined standard values of serum parameters, particularly vitamin A, and values measured in diseased subjects are not appropriate. As a wide variation in reported normal ranges have been derived by a number of methods used in different laboratories, it is essential that control subjects be used to provide normal values of vitamin A using the same method and conditions involved in determination of serum vitamin A levels in the patient group.

At this point, it is not known if low vitamin A levels precede the manifestation of cancer or conversely, if the presence of neoplastic disease affects vitamin A status. Prospective studies may overcome the possible effects of the disease on serum levels of retinol in that blood samples are taken several years before the diagnosis of cancer. Generally prospective studies indicate that low vitamin A concentrations precede cancer development, however this has recently been disputed (Wald et al., 1984; Nomura et al., 1985). This question can not be answered by case-control studies, but, with the use of appropriate controls, such investigations can indicate whether or not the effect of subnormal vitamin A status is related to disease in general

or specifically to cancer.

In the majority of retrospective studies which demonstrated subnormal serum retinol levels in cancer patients, healthy subjects or non-specified hospital patients were used as a control group. Many factors including stress, infections, nutritional status and diseases other than cancer are known to depress the levels of circulating vitamin A. Therefore, if the effect of cancer on biochemical indices is being studied, exogenous factors must be controlled for by using a suitable control population. When investigating vitamin A status in patients with cancer of a particular site, subjects with benign disease affecting the same site should be used as controls. The use of proper control groups is of particular importance when gastrointestinal cancer is being investigated. Disruption of the intestinal mucosa due to the presence of any localized disease may result in impaired absorption of vitamin A or other nutrients involved in its transport or metabolism." Irrespective of the site of cancer or disease in question, food intake may be altered in patients due to anorexia associated with illness.

In the first retrospective study investigating the relationship between vitamin A and cancer (Abels et al., 1941), appropriate control groups were used. Healthy individuals were used in order to establish normal levels of plasma vitamin A and beta-carotene in a New York population. Serum parameters in 51 patients with GI cancer were compared

to normal values as well as to values obtained from 33 patients with precancerous lesions and 14 patients with benign intestinal disorders marked by persistent vomiting or diarrhea. In contrast to the subnormal levels detected in the cancer patients, non-cancer patients did not show abnormally low plasma vitamin A or beta-carotene levels. In addition, low plasma vitamin A concentrations were found in patients with other neoplastic diseases including leukemia, bone sarcomas and pancreatic cancer. By using these various control groups, the authors were able to conclude that low vitamin A levels were not a phenomenon general to gastrointestinal disease but specific to cancer. Unfortunately, the small patient numbers and simplistic analytical methodology involved make this data difficult to interpret.

In another early retrospective study (Wahi et al., 1965), serum levels of vitamin A in 60 healthy subjects were compared to those in 409 patients with oral cancer and 127 patients with oral leukoplakia, a precancerous condition. In contrast to the findings of Abels et al. (1941), serum vitamin A levels were low in cases with oral leukoplakia when compared to healthy subjects and the mean serum vitamin A concentration was even lower in patients with oral cancer.

Atukorala et al. (1979) found that the mean serum vitamin A, RBP and zinc concentrations from 26 lung cancer patients were lower than mean values obtained from patients with either non-malignant lung or non-lung diseases. As

normal values for these parameters were not determined from healthy subjects, it is not known whether the non-malignant diseases had any effect on vitamin A status. Without this information, it again can not be concluded that low serum vitamin A levels are specific to malignant disease.

The present study was undertaken to investigate the influence of colorectal cancer and benign GI disease on vitamin A status. Serum samples from 41 colorectal cancer patients, 40 benign GI disease patients and 31 healthy subjects were obtained from the sera bank of the Mayo Clinic in Rochester. The benign GI disease category consisted of patients with polyps (10), gastric ulcers (6), inflammatory bowel disease (10), diverticulosis (5), chronic liver disease (4) and cholithiasis (5). The healthy subjects were used not as true controls but rather to provide normal values of specific serum parametes. Serum parameters measured for assessment of vitamin A status included retinol, its transport proteins (RBP and prealbumin) as well as zinc, which is required for the synthesis of the proteins.

Results from this study demonstrated that patients with either benign or malignant GI disease had subnormal levels of retinol, RBP and prealbumin when compared with healthy subjects (Table 3.5). Low retinol levels were significantly correlated with serum levels of both RBP and prealbumin, thus reflecting compromised metabolic availability of vitamin A. While circulating levels of vitamin A

and its transport proteins were depressed to approximately the same extent in these two patient groups, zinc levels were subnormal only in benign GI disease patients. The discrepancy in mean zinc levels between benign and malignant patients may reflect a difference in the mechanism responsible for low vitamin A levels in these two patient groups.

Benign GI diseases such as adenomatous polyps, diverticulitis and inflammatory bowel disease can be considered either as precancerous lesions or factors which predispose to colorectal cancer. If such conditions precede cancer development, they may be responsible for the subnormal serum retinol concentrations demonstrated in patients with colorectal cancer.

It cannot be stated with certainty that these GI diseases actually cause depressed vitamin A status. The subnormal retinol levels may in fact predispose individuals to these various intestinal disorders. Vitamin A deficiency may be a common etiologic factor in benign and malignant gastrointestinal diseases. During vitamin A deprivation, normal epithelial tissue undergoes metaplastic change with altered growth activity that parallels early neoplastic alterations. Several other disorders of the intestinal system also involve disruption of epithelial tissue. Benign polyps, particularily adenomas, display a serious disturbance in cellular division with loss of control of mitotic processes. Alterations in these hyperplastic

polyps, which are considered to be precancerous lesions, might render the intestinal mucosa more vulnerable to carcinogens (Painter and Burkitt, 1971).

Ulcerative colitis and regional enteritis are diseases of unknown etiology characterized by inflammation of the colonic mucosa. It has been suggested that similar etiologic factors are involved in the development of both inflammatory bowel disease and colorectal cancer. For example, bile acid secretion is elevated by high levels of dietary meat and fat. In addition to increasing the incidence of colon cancer in experimental animals, bile acids and their metabolites are capable of producing degenerative changes in colonic mucosa which may lead to inflammatory bowel disease (Bennet, 1986).

Factors that protect the stomach mucosal lining, such as mucus production and replacement of shed or damaged cells, normally prevent ulcer formation. Because of its integral role in the replication of intestinal epithelial cells, mucus production and the synthesis and secretion of glycoproteins, a deficiency in vitamin A may promote ulcer formation. The development of several diseases of the gastrointestinal system appear to involve degradation of the protective mucosal cells. As vitamin A deficiency compromises the integrity of the intestinal mucosa, it may be a causative factor in these diseases.

4.1. MALNUTRITION AND MALABSORPTION.

Prospective epidemiolgic studies suggest that low dietary intakes of vitamin A increase the risk for subsequent development of cancer in humans (Bjelke, 1975; Hirayama, 1979; Shekelle et al., 1981; Colditz et al., 1985). As these studies largely measured the intake of carotene-containing foods, any protective effect of total vitamin A intake may reflect the anti-oxidant activity of carotenoids (Krinsky, 1979). While a low dietary intake of preformed or pro-vitamin A may precede the development of epithelial cancers by several years, it will not necessarily result in depressed serum retinol levels. Liver reserves of vitamin A must be depleted before serum retinol levels will fall (Olson, 1984).

Case-control studies give inconsistent findings regarding the relationship between vitamin A consumption and cancer risk (Graham, 1984). These retrospective studies must be viewed with caution due to difficulties in obtaining dietary histories from patients relevant to the time period before the onset of their disease.

In this study, depressed levels of both RBP and prealbumin in benign and malignant GI patients suggest that concurrent low serum retinol levels were not solely the result of dietary vitamin A deficiency. During vitamin A depletion in rats serum RBP levels are markedly reduced, likely due to a blockage in secretion of hepatic RBP, while

prealbumin levels remain unchanged (Nevab et al., 1977). Thus a deficiency of only vitamin A would tend to result in low serum levels of retinol and RBP but normal prealbumin levels.

Patients with inflammatory bowel disease are at risk of protein and fat malabsorption as a result of a diseased or resected intestinal tract. Malabsorption of fat-soluble vitamins is a known complication of inflammatory bowel disease which was suggested to be the cause of low blood retinol levels in patients with ulcerative colitis (Sharman et al., 1979).

Hypoalbuminemia is also known to be a nutritional consequence of both inflammatory bowel disease and benign polyps, possibly resulting from blood, protein and fluid loss due to persistent diarrhea and vomiting (Gilinsky et al., 1986). Main et al. (1983) found that low plasma retinol concentrations were associated with depletion of plasma proteins, especially RBP and prealbumin, in patients with regional enteritis. Patients with cancer of the large intestine have also demonstrated depressed levels of serum albumin, RBP and prealbumin (Milano et al., 1978).

Various studies have indicated that depressed vitamin A and RBP levels may be associated with protein deficiency. Because of their short half lives RBP and prealbumin are very sensitive indicators of alterations in protein or energy status (Gofferje, 1978). In children with protein-energy malnutrition serum levels of RBP and prealbumin were

markedly decreased, while dietary treatment resulted in a rapid restoration of these two proteins to normal levels (Ingenbleek et al., 1975). The effect of acute malnutrition on protein status was attributed to diminished hepatic synthesis of plasma proteins.

It is possible that zinc deficiency might also contribute to the pathogenesis of vitamin A deficiency through depression of protein synthesis, particularly the synthesis of RBP and prealbumin (Smith et al., 1974). Among the many nutritional consequences of inflammatory bowel disease, a recently recognized complication is zinc deficiency. Insufficient zinc intake may contribute to this complication, however, dietary intake of zinc was found to be adequate in apparently zinc deficient patients with regional enteritis (McClain et al., 1980). Valberg et al. (1986) found that zinc absorption was significantly decreased in patients with moderate or severe inflammatory bowel disease. In addition to the possibility of impaired absorption of zinc by mucosal cells, ingested zinc may be lost due to diarrhea or drainage from intestinal fistulas. Evidence suggests that low zinc absorption could also be related to decreased protein intake in that rats fed low-protein diets had impaired zinc absorption (Van Campen and House, 1974).

It should be pointed out that no information regarding the usual dietary habits of patients included in this study was available. It is therefore impossible to determine

whether or not the decreased serum levels of vitamin A, RBP, prealbumin and zinc were the result of dietary insufficiencies. It is conceivable that benign GI disease patients with cholithiasis suffered from fat malabsorption resulting in the malabsorption of vitamin A. Futhermore, the 4 patients with chronic liver disease likely experienced impaired hepatic synthesis of RBP and prealbumin resulting in concomitant low serum vitamin A levels. Indeed, of all benign GI disease patients, those subjects with chronic liver disease had the most severely depressed serum RBP and prealbumin values.

4.2. STRESS

Physical stress, trauma and infections are associated with depressed levels of circulating retinol and presumably its carrier proteins. Several stress-induced alterations in the absorption or metabolism of vitamin A have been suggested as possible causes of this effect.

As stress stimulates the adrenal glands, vitamin A depletion may be the result of increased adrenal cortical activity. In fact, studies have demonstrated that the administration of cortisone results in lower vitamin A levels in the liver, kidney, adrenals, thymus and plasma in rats fed deficient, normal or excessive quantities or vitamin A or beta-carotene (Atukorala et al., 1981; Clark and Colburn, 1955).

Sivakumar and Reddy (1972) found that children with acute febrile infections had an excessive urinary loss of labeled vitamin A resulting in increased body requirements. Gross impairment of vitamin A absorption was also demonstrated in these children; an effect attributed to elevations in adrenal cortical activity.

Elevated plasma levels of corticosteroids have been detected in patients with epithelial cancer of the breast (Desphande et al., 1969) prostate (Doe et al., 1969) and lung (Lichter and Sirelt, 1968). These findings suggest that vitamin A deficiencies in cancer patients may be the result of an increased requirement for the vitamin associated with elevated plasma corticosteroid levels.

In addition, an elevated rate of tissue catabolism relative to the rate of hepatic mobilization is thought to play a role in the lowered retinol levels . Infections, fever and stress are associated with elevated levels of thyroxine, which increases the catabolic activity of tissues. Patients with rheumatic fever and other infectious diseases showed a decrease in serum vitamin A concentrations, with development of the disease, that was accompanied by a parallel change in RBP (Nutr. Rev., 1981). As no change in vitamin A intake occurred in these patients, it was suggested that the infection resulted in depressed serum retinol levels due to an impaired release of the vitamin from the liver or due to an increased turnover of RBP.

Burn patients demonstrated reduction in serum vitamin A and RBP levels which closely reflected the severity of burn injury (Cynober et al., 1985). The maximal decrease in these parameters took place 4 to 9 days after the burn injury during the hypermetabolic stage associated with decreased serum amino acid levels. Low RBP levels may have been the result of increased breakdown or decreased synthesis of the protein . Return to normal values was more rapid for RBP than for vitamin A, suggesting that the subnormal vitamin A levels were caused by alterations in RBP metabolism.

It is possible that the low serum values of vitamin A and related parameters demonstrated in the present investigation are associated with physiological stress induced by either benign or malignant disease. As serum corticosteroid concentrations were not measured in these patients, it is difficult to assess the presence or effect of stress.

In this study, patients with either benign or malignant disease who underwent surgery had somewhat lower values of vitamin A, its carrier proteins and zinc than did patients who were not treated by surgery. Cancer patients undergoing resection of the colon or rectum rarely exhibit malnutrition due to loss of absorptive area of the intestine (Lawrence, 1977). However, stress associated with surgery may provoke a metabolic response resulting in a vitamin A deficency. Seifter et al. (1973) demonstrated that stress induced by

surgery exacerbated a marginal vitamin A deficiency in rats. Serum concentrations of vitamin A, RBP and prealbumin were also reduced significantly, from normal values, in patients following surgery (Kasper et al., 1975; Ramsden et al., 1978). Postoperative reductions in serum values of these parameters occurred despite an optimal supply of essential nutrients. The immediate post-surgical period was also characterized by an increase in urinary excretion of retinol and RBP. The fall in serum prealbumin levels, however, was shown to be the result of decreased hepatic synthesis of this protein rather than increased catabolism or urinary excretion (Ramsden et al., 1978). Reduced circulating quantities of prealbumin available for complexing with RBP would result in increased glomerular filtration and urinary loss of RBP and retinol.

These data suggest that stress markedly increases the body's requirement for vitamin A. In summary, the reduction in body retinol levels observed under conditions of stress may be mediated by a decreased mobilization from hepatic stores, increased cellular need leading to tissue depletion, decreased absorption and/or increased renal loss of the vitamin.

In this study, lower mean serum vitamin A, RBP and zinc levels in samples collected shortly after surgery (less than one month) versus those collected approximately one month later, reflect the known transient effect of stress on circulating vitamin A. These results suggest that surgery

did contribute to the depression of serum vitamin A levels in GI patients.

In order to further investigate the effect of time lapse between surgery and blood collection on these parameters, serum from post-operative colorectal cancer patients were examined. Frozen serum samples from 134 patients were obtained from the Cross Cancer Institute in Edmonton. Blood samples analyzed from these patients had been collected over longer time intervals following surgery (between 1 week to 8 months). In contrast to the cancer patients from the Mayo Clinic, these subjects were apparently disease-free after surgery.

A significant difference was detected for serum retinol with the lowest average values in blood samples collected less than one month after surgery and the highest average value in samples collected more than 6 months after surgery. Similarily, the highest values for serum RBP, prealbumin and zinc were found in samples with the greatest time lag between surgery and blood sample collection. Serial blood samples were analyzed in 35 of the CCI patients; the mean time span between the two blood collections was 79 days. Increased mean values of all four parameters were found in the second serum sample taken from these patients, the difference being greatest for vitamin A. Because surgery apparently cured these patients of colorectal cancer, the trend towards normalization of serum values of these parameters may reflect recovery from surgery or recovery

from the presence of neoplastic disease.

4.3. - ADJUVANT TREATMENT

The treatment of cancer with chemotherapeutic agents or radiation commonly causes side effects which adversely affect nutritional status. Taste aberration, nausea and vomiting may depress food intake, while diarrhea and damage to the gastrointestinal tract result in malabsorption of several nutrients (Donaldson and Lenon, 1979: Ohnuma and Holland, 1977). In addition, liver damage is often induced by chemo- or immunotherapeutic drugs leading to serious nutritional problems.

Information regarding the effect of adjuvant cancer therapy on vitamin A status is scarce. However, one study showed that patients with testicular teratoma had a transient drop in plasma vitamin A, RBP and prealbumin levels upon treatment with the vinca alkaloid, vinblastine (Atukorala, 1980).

When comparing patients from the Mayo Clinic who did not receive adjuvant treatment to those who were undergoing chemo-, immuno- and/or radiotherapy, no statistically significant differences existed between serum levels of the parameters measured. But because no information was available outlining the specific type or duration of treatment given, no conclusions can be made about the effect of cancer therapy on vitamin A status in these patients.

4.4. DIRECT EFFECT OF TUMOURS

The presence of cellular binding protein for retinol (CRBP) and retinoic acid (CRABP) in tumours suggests that neoplastic tissue has an increased requirement for vitamin A. In comparison to normal adjacent tissue, the levels of CRBP and CRABP are higher in human neoplastic tissue of the oral cavity (Ong et al., 1982), lung and breast (Ong et al., 1975). An increased requirement of vitamin A by tumour cells may result in a greater demand for RBP synthesis and lead to depressed RBP and vitamin A status.

Since zinc is required for nucleic acid and protein synthesis (Vallee, 1977), rapidly growing tumour tissue may increase the body's requirement for zinc. The subnormal serum zinc levels detected in patients with cancer may reflect the zinc requirements of neoplastic tissue. Davies et al. (1968) found markedly depressed plasma zinc levels in patients with carcinoma of the bronchus as compared to healthy subjects and subjects with chronic bronchitis or other illness of the pulmonary system. Malignant breast tissue was found to have a mean concentration of zinc 5.7 times that of normal adjacent breast tissue in human subjects (Schwartz et al., 1974). The authors concluded that unless neoplasms develop in tissues rich in zinc, subnormal serum zinc concentrations will result in cancer patients.

As all cancer patients from the Mayo Clinic had distant

metastases at the time of blood sample collection, effects of the primary colorectal tumour on vitamin A status cannot be distinguished from the possible effects of a secondary tumour. It would appear that distant metastases did have some affect on vitamin A status in that patients with metastatic disease in 2 or more sites had lower levels of vitamin A, RBP and prealbumin than did patients with only 1 secondary tumour. In contrast, the extent of spread of the disease did not have any influence on zinc levels.

It might be expected that liver metastases would result in low vitamin A levels in association with depressed hepatic synthesis of RBP and prealbumin. However mean values of the serum parameters were not significantly different in patients with liver metastases when compared to the mean from all cancer patients. While no significant differences in levels of vitamin A and its related factors were detected in patients with different metastatic sites, the small number of patients in each group precluded a meaningful comparison. Larger numbers of metastatic cancer patients are needed in order to evaluate possible differences between the sites.

In order to assess the effect of metastic disease on serum vitamin A status, a comparison should be made between colorectal cancer patients with and without distant recurrences. None of the CCI patients had distant metastases at the time at which their blood was collected, but a meaningful comparison cannot be made between CCI and

Mayo Clinic patients because of differences in storage conditions of serum samples. Also the possible direct effect of the colorectal tumour in CCI patients is not fully present as these patients were considered to be free of cancer when their blood samples were collected.

The stage of progression of colorectal cancer appeared to have some effect on serum levels of vitamin A, RBP, prealbumin and zinc in the CCI patients. Serum levels of all parameters measured were lower in patients in which the colorectal tumour had invaded the lymph nodes (Dukes C) as compared to patients without lymph node involvement (Dukes B2). This difference was particularily large for vitamin A and zinc. It would appear from this data that vitamin A levels reflect the severity of the disease. To further investigate this question studies should be conducted using patients with different stages of colorectal tumour development without metastatic spread.

4.5. DIAGNOSTIC AND PROGNOSTIC INDICATORS

Prospective serum studies have demonstrated that low serum retinol existed long before the development of various epithelial cancers. In the first of such studies, Wald et al. (1980) proposed that serum retinol levels in man may have predictive value for subsequent cancer development, particularily for lung and gastrointestinal cancers.

Similarly, retrospective studies have generally shown that patients with epithelial cancer have subnormal vitamin A status. This suggests that serum values of retinol and its related factors may be used as early diagnostic indicators for epithelial cancers. Because colorectal cancer has a slow growth rate and a correspondingly long interval before reaching symptom-producing size, it would be of great benefit if the diagnosis of the disease could be made during this asymptomatic stage. It has been suggested that survival from colorectal cancer would be significantly improved if adequate screening tests were used (Van de Velde et al., 1986).

In the present study, serum values of retinol, RBP, prealbumin and zinc from the Mayo Clinic subjects were tested for their ability to distinguish between healthy subjects, patients with benign GI disease and colorectal cancer patients. In view of the fact that serum vitamin A, RBP and prealbumin levels were very similar for patients with benign or malignant disease, it cannot be expected that these parameters could distinguish between the two patient groups. Also, the considerable overlap of all serum parameter levels between the healthy and patient groups warrants caution in proposing that these biochemical indices could be used as diagnostic tools for colorectal cancer. According to their serum levels of these four parameters, 56.6% of the subjects were correctly catagorized as being healthy or diseased. While this is an improvement over the

42% of subjects who would be correctly categorized through random distribution into 1 of the 3 groups, the high level of error in this test would appear to preclude the use of these parameters as a diagnostic tool. This test lacks the degree high of sensitivity and specificity that is needed in a screening diagnostic tool for the diagnosis of cancer (Steinburg et al., 1986).

Little information is available as to whether the status of vitamin A , its carrier proteins and zinc can be used to predict the survival outcome of cancer patients. Serial measurements of carcinoembryonic antigen (CEA) levels have prognostic value in relation to recurrence of colorectal cancer or the development of matastases, but, they are of little value with respect to duration of survival of cancer patients (Aabo, et al., 1986). Because abnormalities in hepatic protein synthesis often accompany advanced cancer, Milano et al. (1978) investigated the use of serum prealbumin, RBP and albumin levels as prognostic indicators in postoperative, colorectal cancer patients. Prealbumin proved to be a sensitive indicator of tumour load in that serum levels of prealbumin dropped with tumour progression. As a rapid fall in levels of this serum protein often occurred 2 to 3 months prior to a patients's death, serial measurements of prealbumin also appeared to have short-term prognostic significance.

A further study was undertaken by Basu et al.(1985) to investigate whether lower plasma retinol is associated with

an increased risk of cancer recurrence in post-operative, apparently disease-free colorectal cancer patients. Plasma levels of retinol and RBP were found to be lower in 12 patients who subsequently developed a cancer recurrence as compared to the 91 patients who remained disease-free. Because the followup of these patients was less than 1 year, a subsequent 5 year followup of subjects from this colorectal cancer patient population was undertaken in the present study.

During this followup period, 32.2% of Duke's B2 and 65.9% of Duke's C patients developed a recurrence of cancer, while 21.3% of Duke's B2 and 50.8% of Duke's C patients died of their cancer recurrence. Values of vitamin A, RBP, prealbumin and zinc from serum samples taken within 6 months of supposedly curative surgery did not significantly differ between patients who remained disease-free and patients who subsequently developed a cancer recurrence. Using these values, only 43.1% of patients were correctly classified according to their survival status after approximately 5 years. This strongly suggests that serum values of vitamin A and its related factors, measured in apparently disease-free subjects soon after resection of colorectal tumours, do not aid in predicting long-term survival status. Rather, serial postoperative measurements of these parameters may be of use in detecting cancer recurrences within months of their development (Milano et al, 1978).

4.6. CONCLUSION

In accordance with findings from previous studies, patients with colorectal cancer were found to have subnormal vitamin A status as compared to healthy subjects. In an attempt to compensate for the influence of non-specific disease on vitamin A levels, subjects with benign GI disease were used as controls. The biochemical availability of vitamin A, reflected by serum retinol, RBP, and prealbumin levels, was depressed to the same extent in patients with either benign or malignant GI disease. Thus, subnormal circulating levels of vitamin A and its transport proteins appear to be associated with several diseases involving disruption of intestinal epithelium. Depressed zinc levels in benign but not malignant disease patients may reflect a difference in the mechanism responsible for subnormal vitamin A status.

Whether subnormal vitamin A status is a cause or effect of epithelial cancer or other intestinal disorders is not known. Numerous factors are involved in the regulation of serum retinol levels, including the dietary intake, absorption and metabolic requirement of several nutrients. Stress, related to the presence of disease or from surgery, may lead to a transient reduction in circulating vitamin A levels. Surgery did appear to depress serum levels of all parameters measured, particularily zinc, in both benign and malignant disease patients.

Vitamin A deficiency may be an etiologic factor in the development of disease affecting the intestinal epithelium. Findings of low serum levels of vitamin A and its related factors in patients with benign or malignant GI disease suggest that the dietary intake of vitamin A should be increased to, at least, meet the recommended nutrient intake for Canadians of 800 to 1000 RE per day. The possibility of increased requirement for vitamin A and zinc by tumour cells preclude suggestions for supplementation with excessive amounts of these nutrients. Should the need for vitamin A by these tissues be increased, large doses could possibly enhance the growth of neoplastic tissue.

Patients with colorectal cancer or benign GI disease could not be accurately distinguished from healthy subjects according to their serum levels of retinol, RBP, prealbumin and zinc. Similarily, postoperative levels of these parameters did not aid in making predictions regarding the long-term survivial status of colorectal cancer patients. Because their circulating concentrations are influenced by several factors, these biochemical indices do not provide the sensitivity or specificity required of tumour markers.

REFERENCES

AABO, K., PEDERSON, H., KJAER, M. Carcinoembryonic antigen (CEA) and alkaline phosphatase in progressive colorectal cancer with special reference to patient survival. Eur. J. Cancer Clin. Oncol. 1986; 22: 211-217.

ABELS, J.C., GORHAM, A.T., PACK, G.T., RHOADS, C.P. Metabolic studies in patients with cancer of the gastrointestinal tract. I. Plasma vitamin A levels in patients with malignant neoplastic disease, particularily of the gastrointestinal tract. J. Clin. Invest. 1941; 45: 2369-2372.

ALBERTA CANCER BOARD. Incidence Rates By Age and Sex, 1985 Unpublished.

ATUKORALA, S., BASU, T.K., DICKERSON, J.W.T. Effects of corticosterone on the plasm and tissue concentrations of vitamin A in rats. Ann. Nutr. Metab. 1981;25:234-238.

ATUKORALA, S., BASU, T.K., DICKERSON, J.W.T., DONALDSON, D., SAKULA, A. Vitamin A, zinc and lung cancer. Br. J. Cancer. 1979; 40: 927-931.

ATUKORALA, T.M.S. Vitamin A and lung cancer. Thesis. 1980

BANJI, M.S., AHMED, F. Effect of oral contraceptive steroids on vitamin status of women and female rats. World Rev. Nutr. Diet. 1978; 31: 135-140.

BASHOR, M., TOFT, D.D., CHYTIL, F. In vitro binding of retinol to rat tissue components. Proc. Natl. Acad. Sci. USA. 1973; 70: 3483-3487.

BASU, T.K., CHAN, U.M., FIELDS, A.L.A., McPHERSON, T.A. Retinol and postoperative colorectal cancer patients. Br. J. Cancer. 1985; 51: 61-65.

BASU, T.K., ROWLANDS, L., JONES, L., KOHN, J. Vitamin A and retinol-binding protein in patients with myelomatosis and cancer of epithelial origin. Eur. J. Cancer Clin. Oncol. 1982; 18: 339-342.

BENNET, J.D. Ulcerative colitis: The result of an altered bacterial metabolism of bile acids or cholesterol. Med. Hypotheses. 1986; 20: 125-132.

BICHLER, E., DAXENBICHLER, G., MARTH, C.H. Vitamin A status and retinol-binding proteins in carcinomas of the head

neck region. Oncology. 1983; 40: 336-339.

BJELKE, E. Dietary vitamin A and human lung cancer. Int. J. Cancer. 1975; 15: 561-565.

BREVARD, P.B., ANDERTON, L.G., MAGEE, A.C. In vivo effects of retinoids on the histological changes in colorectal tissue. Nutr. Rep. Inter. 1985; 31: 635-648.

BROWN, E.D., CHEN, W., SMITH, J.C. Vitamin A metabolism during the repletion of zinc deficient rats. J. Nutr. 1976; 106; 563-568.

CHEN, C.C., HELLER, J. Uptake of retinol and retinoic acid from serum retinol-binding protein by retinal pigment epithelial cells. J. Biol. Chem. 1977; 252: 5216-5221.

CHOPRA, D.P., WILKOFF, L.J. Inhibition and reversal of carcinogen-induced lesions in mouse prostate in vitro by all trans-retinoic acid. Proc. Am. Assoc. Cancer Res. 1975; 16: 35.

CLARK, I., COLBURN, R.W. A relationship between vitamin A metabolism and cortisone. Endocrinology. 1955; 56: 232-238.

CLIFFORD, P. Carcinogens in the nose and throat. Naso-pharyngeal carcinoma in Kenya. Proc. Roy. Soc. Med. 1972; 65: 682-686.

COHEN, M.H., PRIMACK, A., BRODER, L.E., WILLIAMS, L.R. Vitamin A serum levels and dietary vitamin A intake in lung cancer patients. Cancer Letts. 1977; 4: 51-54.

COHEN, S.M., WITTENBERG, J.F., BRYON, G.T. Effect of avitaminosis A and hypervitaminosis A on urinary bladder carcinogenicity of N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. Cancer Res. 1976; 36: 2334-2339.

COLDITZ, G.A., BRANCH, L.G., LIPNICK, R.J., WILLETT, W.C., ROSNER, B., POSNER, B.M., HENNEKENS, C.H. Increased green and yellow vegetable intake and lowered cancer deaths in an elderly population. Am. J. Clin. Nutr. 1985; 41: 32-36.

CYNOBER, L., DESMOULINS, D., LIORET, N., AUSSEL, C., HIRSCH-MARIE, H., SAIQY, R. Significance of vitamin A and retinol-binding protein serum levels after burn injury. Clin. Chim. Acta. 1985; 148: 247-253.

DALIAM, A., SAVOURE, N., COTTENCIN, C., NICOL, M. Cancerogenese experimentale. Stimulaton de l'ornithine decarboxylase de la muqueuse colique chez le rat carence en vitamine A par le desoxycholate de sodium. Bull. Cancer (Paris). 1986; 73: 243-250.

DAVIES, I.J.T., MUSA, M., DORMANDY, T.L. Measurement of plasma zinc J. Clin. Path. 1968; 21: 359-365.

DELUCA, L., ANDERSON, H., WOLF, G. The in vivo and in vitro biosynthesis of lung tissue glycopeptide. Arch. Intern. Med. 1971; 127: 853-857.

DELUCA, L., LITTLE, E.P., WOLFE, G. Vitamin A and protein synthesis by rat intestinal mucosa. J. Biol. Chem. 1969: 244: 701-708.

DELUCA, L., MAESTRI, N., BONANNI, F., NELSON, D. Maintenance of epithelial cell differentiation: The mode of action of vitamin A. Cancer. 1972; 30: 1326-1331.

DELUCA, L.M. The direct involvement of vitamin A in glycosyl transfer reactions of mammalian membranes. Vitam. Horm. 1977; 35: 1-57.

Depression of serum levels of retinol and retinol-binding protein during infection. Nutr. Rev. 1981; 39: 165-167.

DESPHANDE, N., JENSEN, V., CARSON, P., BULBROOK, R.D. Some aspects of the measurement of cortisol production in patients with breast cancer. J. Endocrinol. 1969; 45: 571-578.

DOE, R.P., DICKERSON, P., ZINNEMAN, H.H., SEAL, U.S. Elevated nonprotein bound cortisol (NPC) in pregnancy, during estrogen administration, and in carcinoma of the prostate. J. Clin. Endocrin. 1969; 29: 757-766.

DOLL, R., PETO, R. The causes of cancer-quantitative estimates of avoidable risks of cancer in the United States today. J.N.C.I. 1981; 66: 1191-1308.

DONALDSON, S.S., LENON, N. Alterations of nutritional status. Impact of chemotherapy and radiation therapy. Cancer. 1979; 43: 2036-2052.

DOWLING, J.E., WALD, G. The biological function of vitamin A acid. Proc. Natl. Acad. Sci. USA. 1960; 46: 587-608.

FLAIM, E., WILLIFORD, W.O., MULLEN, J.O., BUZBY, G.P., CROSBY, L.O. The relationship of serum cholesterol and

vitamin A in hospitalized patients with and without cancer. Am. J. Clin. Nutr. 1986; 44: 370-378.

FUJIMAKI, Y. Formation of carcinoma in albino rats fed on deficient diets. J. Cancer Res. 1926; 10: 469-477.

GAL, I., PARKINSON, C., CRAFT, I. Effects of oral contraceptives on human plasma vitamin-A levels. Br. Med. J. 1971; 2: 436-438.

GANGULY, J. Absorption of vitamin A. Am. J. Clin. Nutr. 1969; 22: 923-933.

GENTA, V.M., KAUFMAN, D.G., HARRIS, C.C., SMITH, J.M., SPORN, J.M., SAFFIOTTI, U. Vitamin A deficiency enhances the binding of benzo(a)pyrene to tracheal epithelial DNA. Nature. 1974; 247: 48-49.

GILINSKY, N.H., ELLIOT, M.S., PRICE, S.K., WRIGHT, J.P. The nutritional consequences and neoplastic potential of juvenile polyposis coli. Dis. Colon Rectum. 1986; 29: 417-420.

GLOVER, J., JAY, C., WHITE, G.H. Distribution of retinol-binding protein in tissues. Vitam. Horm. 1974; 32: 215-235.

GOFFERJE H. Prealbumin and retinol-binding protein- highly sensitive parameters for the nutritional state in respect of protein. Med. Lab. 1978; 5: 38-44.

GOODMAN, D.S., HUANG, H.S., SHIRATORI, T. Mechanism of the biosynthesis of vitamin A from B-carotene. J. Biol. Chem. 1966; 241: 1929-1932.

GOODMAN, D.S., HUANG, H.S., SHIRATORI, T. Tissue distribution and metabolism of newly absorbed vitamin A in the rat. J. Lipid Res. 1965; 6: 390-396.

GOODMAN, G.E., ALBERTS, D.S., PENG, Y.M., BEAUDRY, J., LEIGH, S.A., MOON, T.E. Plasma kinetics in oral retinol in cancer patients. Cancer Treat. Rep. 1984; 68: 1125-1133.

GRAHAM, S., DAYAL, H., SWANSON, M., MITTELMAN, A., WILKINSON, G. Diet in the epidemiology of cancer of the colon and rectum. J.N.C.I. 1978; 28: 421-424.

GRAHAM, S. Epidemiology of retinoids and cancer. J.N.C.I. 1984; 73: 1423-1428.

GREGOR, A., LEE, P.N., ROE, F.J.C., WILSON, M.J., MELTON, A. Comparison of dietary histories in lung cancer cases and controls with special reference to vitamin A.

Nutr. Cancer. 1980; 2: 93-97.

HAINES, A.P., THOMPSON, S.G., BASU, T.K., HUNT, R. Cancer, retinol binding protein, zinc and copper. Lancet. 1982; 1: 52-53.

HANKIN, J.H., KOLONEL, L.N., HINDS, M.W. Dietary history methods for epidemiologic studies: Application in a case-control study of vitamin A and lung cancer. J.N.C.I. 1984; 73: 1417-1421.

HANSEN, L.G., WARWICK, W.J. A fluorometric method for the determination of serum vitamin A. Am. J. Clin. Pathol. 1968; 50: 525-529.

HARRIS, R.W.C., FORMAN, D., DOLL, R., VESSEY, M.P., WALD, N.J. Cancer of the cervix uteri and vitamin A. Br. J. Cancer. 1986; 53: 653-659.

HELLER, J. Characterization of bovine plasma retinol-binding protein and evidence for lack of binding between it and other bovine plasma proteins. J. Biol. Chem. 1975; 250: 6549-6554.

HILL, D.L., GRUBBS, C.J. Retinoids as chemopreventive and anticancer agents in intact animals (review). Anticancer Res. 1982; 2: 111-124.

HIRAYAMA, T. Diet and cancer. Nutr. Cancer. 1979; 1: 67-81.

HUBER, A.M., GERSHOFF, S.N. Effect of zinc deficiency on the oxidation of retinol and ethanol in rats. J. Nutr. 1975; 105: 1486-1490.

IBRAMIN, K., JAFAREY, N.A., ZUBERI, S.J. Plasma vitamin A and carotene levels in squamous cell carcinoma of oral cavity and oro-pharynx. Clin. Oncol. 1977; 3: 203-207.

INGENBLEEK, Y., VAN DEN SCHRIEK, H.G., DE NAYER, R., DE VISSCHER, M. The role of retinol-binding protein in protein-calorie malnutrition. Metabolism. 1975; 5: 38-44.

JOHNSON, B.C., KENNEDY, M., CHIBA, N. Vitamin A and nuclear RNA synthesis. Am. J. Clin. Nutr. 1969; 22: 1048-1058.

KANAI, M., RAZ, A., GOODMAN, D.S. Retinol-binding protein: The transport protein for vitamin A in human plasma. J. Clin. Invest. 1968; 47: 2025-2044.

KANDA, Y., GOODMAN, D.S., CANFIELD, R.E., MORGAN, F.J. The amino acid sequence of human plasma prealbumin. J. Biol. Chem. 1974; 249: 6796-6805.

KARK, J.D., SMITH, A.H., SWITZER, B.R., HAMES, C.G. Serum vitamin A (retinol) and cancer incidence in Evans County, Georgia. J.N.C.I. 1981; 66: 7-16.

KASPER, H., BRODERSON, M., SCHEDAL, R. Concentration of vitamin A, retinol-binding protein and prealbumin in serum in response to stress. A contribution to the prevention of stress ulcers by means of vitamin A. Acta. Hepatogastroenteral. 1975; 22: 403-408.

KRINSKY, N.I. Carotenoid protection against oxidation. Pure Appl. Chem. 1979; 51: 649-660.

KUMMET, T., MEYSKENS, F.L. Vitamin A: A potential inhibitor of human cancer. Sem. Oncol. 1983; 3: 281-289.

LAMBERT, B., BRISSON, G., BIELMANN, P. Plasma vitamin A and precancerous lesions of cervix uteri: A preliminary report. Gynecol. Oncol. 1981; 11: 136-139.

LAWRENCE, W. Nutritional consequences of surgical resection of the gastrointestinal tract for cancer. Cancer Res. 1977; 37: 2379-2386.

LICHTER, I., SIRETT, N.E. Plasma cortisol levels in lung cancer. Br. J. Med. 1968; 2: 154-156.

LOFTI, A.M., SPENCER, R.J., ILSTRUP, D.M., MELTON, L.J. Colorectal adenomas: Distribution, incidence of malignant transformation and rate of recurrence. Dis. Colon Rectum. 1986; 29: 383-387.

LOPEZ, S.A., LE GARDEUR, B.Y., JOHNSON, W.D. Vitamin A status and lung cancer. Am. J. Clin. Nutr. 1981; 34: 641.

MAIN, A.N.H., MILLS, P.R., RUSSELL, R.I., BRONTE-STEWART, J., NELSON, L.M., McLELLAND, A., SHENKIN, A. Vitamin A deficiency in Crohn's disease. Gut. 1983; 24: 1169-1175.

MAHADEVEN, S., MALATHI, P., GANGULY, J. Influence of protein on absorption and metabolism of vitamin A. World Rev. Nutr. Diet. 1965; 5: 209-236.

MAHMOUD, L.A.N., ROBINSON, W.A. Vitamin A levels in human bladder cancer. Int. J. Cancer. 1982; 30: 143-145.

MANCINI, G., CARBONARA, A.O., HEREMAN, J.I. Immunochemical quantiation of antigens by single radial immunodiffusion. Immunochem. 1965; 2: 235-254.

MATHEWS-ROTH, M.M. Antitumor activity of B-carotene, canthaxanthin and phytoene. Oncology. 1982; 39: 33-37.

McCLAIN, C., SOUTOR, C., ZIEVE, L. Zinc deficiency: A complication of Crohn's disease. Gastroenterolgy. 1980; 78: 272-279.

McCOLLUM, E.V., DAVIS, M. The nature of the dietary deficiencies of rice. J. Biol. Chem. 1915; 23: 181-246.

McLAREN, D.S., SHIRAJIAN, E., LOSHKAJIAN, H., SHADAREVIAN, S. Short-term prognosis in protein-calorie malnutrition. Am. J. Clin. Nutr. 1969; 22: 863-870.

MELLOW, M.H., LAYNE, E.A., LIPMAN, T.O., KAUSHIK, M., HOSTETLER, C., SMITH, J.C. Plasma zinc and vitamin A in human squamous esophageal cancer. Gastroenterology. 1981; 80: 1229- 1232.

MILANO, G., COOPER, E.H., GOLIGHER, J.C., GILES., G.R., NEVILLE, A.M. Serum prealbumin, retinol-binding protein, transferrin and albumin levels in patients with large bowel cancer. J.N.C.I. 1978; 61: 687-691.

MODAN, E., CUCKLE, H., LUBIN, F. A note on the role of dietary retinol and carotene in human gastrointestinal cancer. Int. J. Cancer. 1981; 28: 421-424.

MOORE, T. Vitamin A and carotene. VII. The distribution of vitamin A and carotene in the body of the rat. Biochem. J. 1931; 25: 275-286.

MORRISON, S.A., RUSSELL, R.M., CARNEY, E.A., OAKS, E.V. Zinc deficiency: A cause of abnormal dark adaptation in cirrhotics. Am. J. Clin. Nutr. 1978; 31: 276-281.

MORSON, B.C. Evolution of cancer of the colon and rectum. Cancer. 1974; 34: 845-849.

MUTO, Y., MORIWAKI, H. Antitumor activity of vitamin A and its derivatives. J.N.C.I. 1984; 73; 1389-1393.

MUTO, Y., SMITH, J.E., MILCH, P.O., GOODMAN, D.S. Regulation of retinol-binding protein metabolism by vitamin A status in the rat. J. Biol. Chem. 1972; 247: 2542-2550.

MUNOZ, N., WAHRENDORF, J., BANG, L.J., CRESPI, M., THURNHAM, D.I., DAY, N.E., JI, Z.H., GRASSI, A., YAN, L.W., LIN, L.G., QUAN, L.Y., YUN, Z.C., FANG, Z.S., YAO, L.J., CORREA, P., O'CONNER, G.T., BOSCH, X. No effect of riboflavine, retinol, and zinc on prevalence of precancerous lesion of oesophagus. Lancet. 1985; 2: 111-114.

NANJI, A.A., FREEMAN, J.B. Gastric by-pass surgery in morbidly obese patients markedly decreases serum levels of vitamins A and C and iron in the peri-operative period. Int. J. Obesity. 1985; 9: 177-179.

NETTESHEIM, P., SNYDER, C., WILLIAMS, M.L., CONE, M.L., KIM, J.C. Effect of vitamin A on lung tumor induction in rats. Proc. Am. Assoc. Cancer Res. 1975; 16: 54.

NEVAB, M., SMITH, J.E., GOODMAN, D.S. Rat plasma prealbumin metabolic studies on effects of vitamin A status and on tissue depletion. J. Biol. Chem. 1977; 252: 5107-5114.

NEWBERNE, P.M., ROGERS, A.E. Rat colon carcinomas associated with aflatoxin and marginal vitamin A. J.N.C.I. 1973; 50: 439-448.

NEWBERNE, P.M., SUPHAKAN, V. Preventative role of vitamin A in colon carcinogenesis in rats. Cancer. 1977; 40: 2553-2556.

NORMURA, A.M.Y., STEMMERMANN, G.N., HEILBRUN, L.K., SALKELD, R.M., VUILLEMIER, J.P. Serum vitamin levels and the risk of cancer of specific sites in men of Japanese ancestry in Hawaii. Cancer Res. 1985; 45: 2369- 2372.

OHNUMA, T., HOLLAND, J.F. Nutritional consequences of cancer chemotherapy and immunotherapy. Cancer Res. 1977; 37: 2395-2406.

OLSON, J.A. Serum levels of vitamin A and carotenoids as reflectors of nutritional status. J.N.C.I. 1984; 73: 1439-1444.

ONG, D.E., CHYTIL, F. Retinoic acid-binding protein in rat tissue. J. Biol. Chem. 1975; 250: 6113.

ONG, D.E., CHYTIL, F. Presence of cellular retinol and retinoic acid binding proteins in experimental tumors. Cancer Letters. 1976; 2: 2530- 2536.

ONG, D.E., PAGE, D.L., CHYTIL, F. Retinoic acid-binding protein: Occurence in human tumor. Science. 1975; 190: 60-61.

ORR, J.W., WILSON, K., BODIFORD, C., CORNWELL, A., SOONG, S.W., HONEA, K.L., HATCH, K.D., SHINGLETON, H.M. Nutritional status of patients with untreated cervical cancer. II. Vitamin assessment. Am. J. Obstet. Gynecol. 1985; 151: 632-635.

PAINTER, N.S., BRKITT, D.P. Diverticular disease of the colon: A deficiency disease of Western civilization. Br. Med. J. 1971; 2: 450-454.

PETERSON, P.A., NILSSON, S.F., OSTBERG, L., VAHLQUIST, A. Aspects of the metabolism of retinol-binding protein and retinol. Vitam. Horm. 1974; 32: 181-214.

RAMSDEN, D.B., PRINCE, H.P., BURR, W.A., BRADWELL, A.R., BLACK, E.G., EVANS, A.E., HOFFENBURG, R. The inter-relationships of thyroid hormones, vitamin A and their binding proteins following acute stress. Clin. Endocrinol. 1978; 8: 109-122.

RASK, L., PETERSON, P.A. In vitro uptake of vitamin A from the retinol-binding plasma protein to mucosal epithelial cells from the monkey's small intestine. J. Biol. Chem. 1976; 251: 6360-6366.

RASK, L., VAHLQUIST, A., PETERSON, P.A. Studies on two physiological forms of the human retinol-binding, protein differing in vitamin A and arginine content. J. Biol. Chem. 1971; 241: 6638-6646.

RETTURA, G., DUTTAGUPTA, C., LISTOWSKY, P., LEVENSON, S.M., SEIFTER, E. Dimethylbenz(a)anthracene (DMBA) induced tumors: Prevention by supplemental B-carotene. (Abstract no. 2891). Fed. Proc. 1983; 42: 786.

RIETZ, P., WISS, O., WEBER, F. Metabolism of vitamin A and the determination of vitamin A status. Vitam. Horm. 1974; 32: 237-249.

ROELS, O.A., ANDERSON, O.R., LIU, N.S.T., SHAH, D.O., TRENT, M.E. Vitamin A and membrane. Am. J. Clin. Nutr. 1969; 22: 1020-1032.

ROWE, N.H., GORLIN, R.J. The effect of vitamin A deficiency upon experimental oral carcinogenesis. J. Dent. Res. 1959; 38: 72-83.

SALONEN, J.T., SALONEN, R., LAPPETELAINEN, R., MAENPAA, P.H., ALFTHAN, G., PUSKA, P. Risk of cancer in relation to serum concentrations of selenium and vitamins A and E: matched case-control analysis of prospective data. Br. Med. J. 1985; 290: 417-420.

SAWICKI, J., OSTROWSKI, J., SWIETOCHOWSKA, B., JANIK, P., SIKORA,J., SLOWIK-GABRYELSKA, A., BATURA-GABRYEL, H., NOWACKI, M., PRZYBYSZEWSKA, M. Vitamin A (retinol) level in colon and lung cancer patient sera. Neoplasma. 1985; 32: 225-227.

SCHWARTZ, A.E., LEDDICOTTE, G.W., FINK, R.W., FRIEDMAN, E.W. Trace elements in normal and malignant human breast tissue. Surgery. 1974; 76: 325-329.

SEIFTER, E., RETTURA, G., SEIFTER, J., DAVIDSON, H., LEVENSON, S.M. Thymotropic action of vitamin A (Abstract). Fed. Proc. 1973; 32: 947.

SHARMAN, I.M., DICK, A.P., FARTHING, M.J.G., BRYANT, J.R., BEEVAR, S. Carotenoid and retinol levels in the blood of ulcerative colitis patients and controls. Proc. Nutr. Soc. 1979; 38: 54A.

SHEKELLE, R.B., LIN, S., RAYNOR, W.J., LEPPER, M., MALIZA, C., ROSSOF, A.H. Dietary vitamin A and risk of cancer in the Western Electric Study. Lancet. 1981; 2: 1185-1190.

SIVAKUMAR, B., REDDY, V. Absorption of labelled vitamin A in children during infection. Br. J. Nutr. 1972; 27: 299-304.

SMITH, F.R., GOODMAN, D.S. The effects of diseases of the liver, thyroid and kidneys on the transport of vitamin A in human plasma. J. Clin. Invest. 1971; 50: 2426-2435.

SMITH, F.R., GOODMAN, D.S., ZAKLAMA, M.S. Serum vitamin A, retinol-binding protein and prealbumin concentrations in protein-calorie malnutrition. Am. J. Clin. NUtr. 1973; 26: 973-987.

SMITH, J.C., McDANIEL, E.G., FAN, F.F., HALSTEAD, J.A. Zinc: a trace element essential in vitamin A metabolism. Science. 1973; 181: 954-955.

SMITH, J.E., BROWN, E.D., SMITH, J.C. The effect of zinc deficiency on the metabolism of retinol-binding protein in the rat. J. Lab. Clin. Med. 1974; 84: 692-698.

SMITH, J.E., GOODMAN, D.S. Retinol-binding protein and the regulation of vitamin A transport. Fed. Proc. 1979; 38: 2504-2509.

SPORN, M.B., ROBERTS, A.B. Role of retinoids in differentiation and carcinogenesis. J.N.C.I. 1984; 73: 1381-1387.

SPSSX User's Guide, Edition 2. McGraw-Hill Company. USA, 1986.

STAHLEIN, H.B., BUESS,E., ROSEL, F., WIDMER, L.K., BRUBACHER, G. Vitamin A, cardiovascular risk factors and mortality. Lancet. 1982; 1: 394-395.

STEINBURG, S.M., BARKIN, J.S., KAPLAN, R.S., STABLEIN, D.M. Prognostic indicators of colon tumors. The gastro-intestinal tumor study group experience. Cancer. 1986; 57: 1866-1870.

STEMMERMANN, G.N., YATANI, R. Diverticulosis and polyps of the large intestine. Cancer. 1973; 31: 1260-1270.

STICH, H.F., STICH, W., ROSIN, M.P., VALLEJERA, M.O. Use of the micronucleus test to monitor the effect of vitamin A, beta-carotene and canthaxanthin on the buccal mucosa of betal nut tobacco chewers. Int. J. Cancer. 1984; 34: 745-750.

SWENERTON, H., SCHRADER, R., HURLEY, L.S. Zinc-deficient embryos: reduced thymidine incorporation. Science. 1969; 166: 1014-1015.

TAKASE, S., ONG, D.E., CHYTIL, F. Cellular retinol-binding protein allows specific interaction of retinol with the nucleus in vitro. Proc. Natl. Acad. Sci. USA. 1979; 76: 2204-2208.

THOMPSON, J.N., ERODDY, P., MAXWELL, W.B. Simultaneous fluorometric determination of vitamin A and E in human serum and plasma. Biochem. Med. 1973; 8: 403-414.

THOMPSON, J.N., HOWELL, J.M., PITT, G.A.J. Vitamin A and reproduction in rats. Proc. Roy. Soc. London. 1964; 159: 510-535.

THOMPSON, J.N. Interference from carotenoids in the fluorometric analysis of serum vitamin A in cancer studies. Eur. J. Cancer Clin. Oncol. 1983; 19: 1645-1646.

TRICHOPOULOS, D., POLYCHROMOPOULOU, A. Epidemiology, diet and colorectal cancer. Eur. J. Cancer Clin. Oncol. 1986; 22: 335-338.

TYLER, H.A., BARR, L.C., KISSIN, M.W., WESTBURG, G., DICKERSON, J.W.T. Vitamin A and non-epithelial tumours. Br. J. Cancer. 1985; 51: 425-427.

TYLER, H.A., DICKERSON, J.W.T. Determination of serum retinol in cancer studies. Eur. J. Cancer Clin. Oncol. 1984; 20: 1205-1206.

UNDERWOOD, B.A. The determination of vitamin A and some aspects of its distribution, mobilization and transport in health and disease. World Rev. Nutr. Diet. 1974; 19: 123-172.

UNDERWOOD, B.A. Vitamin A and cancer prevention conference- an introduction. J.N.C.I. 1984; 73: 1371-1372.

VALBERG, L.S., FLANAGAN, P.R., KERTESZ, A., BONDY, D.C. Zinc absorption in inflammatory bowel disease. Dig. Dis. Sci. 1986; 31: 724-731.

VALLEE, B.L. Zinc biochemistry in normal and neoplastic growth processes. Experientia. 1977; 33: 600-601.

VAN CAMPEN, D., HOUSE, W.A. Effect of a low protein diet on an oral dose of Zn and on tissue concentrations of zinc and copper in rats. J. Nutr. 1974; 104: 84-90.

VAN DE VELDE, C.J.H., BLOEM, R.M., ZWAVELING, A. Management of colorectal cancer. Eur. J. Cancer Clin. Oncol. 1986; 22: 339-344.

VAN STEVENICK, J., DE GOEIJ, A.F.P.M. Determination of vitamin A in blood plasma of patients with carotenaemia. Clin. Chim. Acta. 1973; 49: 61-64.

WAHI, P.N., KEHAR, U., LAHIR, B. Factors influencing oral and oropharyngeal cancers in India. Br. J. Cancer. 1965; 19: 642-660.

WALD, G. Molecular basis of visual excitement. Science. 1968; 162: 230-239.

WALD, N.J., BOREHAM, J., HAYWARD, J.L., BULBROOK, R.D. Plasma retinol, B-carotene and vitamin E levels in relation to the future risk of breast cancer. Br. J. Cancer. 1984; 49: 321-324.

WALD, N.J., IDLE, M., BOREHAM, J. Low serum vitamin A and subsequent risk of cancer: Preliminary results of a prospective study. Lancet. 1980; 2: 813-815.

WATSON, D.W. Ulcerative colitis, autoimmune epiphenomena, and colonic cancer. Cancer. 1974; 34: 867-871.

WEGENER, M., BORSCH, G., SCHMIDT, G. Colorectal adenomas: Distribution, incidence of malignant transformation, and rate of recurrence. Dis. Colon Rectum. 1986; 29: 383-387.

WEGENER, W.S., ROMANO, A.H. Zinc stimulation of RNA and protein synthesis in Rhizopus nigricans. Science. 1973; 142: 1669-1670.

WEISS, N.S., DALING, J.R., CHOW, W.H. Cholecystectomy and the incidence of cancer of the large bowel. Cancer. 1982; 49: 1713-1715.

WILLETT, W.C., POLK, B.F., UNDERWOOD, B.A., STAMPFER, M.J., PRESSEL, S., ROSNER, B., TAYLOR, J.O., SCHNEIDER, K., HAMES, C.G. Relation of serum vitamins A and E and carotenoids to the risk of cancer. N. Eng. J. Med. 1984a; 310: 430-434.

WILLET, W.C., STAMPFER, M.J., UNDERWOOD, B.A., SAMPSON, L.A., HENNEKENS, C.H., WALLINGFOED, J.C., COOPER, L., HSIEH, C.C., SPEIZER, F.E. Vitamin A supplementation and plasma retinol levels: A randomized trial among women. J.N.C.I. 1984b; 73: 1445-1448.

WILLETT, W.C., STAMPFER, M.J., UNDERWOOD, B.A., TAYLOR, J.O., HENNEKENS, C.H. Vitamins A, E, and carotene: Effects of supplementation on their plasma levels. Am. J. Clin. Nutr. 1983; 38: 559-566.

WOLBACH, S.B., HOWE, P.R. Epithelial repair in recovery from vitamin A deficiency. J. Exp. Med. 1933; 57: 511-527.

WOLBACH, S.B., HOWE, P.R. Tissue changes following deprivation of fat-soluable vitamin A. J. Exp. Med. 1925; 42: 753-777.

ZACHMAN, R.D. The stimulation of RNA synthesis in vivo and in vitro by retinol (vitamin A) in the intestine of vitamin A deficient rats. Life Sci. 1967; 6: 2207-2213.

ZIEGLER, R.G., MASON, T.J., STEMHAGEN, A., HOOVER, R., SCHOENBURG, J.B., GRIDLEY, G., VIRGO, P.W., FRAUMENI, J.F. Caotenoid intake, vegetables and the risk of lung cancer among white men in New Jersey. Am. J. Epidemiol. 1986; 123: 1080-1093.

ZILE, M., DE LUCA, H.F. Vitamin A and ribonucleic acid synthesis in rat intestine. Arch. Biochem. Biophys. 1970; 140: 210-214.