

AN INVESTIGATION OF THE ASSOCIATION OF COLONIC ADENOMATOUS  
POLYPS AND NUTRITIONAL STATUS OF RETINOL AND CAROTENE

by

Cecilia Ann Magnetti

Thesis submitted to the Graduate Faculty of the  
Virginia Polytechnic Institute and State University  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Nutrition

APPROVED:

---

R. Webb, Chairman

---

P. E. Bowen

---

J. Donohue

---

G. Bunce

December, 1985  
Blacksburg, Virginia

AN INVESTIGATION OF THE ASSOCIATION OF COLONIC  
ADENOMATOUS POLYPS AND NUTRITIONAL STATUS OF  
RETINOL AND CAROTENE

by

Cecilia Ann Magnetti

(ABSTRACT)

The hypothesis was evaluated that lower dietary consumption of carotene or retinol or lower serum levels of beta carotene or retinol are associated with the development of colonic adenomas. To evaluate this hypothesis, selected patients who were to undergo colonoscopy to determine polyps status were asked to undergo a battery of tests to assess nutritional status. These tests included a dietary and demographic questionnaire, serologic assessment of beta carotene and retinol, and a dark adaptation test.

One hundred male subjects were evaluated. Fifty-seven were found to have colonic adenomatous polyps. Cases and controls appeared to be well matched for demographic characteristics. There were no statistically significant

differences for any nutritional parameter between cases and controls, but cigarette smoking was more prevalent among cases than controls ( $p < 0.05$ ).

Because nonsignificant negative associations with colonic adenomas were observed for some of the nutritional parameters, it is concluded that additional subjects should be studied, as planned.

## ACKNOWLEDGEMENTS

The author owes a debt of gratitude to several persons. First there is my husband, J. Walter Kikendall, who not only recruited the subjects but acted as tutor, proof-reader, and as a source of emotional support. My father has always stressed the importance of education, and he and other members of my family receive special thanks for their life-long encouragement and support. My son William C. Opdyke also helped by entering much of the dietary data into the computer.

Two members of my committee receive special acknowledgement. If Dr. Joyce Donohue hadn't made biochemistry so interesting and challenging, I might never have progressed beyond my first course in graduate school. I will never forget her inviting me to her home to study for my "Vitamins" course. And Dr. Phyllis Bowen has succeeded in making the project much more than I initially had intended. She secured the funding, prodded me, corrected me, and acted as my friend.

Dr. Maria Sapuntzakis is to be thanked for performing the biochemical analysis and for explaining her procedures

to me. Mary Burgess is also to be thanked for administering the questionnaire, processing the blood, programming the computer and entering the questionnaire data.

Dr. Patricia Guenther has served as a constant advisor, source of information, and friend. Without her constant encouragement, I might have discontinued. She receives my heart-felt thanks. Dr. Sam Bowen helped to salvage a "Lost Weekend" with his computer wizardry and good humor, both of which were appreciated.

Finally, I wish to thank all of the committee members for their time, thought, helpful comments, advice, and, above all their patience.

## TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
Chapter	page
I. THE PROBLEM AND ITS SETTING	1
1.1 INTRODUCTION	1
1.2 HYPOTHESIS	3
1.3 RESEARCH OBJECTIVES	3
1.4 TERMINOLOGY	4
II. THE REVIEW OF LITERATURE	7
2.1 ANIMAL STUDIES	8
2.2 EPIDEMIOLOGIC DIETARY STUDIES IN HUMANS	15
2.3 SEROLOGIC STUDIES IN HUMANS	24
2.4 CLINICAL TRIALS IN HUMANS	30
2.5 SUMMARY OF AVAILABLE HUMAN DATA	31
2.6 POSSIBLE MECHANISMS OF ACTION	32
III. MATERIALS AND METHODS	35
3.1 PATIENT POPULATION	35
3.2 RECRUITMENT	37
3.3 STUDY PROCEDURES	38
3.3.1 SERUM SAMPLES	39
3.3.2 DEVELOPMENT AND ADMINISTRATION OF THE DIETARY QUESTIONNAIRE	40
3.3.3 DARK ADAPTATION TEST	43
3.3.4 SERUM ANALYSIS	44
3.3.5 CLASSIFICATION OF PARTICIPANTS AS CASES OR CONTROLS	47
3.3.6 CLASSIFICATION OF PARTICIPANTS AS SMOKERS OR NONSMOKERS	49
3.3.7 VALIDITY OF THE EXPERIMENTAL PROCEDURES	49

Chapter	page
3.4 STATISTICS	52
IV. RESULTS	54
4.1 DESCRIPTION OF THE STUDY POPULATION	54
4.2 DEMOGRAPHIC DATA FOR CASES AND CONTROLS	57
4.3 COMPARISON OF STUDY PARAMETERS FOR CASES AND CONTROLS	59
4.4 CORRELATIONS OF STUDY PARAMETERS WITH EACH OTHER	61
4.5 COMPARISON OF CIGARETTE SMOKERS AND NON-SMOKERS	65
4.6 LOGISTIC REGRESSION	68
4.7 ODDS RATIOS FOR PARAMETERS OTHER THAN SMOKING	69
V. DISCUSSION	74
VI. SUMMARY AND CONCLUSIONS	78
LITERATURE CITED	81
APPENDIX	
1. CHECK-LIST OF EXCLUSIONS	90
2. DOCUMENTS FOR INFORMED CONSENT	91
3. DIETARY QUESTIONNAIRE	94
4. RAW DATA FOR THE STUDY SAMPLE	101
VITA	112

## Chapter I

### THE PROBLEM AND ITS SETTING

#### 1.1 Introduction

Approximately 120,000 new cases of adenocarcinoma of the colon develop in this country each year (1). Colon cancer now ranks second as a cause of cancer deaths in American males and third in American females. Apart from surgery which is effective for early colon cancer, no other therapy has proven more than minimally effective for this disease. Approximately 60% of those who develop colon cancer will ultimately die of this disease (1).

Current evidence suggests that virtually all colon cancer develops within previously benign adenomatous polyps of the colon (2). For this reason adenomatous polyps are often referred to as premalignant lesions. A study of the cause of colonic adenomatous polyps may also help to elucidate the cause of colon cancer, and an inhibitor of colon polyps might also inhibit the formation of colon cancer.

A number of previous investigations, which will be reviewed in Section 2.2, have suggested that vitamin A may protect against certain forms of cancer. Some of these investigations have documented lower serum levels of vitamin A in patients with cancer than in control patients without cancer. Several other retrospective studies have found that cancer patients report having consumed lesser amounts of foods containing vitamin A and/or carotenoids than control subjects without cancer. Unfortunately these results must be interpreted with caution as the presence of cancer may have depressed the serum levels and may have altered the cancer patients' perception of past dietary consumption.

A number of well-controlled studies involving experimental animals (Section 2.1) have demonstrated a protective effect for vitamin A or beta carotene against carcinogenesis. This effect has not been shown in some studies, however, suggesting that the protective effect may be specific for certain animals, organs, or carcinogens. Because of the conflicting results in previously performed animal studies, it is doubtful that additional animal studies will unequivocally answer the question of whether vitamin A may protect humans from cancer.

Because the evidence to date suggests, but does not

conclusively demonstrate, that vitamin A or certain carotenoids may inhibit cancer in humans, additional evidence is needed. This study has investigated the association between dietary and serum vitamin A and carotene and the presence of colonic adenomatous polyps, taking advantage of the fact that these polyps are premalignant lesions but cause no systemic illness which might alter serum levels or dietary consumption of the provitamins or retinol.

## 1.2 Hypothesis

That lower consumption of vitamin A or carotene or marginal or lower serum levels of vitamin A or beta carotene are associated with the presence of colonic adenomatous polyps.

## 1.3 Research Objectives

This research has attempted to answer the following specific questions:

- 1) Is the current level of consumption of total vitamin A or retinol (animal sources and pills only) or carotene (vegetable sources) associated with the presence

or absence of adenomatous colonic polyps?

2) Is the level of consumption of these nutrients 5-10 years ago associated with the presence or absence of adenomatous colonic polyps?

3) Is the serum level of retinol or beta carotene associated with the presence or absence of colonic adenomatous polyps?

4) Is the result of a simple dark adaptation test (a functional test of vitamin A sufficiency) associated with the presence or absence of colonic polyps?

#### 1.4 Terminology

Fruits and vegetables contain a wide variety of carotenoids which widely vary in their convertibility into vitamin A (3). Until recently, it was assumed that any protective effect of these compounds against cancer was due to their conversion into retinol. Currently available food composition tables derive their vitamin A data primarily from modifications of the methods (4) of the Association of Official Analytical Chemists (AOAC). These methods distinguish the relatively nonpolar carotenoids such as alpha and beta carotene and lycopene from more polar compounds. But lycopene which has no provitamin A

activity is measured while some more polar compounds having provitamin A activity are not measured by the AOAC methods (3, 5). An example of the latter is beta cryptoxanthin which has provitamin A activity but is incompletely measured by the AOAC procedures. Since these compounds do vary in their provitamin A activity, the assumptions adopted to convert the resulting analytical data into retinol equivalents (RE) or international units (IU) introduce error. For the purposes of conversion, 1 RE is considered to be equivalent to 3.3 IU of vitamin A activity when the source is retinol, 10 IU when the source is beta carotene, or 5 IU when the source is unknown (6).

Since the food tables express the data only in terms of vitamin A activity (IU or RE), a problem in terminology is also created. There is no generally accepted term to designate the compounds with provitamin A activity but to exclude carotenoids without provitamin A activity. Since it is modern technology such as high performance liquid chromatography which has increased the awareness of these distinctions, most previous dietary cancer surveys have understandably used the term "carotene" or "beta carotene" to designate the compound(s) presumed to be active against cancer. For lack of a better term, this paper will also adopt the term "carotene" to refer to all dietary

carotenoids which are measured by the AOAC procedures and therefore included in nutrient composition tables. It is recognized that this term is imprecise. The more precise term "beta carotene" will be employed when it is appropriate in the context. Recently it has been proposed that the mechanism for protection against cancer may be unrelated to the provitamin A activity of carotenoids (Section 2.6). Thus, the truly protective compounds may or may not be measured by the AOAC methods and may or may not be included in the "carotene" values listed in currently available nutrient analysis tables. The future availability of more accurate food composition data may permit reassessment of the results of this study, testing the associations between individual dietary carotenoids and xanthophylls and the incidence of colonic adenomas.

## Chapter II

### THE REVIEW OF LITERATURE

The evidence to support a protective effect for retinol or carotene against human carcinogenesis comes primarily from four sources: prospective epidemiological studies of dietary intake and future cancer incidences; case-control studies comparing dietary intake of cancer patients and controls; biochemical studies comparing retinol or carotene levels in cancer patients and controls; and a few prospective biochemical studies utilizing blood stored years previously to compare retinol or carotene levels from individuals who ultimately developed cancer and matched controls. In addition, a number of controlled trials involving experimental animals have shown that either retinol or beta carotene can prevent or inhibit experimental carcinogenesis in these animals. Finally an uncontrolled trial has demonstrated inhibition of the formation of buccal mucosal cell micronuclei following the administration of vitamin A and beta carotene to a group of

people with a high incidence of this lesion (7). This section will review these various types of evidence and will conclude with a summary of the proposed mechanisms whereby retinol or beta carotene might act to inhibit carcinogenesis.

### 2.1 Animal Studies

The hypothesis that vitamin A might inhibit carcinogenesis is hardly new. Investigators had already discovered that a deficiency of vitamin A led to metaplastic changes in the urinary bladder, respiratory tract, and gastrointestinal tract even prior to the discovery of the role of vitamin A in the visual cycle (8). As early as 1926, it was noted that vitamin A-deficient rats were prone to develop carcinoma of the stomach (9). Many investigators since then have repeatedly studied the effect of vitamin A deficiency or supplementation on experimental carcinogenesis in animals. A number of these studies (10-22) are summarized in Table 1. These will not be discussed individually as the general design is similar for all studies. In each study summarized in Table 1, different groups of animals received different amounts of retinol (or other retinoid or carotenoid). All animals or

Table 1. Animal Studies of the Effect of Retinoids on Carcinogenesis.

Animal(Reference)	Carcinogen	Site	Supplement	Controls	Timing*	Frequency of Tumors Supplemented	Controls
Hamster(10)	BAP(a)	forestomach	retinyl palmitate	replete	after	9/72	16/72
Hamster(10)	BAP	lung	retinyl palmitate	replete	after	1/46	13/53
Rats(11)	DMH(b)	colon	retinyl palmitate	replete	both	9/20	13/20
Rats(11)	DMH	small bowel	retinyl palmitate	replete	after	14/20	12/20
Rats(12)	AFB(c)	colon	retinyl palmitate	deficient	both	0/50	6/50
Rats(12)	AFB	liver	retinyl palmitate	deficient	both	19/50	11/50
Rats(13)	DMH	intestine	retinyl palmitate	replete	both	15/25	21/37
Rats(14)	DMBA(d)	breast	retinyl methyl ether	replete	after	11/30	35/50
Rats(14)	DMBA	breast	retinyl acetate	replete	after	7/20	35/50
Rats(15)	AFB(c)	liver	retinyl acetate	deficient	both	50/100	91/108
Rats(15)	AFB	colon	retinyl acetate	deficient	both	5/100	31/108
Rats(15)	DMH	colon	13-cis-retinoic acid	replete	both	8/20	20/20
Rats(16)	MNNG(e)	colon	retinyl palmitate	deficient	both	17/24	5/20
Rats(17)	BAP	lung	13-cis-retinoic acid	not stated	after	0/275	6/109
Rats(18)	OH-BEN(f)	bladder+	13-cis-retinoic acid	replete	after	57%	87%
Hamster(19)	BCP(g)	pancreas	various retinoids	replete	after	109/392	18/50
Rats(20)	azaserine	pancreas	various retinoids	replete	after	30/390	10/48
Rats#(20)	azaserine	liver	various retinoids	replete	after	28/196	2/24
Mouse(21)	OH-BEN	bladder+	13-cis-retinoic acid	replete	after	7/44	18/46
Hamster(22)	BAP	lung	retinyl acetate	replete	after	59/73	48/83

\* Timing of Supplement relative to initiation of carcinogen

+ urinary bladder

(e)N-methyl-N'-nitro-N-nitrosoguanidine

# female rats

(f)N-butyl-N-(4-hydrobutyl)nitrosamine

(a)benzo[a]pyrene

(g)N-nitrosobis(2-oxopropyl)amine

(b)1,2-dimethylhydrazine(a)anthracene

(c)afatoxin-B

(d)7,12-dimethylbenz(a)anthracene

a subset of animals were also treated with a carcinogen. Animals were then observed for the development of carcinoma. As can be seen in Table 1, several (but not all) of these studies have demonstrated that the incidences of tumors in response to carcinogens can be increased by vitamin A deficiency or decreased by supplementation with retinol or a carotenoid. Of possible practical importance for human applications, vitamin A was found to inhibit carcinogenesis even when administered days or weeks subsequent to the administration of the carcinogen (10, 18).

The work of Rettura et al. (23) and Seifter et al. (24) differs in design from the papers included in Table 1 and will be reviewed in detail because these efforts present dramatic evidence that retinol or beta carotene can inhibit even an established cancer. These investigators performed several experiments using C3HBA adenocarcinoma cells. These cells are maintained as tumors growing on live mice and can be transplanted directly to other mice to produce cancer in the recipient. In the first set of experiments (23), recipient mice were all fed diets which provided approximately double the National Research Council's recommended dietary allowance for vitamin A for mice. In addition, groups of mice also received supplemental retinol (150,000 IU/kg diet) or beta carotene

(90 mg/kg diet). On the fourth day all animals were inoculated with tumor cells under the skin. Both retinol ( $p < 0.02$ ) and beta carotene ( $p < 0.05$ ) reduced the incidence of tumors which grew to detectable size. Both retinol and beta carotene also increased the latent period prior to the development of detectable tumors.

In another set of experiments by Rettura et al. (23), mice were first inoculated with tumor cells. Then 13 days later when the tumors had reached mean diameters of 6.2 mm, some of the animals were switched to diets supplemented with retinol or beta carotene. The two supplemented groups of animals exhibited equal and marked reduction of tumor growth compared to animals receiving no supplement, and these animals survived longer than the animals not receiving supplements (60 vs. 41 days).

In a final set of experiments (24), mice were inoculated on the thigh with tumor cells on day one. On day thirteen, these mice were randomly assigned to one of several groups. Various groups did or did not receive radiation to the tumor, and either continued to receive the standard vitamin A-replete chow or were switched to otherwise identical chow which had been supplemented with additional retinol or beta carotene. In the mice that did not receive radiation, either retinol or beta carotene

retarded tumor growth equally, so that the tumor size doubling time increased from about seven days to about fourteen days. Nevertheless, tumors continued to grow in all animals. Irradiation alone caused tumor regression for approximately eighteen days, but progressive tumor growth was observed thereafter. Irradiation alone increased mean survival time to approximately 84 days. The combination of local irradiation plus either beta carotene or retinol resulted in complete tumor regression in all of the 48 animals. In four animals the tumors recurred and caused death, but 43 of the remaining 44 mice survived without palpable tumor for at least one year. (One mouse suffered an anesthetic death.) These animal experiments demonstrate rather conclusively an anti-cancer effect for both retinol and beta carotene.

Several of the studies listed in Table 1 deal specifically with colon cancer. Newberne and Rogers (12) fed retinyl palmitate to three groups of Charles River rats beginning at weaning. Each group also received aflatoxin B, a known carcinogen, mixed in with rat chow on a chronic basis for as long as twenty-four months. Six of 50 rats receiving only 5 mg/day of retinyl palmitate (marginal vitamin A deficiency) developed colon cancer. No rat receiving a larger dose of retinyl palmitate developed

a colon tumor. Rogers, Herndon, and Newberne (13) performed similar experiments using dimethylhydrazine as the carcinogen and found that high levels of retinyl palmitate in the diet did not reduce the number of rats developing colon cancer (approximately 60%); however, the retinyl palmitate did seem to reduce the number of tumors per rat. Unfortunately, there were several subgroups of rats differing in the dose of dimethylhydrazine as well as the dose of retinyl palmitate, and the number of rats in each subgroup was small, making interpretation of this study difficult. A study by Newberne and Suphakarn (15) showed that 31 of 108 rats marginally deficient in vitamin A developed colon cancer in response to aflatoxin B, but that only 5 of 57 rats given excess retinyl acetate and aflatoxin B developed this cancer. Rogers et al. (13) also reported a protective effect for 13-cis-retinoic acid against colon cancer induced by dimethylhydrazine, with all 20 vitamin A-replete control rats developing cancer compared to only 8 of 20 rats supplemented with 13-cis-retinoic acid. Fleischer et al. (11) found that rats given a low fiber diet and dimethylhydrazine were less likely to develop colon cancer if they were also given a toxic dose of retinyl palmitate, but the reduction in carcinogenesis was not statistically significant. Finally, Narisawa et

al. (16) administered N-methyl-N'-nitro-N-nitrosoguanidine into the rectum of Fischer rats. They found that vitamin A deficiency suppressed, rather than enhanced, carcinogenesis in this model. Thus, only 9 of 20 rats on a vitamin A-free diet developed colonic adenoma or adenocarcinoma compared to 23 of 24 rats receiving a diet supplemented with retinyl palmitate.

In summary, animal experiments show a protective effect for vitamin A or beta carotene against carcinogenesis in several studies involving several different organs in several different experimental animals. Caution is in order, however, because the incidence of cancer was actually higher in some studies (12, 16, 22) in the retinoid-treated animals than in the control animals. As for the colon, a protective effect for vitamin A is suggested but has not been conclusively demonstrated. It is possible that differences in carcinogen, method of carcinogen administration, type of tumors used as an end point, and other factors including the vitamin A status of the control animals may explain the differing results. It is possible that vitamin A protects against some forms of cancer but potentiates the development of others. It is also important to note that the vitamin A-supplemented experimental diets administered in some studies are grossly

over-abundant in vitamin A. Thus the supplemented animals may have been close to toxicity from retinol, so that implications for a protective effect of vitamin A under more natural conditions are less certain.

## 2.2 Epidemiologic Dietary Studies In Humans

Bjelke has performed several studies (25) comparing the dietary habits of cancer patients and controls. In Norway he administered dietary questionnaires to hospitalized patients with cancer of the stomach (n=228), colon (n=162), or rectum (n=116) and to hospitalized age- and sex-matched controls (two controls for each cancer patient). Patients and controls were asked to recall the frequency of consumption a year prior to admission for a variety of foods. The mean consumption frequencies reported by patients with stomach cancer were lower than the control means by more than two standard errors for cabbage, cauliflower, cucumbers, tomatoes, peas, oranges, apples, and alcohol, but not for carrots or for a vitamin A index derived from the consumption data. Consumption reported by the colonic cancer patients was lower than for their controls by more than two standard errors for salted fish, carrots, and coffee. Lesser differences were found

for lamb/mutton, pancakes/waffles, cabbage, cucumber, and milk. As compared to controls, rectal cancer patients reported lower frequencies of use exceeding two standard errors for smoked fish, fresh/frozen fish, bacon/side pork, macaroni/spaghetti, flatbread/rye crisp, rutabagas, and beans. Values for the vitamin A index calculated from the dietary data were more than twice the standard error lower for patients with colorectal cancer (combined data from the colon cancer and rectal cancer series) compared to controls. Carrots and cabbage were the two individual vegetable items that discriminated best between cases and controls. Interestingly, the observed protective effect of dietary vitamin A was confined to individuals less than 60 years of age.

Bjelke (25) used similar techniques to compare 52 esophageal, 83 stomach, 259 colonic, and 114 rectal cancer patients to 1657 controls in Minnesota. Diet did not differentiate cases of esophageal cancer from controls, but dietary factors relative to gastric and colorectal cancer were very similar in Minnesota to those observed in Norway by the same investigator.

These retrospective studies of Bjelke may be criticized for their reliance upon the ability of interviewers to obtain accurate dietary histories from

individuals who have, in many cases, only recently been informed of a diagnosis of cancer and who may already be suffering the symptoms associated with the disease. Since cancer requires several years to evolve and to be diagnosed, it is the past diet rather than the recent diet which is of interest, except to the extent that recent diet is reflective of long-term dietary habits.

Bjelke also performed a prospective study (26) which avoids these problems. He mailed dietary questionnaires to Norwegian males and received usable replies from 8,278 who were 45 years of age or older. After five years, 53 of these men had developed lung cancer. These were age-matched with 106 controls drawn from the original survey. A vitamin A index was derived from the reported consumption of several vegetables, milk, and eggs. Individuals with a vitamin A index exceeding five units had a relative risk of developing lung cancer of 0.31 compared to individuals with a vitamin A index less than five units ( $p < 0.01$ ). The index was negatively associated with cancer incidence at all levels of cigarette smoking.

This study was updated in 1983 to include 153 lung cancer cases among men and women (27). The relative risk for lung cancer was about 0.62 for the highest consumers compared to the lowest consumers of vitamin A. The data

suggested that both total vitamin A and carotene intake were protective. The food items with the strongest negative association with cancer were milk, carrots, cabbage, and tomatoes.

Several additional epidemiologic studies comparing diets of cancer patients and controls may also be divided into prospective and retrospective studies. The largest prospective study (28) was conducted in Japan where more than 265,000 persons were interviewed in 1965. More than 7,000 of these died of cancer in the intervening ten year period. Daily consumers of green-yellow vegetables, the dominant source of carotenoids in Japan, developed fewer cases of lung cancer (relative risk of 0.55-0.85) compared to less frequent consumers of these vegetables. The effect was still observed when smoking was controlled in the analysis. Similar effects were observed for colon cancer, stomach cancer, uterine cancer, and prostate cancer, although only summarized data have been reported for these tumors.

The only other prospective study of diet and cancer risk (29) was conducted in Chicago. Two thousand men were interviewed in 1957. The original raw data have been lost, but the original data had been partially analyzed prior to being lost, so that a value for a retinol index derived

from the reported consumption of eight foods was available for each participant. Additionally, a value for a carotene index was available for each participant, but this index was derived from the reported consumption of only three food groups, "vegetables," "soup," and "fruit." Thus a daily serving of carrots contributed no more weight to this index than a daily serving of green beans or eggplant. Participants were placed into quartiles based on their calculated carotene index and retinol index. Over the next 19 years, 33 men developed lung cancer and 175 men developed other types of cancer. The relative risk of lung cancer was seven times greater for the men in the lowest compared to men in the highest quartile for carotene index ( $p=0.003$ ). The carotene index was not significantly associated with the incidence of other types of cancer lumped together or with any other single type of cancer (including colon cancer). The retinol index was not significantly associated with the incidence of lung cancer or with "other" cancer. That a statistically significant difference for lung cancer could be shown is truly remarkable considering the relatively small population, the crudity of the index, and the low incidence of lung cancer. This may represent only the increased probability of finding at least one statistically significant difference

( $p < 0.05$ ) when multiple tests for significance are performed upon the same data, or it may mean that there is a truly important protective effect for one or more components of the carotene index against lung cancer.

The remaining studies relating diet and cancer are all retrospective. To a large extent, these studies share the problem of reliance upon the accuracy of a dietary history obtained from sick cancer patients. It is possible that such sick patients may uniformly or selectively underestimate their past consumption of foods. Studies involving very early cancer or even precancerous lesions might be preferable in this regard, but only two such studies have been reported. In one of these (30) a dietary history was obtained from Japanese subjects at the time of a screening procedure for early cancer of the stomach. The reported dietary intake of vitamin A was lower in patients with early gastric cancer than in those without. In the other study (31) food diaries reported by patients were analyzed using a computer data base containing 2400 food items. Patients with severe dysplasia of the uterine cervix were found to report lower consumption of foods containing vitamin A ( $p < 0.05$ ) and carotene ( $p < 0.01$ ) than controls matched for age, parity, ethnicity, and socioeconomic status.

All other reported retrospective dietary studies have compared patients with recently diagnosed cancer with controls without cancer. Several such reports have emanated from Roswell Park Institute (32). These investigators found that individuals in the lowest of three groupings for vitamin A consumption had a significantly increased risk for cancer in general compared with the highest vitamin A consumption group. Vitamin A or carotene seemed to protect against cancer of the lung (33), urinary bladder (34), mouth (35), larynx (36), cervix (37), ovary (38) and breast (39). Vitamin A or carotene consumption seemed to have no effect on the incidence of colorectal (40), esophageal (41), or skin (42) cancer and appeared to have an adverse effect on cancer of the prostate (42). In these studies the vitamin A index employed was primarily but not entirely an estimate of carotene consumption.

Preliminary data from a study still in progress in Hawaii (43) indicated that individuals in the lowest quartile for vitamin A consumption have approximately a two-fold increased risk of lung cancer compared to those in the highest quartile. Nonsignificant trends were, thus far, noted for prostate cancer and urinary bladder cancer.

Other retrospective case control studies have shown a decreased risk for lung cancer in Singapore Chinese who

report relatively high carotene intakes (44) and in males (but not females) in London with relatively high reported intakes of vitamin A but not carotene (45). Samet et al. (46) found that the risk of lung cancer was increased in New Mexico among low consumers of carotene but not retinol, but this observation did not hold for Hispanics or for current smokers. Also Ziegler et al. (47) and Ershow et al. (48) have shown in similar studies that relatively low reported consumption of carotene but not retinol is associated with lung cancer in white males in New Jersey (47) and in males and females in Texas (48). Women in North Carolina (49) with oral or pharyngeal cancer reported a lower consumption of fruits and vegetables than controls ( $p < 0.002$ ). A French study (50) showed no difference in the reported consumption of carotene or retinol between 200 esophageal cancer cases and controls, but a study from Iran (51) showed a large relative deficiency in the reported consumption of green vegetables among esophageal cancer patients compared to controls. Finally, a study by Modan et al. (52) in Israel found that 406 patients with gastrointestinal cancer (site not specified) reported that they consumed several carotene-containing foods other than carrots less frequently than controls. However, their consumption of carrots was not different, so that when

total daily carotene intake was estimated, there was no apparent protective effect of carotene. Retinol consumption did not correlate with cancer in this study. These results were interpreted as suggesting that some component of the carotene sources other than carotene itself was protective.

In summary, prospective and retrospective epidemiologic studies indicate that consumers of relatively more carotene and/or retinol are at lower risk for the development of several cancers than are consumers of lesser amounts of these nutrients or the foods that contain them. The data are most compelling for lung cancer because this cancer is most common and has been most studied. Only two groups have studied colon cancer, both retrospectively. Bjelke (25) found that patients with colon cancer were less likely to be avid consumers of foods containing carotene or retinol than controls, but Graham et al. found no difference (40). Graham et al. did find, however, that other vegetables, notably cabbage, broccoli, and cauliflower, seemed to protect against colon cancer. It is probable that individuals who eat carotene-containing vegetables also eat these cruciferous vegetables in large quantity. Wattenberg and Loub (53) and Wattenberg (54) have shown that various indoles contained in cruciferous

vegetables are protective against certain forms of experimental cancer in animals. Thus it is possible that Bjelke's observations (25) are due to the confounding variable of indole consumption. Unfortunately, the study by Modan et al. (52) in Israel does not shed any additional light, in part because the cancer site within the gastrointestinal tract was not specified and also because the food consumption data reported in this study did not include any cruciferous vegetables. It is apparent that additional studies must be performed before the possible effect of dietary vitamin A or carotene on colon cancer can be fully understood.

### 2.3 Serologic Studies In Humans

Another approach to the assessment of a possible protective effect of carotene or retinol against cancer is to compare serum or plasma levels of these compounds in cancer patients and controls or in patients destined to develop cancer and controls. The former type of study is in essence a retrospective study since the current serum or plasma level is used as a reflection or estimate of the level at the time the cancer was initiated or promoted. Obviously this type of study is much easier to perform but

is less meaningful because cancer may conceivably cause depression of serum levels. The truly prospective study is much more costly and time consuming, as serum must be collected from hundreds or even thousands of individuals and saved for several years before adequate numbers of cancer cases are diagnosed from the study population.

Published studies (55-60) in which serum has been drawn from patients with diagnosed cancer and from controls for comparison of retinol and/or carotene are summarized in Table 2. These studies rather uniformly demonstrate lower retinol or total carotene levels in cancer patients, but the meaning of this finding is of doubtful validity as stated above.

Since retinol is relatively stable in frozen serum or plasma, it is possible to analyze frozen samples years later after cancer cases and controls have declared themselves from the larger population which had donated the blood samples. It is not even necessary that the original blood samples were donated with this purpose in mind. Total carotene values are not as stable, but storage at minus 80 degrees centigrade may maintain original serum concentrations (61).

In one such study Wald et al. (62) compared 86 men with cancer and 172 controls drawn from a population of 16,000

TABLE 2. Mean plasma or serum levels of vitamin A or carotene in cancer patients and controls.

COUNTRY*	SITE	SUBSTANCE	CASES	CONTROLS	P
Pakistan(55)	oral	vitamin A	22**	40	<0.01
Pakistan(55)	oral	carotene***	40	62	<0.01
England(56)	lung	vitamin A	47	59	<0.01
England(56)	lung	carotene	105	121	>0.05
U. S. A.(57)	esophagus	vitamin A	33	60	<0.001
England(58)	lung	vitamin A	46	64	<0.01
India(59)	oral	vitamin A	102	160	
India(59)	oral	carotene	68	85	
Austria(60)	head/neck	vitamin A	44	59	<0.001

\* Numbers in parenthesis denote references.

\*\* All data are reported as means in micrograms percent except (59) which has reported the vitamin A data in International Units per 100 milliliters.

\*\*\* All carotene values are for total carotene.

men who had donated blood samples one to four years earlier. Mean serum retinol was 187 IU/dl in individuals who subsequently developed lung cancer and 229 IU/dl in controls ( $p < 0.005$ ). Nonsignificant differences from the control values were noted for cancer of the gastrointestinal tract (mean 216 IU/dl), skin (mean 217 IU/dl), and kidney (mean 215 IU/dl). The risk of developing cancer at any site was 2.2 times greater in the lowest quintile of serum retinol levels than in the highest quintile.

Wald et al. (63) also reported a comparison of 39 women who developed breast cancer and 78 controls drawn from a population of 5004 women in Guernsey who had donated a blood sample seven to fourteen years previously. Controls were matched for age, menopausal status, day of the menstrual cycle, parity, family history of breast cancer, and history of benign breast disease. Retinol and beta carotene were measured by high pressure liquid chromatography (HPLC). Retinol levels were similar among cases and controls. The mean beta carotene level was 36 ug/l for cases and 50 ug/l for controls, but this difference was not statistically significant.

Another similar study was performed by Willett et al. (64, 65), using blood collected in 1973 from 4480 participants in the Hypertension Detection and Follow-Up Program.

Cancer developed in 111 subjects during the subsequent five years. These were matched with 210 control subjects. Serum retinol and total carotenoid levels were compared in the two groups. No statistically significant differences in either serum nutrient level were determined.

Participants in a study (66) of cardiovascular and peripheral arterial disease in Switzerland submitted blood samples in 1971-1973. This study is unique in that vitamin analysis was performed immediately after blood sampling, thus eliminating the possibility that storage might alter the values. Each of 129 subsequent cancer deaths (1971-1980) was matched with two controls. The mean value for plasma beta carotene was lower in patients with each type of cancer (lung, stomach, colorectal, and all others combined) than for the respective control group. The difference reached statistical significance, however, only for the lung cancer group ( $p < 0.05$ ). No significant differences were observed for plasma retinol.

Evans County, Georgia, was the site of two similar studies (67, 68). Blood samples were first drawn from over 3000 individuals in 1960-1962 and sera were frozen (67). Ninety-two of these individuals had developed cancer prior to 1972. These were each matched with two controls for age, race, and sex. Retinol was first measured in the

stored samples from cases and controls by the trifluoroacetic acid method of Neeld and Pearson (69) which gave "unusual and unstable results." For this reason, retinol was then measured by the superior fluorimetric method following alumina column separation. Retinol levels were found to be lower in the cancer group than in controls ( $p=0.003$ ). Unfortunately, the sera used in this study had been thawed and refrozen several times and it is thought that the sera from the cancer patients may have been differentially exposed to light, thawing, and refreezing (68).

A second venipuncture was performed in 1967-1969 on all members of the same cohort who returned for repeat evaluation (92% of the original cohort). Of these, 135 had developed cancer prior to 1981. These were matched with 235 controls. This time no difference in serum retinol levels was seen between the two groups (68). The mean levels for cases and controls in the second Evans County study were more than double the levels in the first study, suggesting that the results in the first study may have been erroneous.

## 2.4 Clinical Trials In Humans

No controlled clinical trials of retinol or any carotenoid in the prevention of human cancers have been reported, although several have been funded by the National Cancer Institute and are in progress. One reason for the lack of reports in this area is the long latency period for cancers to develop. Cancers may take years or decades to be clinically expressed or detected. Stich et al. (7) believe that the formation of micronuclei in the cells of the buccal mucosa represents an early step in carcinogenesis and is, therefore, an appropriate endpoint to study in a clinical chemoprevention trial. Betel nut chewers in the Philippines have a high incidence of oral cancer. The nut chewers who do not have cancer have an increased proportion of buccal mucosal cells with micronuclei. Stich et al. administered both 50,000 IU of vitamin A and 150,000 IU of beta carotene to 40 Philippino betel nut chewers twice weekly for three months. Mucosal scrapings were taken from the inside of the cheek before and after the period of supplementation. The frequency of micronuclei decreased from 4.2% to 1.4% in the supplemented subjects, but did not decrease in a group of unsupplemented subjects. No placebo was used in this trial.

## 2.5 Summary of Available Human Data

The data cited above suggest, but do not prove, that vitamin A, beta carotene, or other carotenoid(s) may inhibit human carcinogenesis. The studies which are weakest in design such as comparison of serum levels or recall of dietary intakes of cancer patients and controls have rather consistently shown lower serum levels and dietary consumption in cancer patients. However, these findings may possibly be explained as the result of the cancer rather than the cause of the cancer. Prospective studies comparing serum levels of patients who eventually developed cancer with matched controls have been less uniform in their results. Discarding the first Evans County study (67) which is of doubtful validity, five prospective studies of retinol levels (62-66, 68) and three of either total carotene or beta carotene (63-66) have been reported. Only one (62) of the five showed statistically significantly lower blood levels of retinol in patients destined to develop cancer, and only one of three (66) showed statistically significantly lower carotene levels.

Prospective studies comparing dietary consumption of patients destined to develop cancer and controls have been more consistent. Studies from Norway (26, 27), Japan (28),

and Chicago (29) have all shown lower consumption of vegetables high in carotenoids in persons destined to develop cancer. The Chicago study found no difference in retinol consumption among the cases and controls, and the design of the Norwegian and Japanese studies does not permit the separate analysis of retinol consumption.

In summary, these data are suggestive that lower carotene consumption and lower carotene blood levels are associated with the development of some types of cancer. The data for retinol are less convincing; however, it should be noted that serum vitamin A levels are not precise indicators of vitamin A nutriture in well-nourished societies (61), and that only one prospective study (29) relating retinol consumption to cancer risk has been performed. Thus a protective effect of dietary retinol should not be discounted. Instead, additional prospective studies should be performed. As an alternative, retrospective studies comparing dietary consumption and/or serum levels in healthy patients with precancerous lesions and controls may be more valid than similar studies involving sick patients with established cancer.

## 2.6 Possible Mechanisms of Action

As yet the mechanism of action of retinoids or

carotenoids in the prevention of cancer is unknown. Several mechanisms have been proposed, however.

First, it appears that retinol may participate in the formation of glycoproteins in the cytoplasm (70, 71). The existence of mannosyl retinyl phosphate in the cytoplasm is known, and its formation from GDP-mannose and retinyl phosphate has been demonstrated (72). Vitamin A deficiency reduces the formation of glycoproteins (73), and it is postulated that this may be important in the promotion of tumorogenesis. While these observations may partially explain the role of retinol in maintaining the normal differentiation of epithelial surfaces, they seem unlikely to explain the role of vitamin A in the suppression of carcinogenesis, as it is difficult to understand how deficient formation of glycoproteins in the cytoplasm could result in the uncontrolled cellular and nuclear division characteristic of cancer.

Other investigators have shown that retinol is transported to specific binding sites in the nucleus by a cellular retinol binding protein (74, 75). It is also known that the alteration of the amount of available retinol results in different rates of production of certain messenger RNA molecules (76). It is therefore conceivable that a nuclear effect of retinol could help to determine

the rate of cell division.

A third mechanism has also been proposed. A body of research (77) suggests that activated forms of oxygen are important promoters of cancer. These activated forms such as superoxide and singlet oxygen may result from ionizing radiation, may be ingested in the form of heated oils, or may be formed within the body as a by-product of respiration in the electron transport chain. It is known that a major function of carotenoids in plants is to quench reactive oxygen (78), and it is possible that beta carotene or other carotenoids or retinol may function in this manner in animals as well.

## CHAPTER III

### MATERIALS AND METHODS

#### 3.1 Patient Population

A common screening test for cancer or adenomatous polyps of the colon is the analysis of a stool sample for occult blood. If subjects with occult blood in the stool undergo colonoscopy to visualize the entire colon, approximately 50% will prove to have at least one adenomatous polyp or cancer (79).

Another group of patients undergo colonoscopy because a barium enema has shown a possible polyp. Many times this is a false-positive finding resulting from the presence of stool in the bowel at the time of the barium enema.

Healthy male patients who reported for colonoscopy at Walter Reed Army Medical Center between November 1983 and July 1985 because of occult blood in the stool or because of a barium enema that was suggestive of colonic polyps

were eligible for participation in this study. Subjects receiving colonoscopy for other reasons were not included.

Female subjects were not eligible because both oral contraceptive agents (80) and naturally occurring hormonal changes in women affect vitamin A metabolism and serum levels of vitamin A.

Subjects with a previous diagnosis of adenomatous colonic polyps or adenocarcinoma of the colon were excluded, partially to maintain the blinded nature of the study and partially to maintain comparability of subjects.

Familial polyps of the colon, Gardner's syndrome and inflammatory bowel disease are colonic diseases which predispose to colonic malignancy (81). Since the mechanisms leading to cancer are probably different in these patients, they were excluded.

Fever, infections, liver disease, thyroid disease, and kidney disease are processes that affect the metabolism of vitamin A (82, 83). Subjects with these disorders were excluded from the study.

Fat malabsorption and ingestion of non-absorbable fats impair vitamin A absorption (82, 84). Subjects in whom fat malabsorption had been diagnosed within three years prior to colonoscopy were therefore excluded. This was done because adenomatous polyps require several years to grow to

a detectable size. It was thought that recent development of malabsorption would have altered the vitamin A levels subsequent to initiation of the polyp. For similar reasons subjects with self-reported weight loss in excess of fifteen pounds in the last six months or subjects who took mineral oil, a nondigestible oil, were excluded.

As a result of eligibility requirements for medical care at Walter Reed Army Medical Center and the exclusion criteria, participants in this study represent a selected group of healthy males, most of whom have retired from the military as senior officers or senior enlisted men and who are engaged in or are retired from second careers. They are predominantly middle-class, well-travelled, and health-conscious. Availability of a wide variety of foods is not a frequent problem.

### 3.2 Recruitment

Male patients who reported to the gastroenterology clinic at Walter Reed Army Medical Center during the months of this study were interviewed by a physician to determine eligibility. A check-list of exclusions was developed and employed to facilitate the screening and recruitment process (Appendix 1). The goals and procedures of the

research project were then explained, and each eligible subject was asked to read and sign forms to document informed consent (Appendix 2).

One hundred and four male subjects were eligible and gave informed consent. Only four eligible subjects refused to participate. Thirteen additional male patients were not interviewed due to absence of the physician or other administrative problems. Two hundred, fifty-nine males were interviewed and found to be ineligible for the following reasons:

Colonoscopy performed for reason other than occult  
 blood in the stool or suspicious barium enema--140  
 Prior colonic adenoma or adenocarcinoma--70  
 Liver disease--16  
 Excessive weight loss in past six months--14  
 Alcoholism--4  
 Inflammatory bowel disease--3  
 Poor general health--3  
 Miscellaneous--9

### 3.3 Study Procedures

Most patients undergoing colonoscopy at Walter Reed Army Medical Center report to the Gastroenterology Clinic

on the morning of the procedure; a few are admitted to the hospital the day prior to the examination. Patients eat a regular meal for supper the night prior to the procedure but do not eat or drink anything on the morning of the procedure day. The bowel purge consists of four to six liters of a balanced, isosmotic electrolyte solution which is consumed orally during the morning prior to performance of colonoscopy in the afternoon. Little of this solution is absorbed, and the bowel is cleaned with a minimum of discomfort.

#### 3.3.1. Serum samples:

After informed consent was granted but either prior to initiation of the purge or very soon thereafter (i.e., always prior to the consumption of one liter), blood was drawn into a foil-wrapped 30-cc polypropylene Sarstedt Monovette syringe. The blood was allowed to clot in the syringe at room temperature for 30-180 minutes prior to centrifugation in the dark for five minutes at 3000 rpm. Following centrifugation, serum was removed into a 15 milliliter polystyrene centrifuge tube (Fisher Scientific) using a polyethylene pipette (Fisher Scientific). The serum sample was then respun at 3000 rpm for 10 minutes. Serum was removed into a second polystyrene tube using another polyethylene pipette and vortexed for 30 seconds.

Serum was then aliquotted into several 1.5 milliliter polypropylene storage tubes (Belart Products), filling each tube to allow only enough head space to permit expansion during freezing. Each tube was labelled and all samples from each participant were wrapped together in a single piece of aluminum foil which was labelled. All transfers of serum to this point were performed in a room lit only with a red light. Serum samples were stored in a freezer at minus 40 degrees centigrade and were subsequently shipped on dry ice by overnight delivery to the University of Illinois at Chicago where they were stored at minus 80 degrees centigrade until analysis.

### 3.3.2. Development and Administration of the Dietary

#### Questionnaire:

The dietary questionnaire was developed by listing all the foods in the United States Department of Agriculture Handbook 456 which contained at least 600 IU of vitamin A or carotene per serving (85). At the time the questionnaire was developed, only a portion of Handbooks 8-9 and 8-11 had been published (86, 87). The USDA had published several pamphlets of provisional tables of nutrient values which ultimately were collated into Handbook 8. Some foods listed in these sources (principally fortified foods such as cereals) did not

appear in the Handbook 456 data base, and these foods were added to the questionnaire list if the average serving contained at least 600 IU of vitamin A or carotene. Several foods containing 150-600 IU per serving (e.g., butter) were added to the questionnaire because of frequency of consumption. The list was later modified when a comparison of the checklist with 30 five-day food records obtained from young men identified several food items that contributed substantive amounts of vitamin A due to frequent consumption or large portion size (e.g., green beans). Cruciferous vegetables were also added to the list as required by another research project.

The dietary questionnaire was administered verbally by a registered dietitian. This interview was performed while the subject drank his purge solution. Neither the dietitian nor the patient was yet aware of the colonoscopy result. The same dietitian administered the questionnaire to all 100 participants.

The dietary questionnaire (Appendix 3) consisted of three parts. The first part consisted of demographic questions, the second part consisted of questions about the recent diet. This portion was intended primarily to serve as a baseline against which a patient could judge his responses to the third part of the questionnaire, but the

responses to the second part were also analyzed. The third portion assessed average dietary consumption for the period five to ten years prior to the interview.

For each food item on the questionnaire, the subject indicated frequency of consumption and usual portion size, as compared to food models demonstrated by the dietitian. The dietitian recorded these responses and subsequently entered the data into a computer. The computer was programmed to convert the quantity and frequency response for each food item into a multiple of the standard portion size per day using a spread sheet program. The value for each food item was then multiplied by a conversion factor to determine average retinol or carotene per day contributed by that particular food item, expressed as retinol equivalents. In most cases this conversion factor was obtained from Handbook 456 or Handbooks 8-9 or 8-11, but in some cases where the value listed in Handbooks 456 or 8-9 or 8-11 had subsequently been proved to be erroneous, an alternative value was obtained from the published literature. Values for each food item were then summed to obtain the average daily consumption for retinol and carotene both expressed as retinol equivalents. A figure for total vitamin A consumption was also generated. The figures for retinol consumption and total vitamin A

consumption include preformed vitamin A in pill form or as fish oil.

The dietary questionnaire also included questions concerning age, race, height, weight, highest military rank (a measure of socioeconomic status), and smoking status. For the purpose of analysis, 5 individuals who smoked cigars but not cigarettes were considered as nonsmokers.

### 3.3.3. Dark Adaptation Test:

The dark adaptation test was performed immediately after the dietary interview according to the method of Vinton and Russell (88). The test was performed in a light-proof room. The work surface was covered with a dark nonreflective material. A light fixture was hung over the work surface so that target brightness on the work surface area measured 0.015-0.017 lux when all other lights in the room were off.

With the lights on, the subject was shown a mixture of red, white, and blue disks on the work surface, and the sorting procedure was explained. The subject was then light-adapted by fixation of vision on a standard x-ray viewbox from a distance of 0.5 meter for one minute. All lights in the room were then turned out except for the test light over the work surface, and timing began. The subject had been instructed to separate the disks into three piles

by color but had been warned that he would not be able to do this until his eyes recovered from the light exposure. Once the subject was able to discern the disk colors, he began to separate them. Any disk sorted into the wrong pile was returned to the original pile by the dietitian. Timing was stopped when 100% accuracy of sorting was achieved. Two consecutive trials were run. Results from both trials were analyzed separately because previous investigators had found that the test time improved from the first to the second trial due to learning the test, but that further improvement was not observed on subsequent trials (88).

#### 3.3.4. Serum Analysis:

Serum was analyzed for retinol, retinol binding protein, beta carotene, and zinc by Dr. Maria Sapuntzakis at the University of Illinois at Chicago. For the retinol and beta carotene analysis, one of the aliquots shipped from the Walter Reed Army Medical Center was allowed to come to room temperature. Then 200 microliters of serum was deproteinized with 100 microliters of absolute ethanol containing the internal standard (1.0 microgram of retinyl acetate per milliliter) and vortexed for ten seconds. Another 100 microliters of absolute ethanol containing one percent L-ascorbic acid was then added. The mixture was

vortexed for thirty seconds and extracted twice with two milliliters of hexane containing 0.01% BHT (w/v). After each addition of hexane, the sample was vortexed for one minute and centrifuged for five minutes at 1750 rpm to separate the phases. Each time the upper phase was withdrawn with a Pasteur pipette to another test tube. After combining both upper phases, the extract (4 ml) was evaporated to dryness in vacuo using a Speed Vac Concentrator (Savant Instruments, Hicksville, NY). Evaporation was accomplished in approximately 15 minutes. The residue was reconstituted with 150 microliters of HPLC mobile phase (50% methanol, 45% acetonitrile, 5% tetrahydrofuran) and 50 microliters of anesthesia-grade ether containing 0.0001 percent BHT (Malinckrodt, Inc., Paris, Ky.). The test tubes containing the retinoid/carotenoid extract were capped and kept on ice until the analysis time. Duplicate extractions were analysed for each serum sample.

Ten microliters of reconstituted extract was injected into the HPLC system (Reodyne injector Model 7125 with a 20 microliter loop, two Model 510 Waters pumps and a Model 680 automated gradient controller) onto a Waters Novapak C18 Column and eluted isocratically with the above mentioned mobile phase (MeOH:Acetonitrile:THF=50:45:5) at a

1 ml/min flow rate. The peaks were detected with a two channel UV/visible absorbance detector (Waters, Model 440), supplied with two filters, 313 nm and 436 nm, and a Kipp and Zonen dual pen recorder (Model B041). Detector sensitivity was set at 0.005 AUFS for the 436 nm channel and 0.02 AUFS for the 313 nm channel.

Retinol and the internal standard, retinyl acetate, were measured on the 313 nm channel. Beta carotene was measured on the 436 nm channel. The peak heights were measured to the nearest 0.5 mm. The retinol level was directly measured by its ratio to the internal standard, while beta carotene was compared with its external standard and then corrected for recovery of the internal standard. Both the retinol and the beta carotene standards were purchased from Sigma Chemical Company, St. Louis, Mo. Their concentrations were determined after solubilization in ethanol using the following extinction coefficients: retinol, 1850 at 325 nm; retinyl acetate, 1565 at 328 nm; beta carotene, 2375 at 450 nm. All standards were greater than 95% pure by HPLC analysis.

Zinc Determination: Serum was analyzed for zinc because zinc deficiency results in depression of serum retinol (89). Duplicate 250 microliter aliquots of each serum sample were diluted five-fold with one milliliter of

distilled water (Dupont ACA water) in zinc-free plastic vials. Zinc standards were prepared from a 1000 ppm (mg/l) standard solution by diluting it with 5% glycerol solution in distilled water to concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, and 1 mg/liter.

A Perkin Elmer 280 atomic absorption spectrophotometer with a zinc hollow cathode lamp was used at the 213.9 nm wavelength setting. The zinc concentrations were read from a standard curve and then corrected for the five-fold dilution of the serum samples.

Retinol Binding Protein: Duplicate twenty microliter aliquots of each serum sample were placed in consecutive wells of the radial immunodiffusion plates (LC-Partigen RBP Kit from Behring Diagnostics). On each plate, the solutions of RBP standard (6 mg/dl, 2.25 mg/dl, 1.125 mg/dl) were also developed. After 72 hours, the precipitin rings were measured with the Calibrating RID Viewer (Transdyne General Corporation) and the concentrations were calculated from the standard curve.

### 3.3.5. Classification of Participants as Cases or Controls:

Following the collection of dietary data and blood samples, all participants underwent colonoscopy to determine whether polyps were present. This procedure

consisted of passing a 165-centimeter-long, flexible, steerable tube through the rectum. The tip of the tube was advanced all the way through the colon to the region of the cecum. Proper position in the cecum was confirmed by fluoroscopy. As the colonoscope was withdrawn, the fiberoptical system within the scope permitted visualization of the entire colonic wall. Any polyp which was seen was biopsied or removed by instruments which were passed through the colonoscope.

Only patients who were discovered to have biopsy-proven adenomas (51 cases) or adenocarcinomas (6 cases) were classified as the 57 cases. Patients found to have carcinoma as a result of the colonoscopy and biopsies were included as cases and were not excluded because they were unaware of the diagnosis at the time of administration of the questionnaire. All of these patients were asymptomatic for cancer and met all the entry criteria including the absence of weight loss. In four of the six cancer patients, the cancer was confined to the polyp. In order to be classified as a control, the participant must have had a colonoscopy complete to the cecum and must have had either no polyps or biopsy evidence that each polyp present was not an adenoma or carcinoma. Colonoscopy successfully classified all but four of the one hundred and four

participants according to these criteria. Data for these four unclassified subjects were not analyzed.

### 3.3.6 Classification of Participants as Smokers or Nonsmokers:

The questionnaire used in this study (Appendix 3) asked only one question concerning smoking: "How many cigarettes do you smoke per day?" Once it became apparent that cigarette smokers were more likely than nonsmokers to have colonic polyps, the questionnaire was altered to obtain a complete smoking history. Prior participants were contacted to determine a smoking history as well. On the basis of the complete history, participants were classified as smokers if they had smoked cigarettes at any time in the last five years. Smokers of cigars who did not smoke cigarettes were classified as nonsmokers.

### 3.3.7 Validity of the Experimental Procedures:

**Serum analysis:** The analytical methodology for measurement of retinol and beta carotene developed by Dr. Sapuntzakis is state-of-the-art. The University of Illinois laboratory is a participant in a joint National Cancer Institute (NCI) effort co-ordinated by the National Bureau of Standards quality assurance program for the measurement of micronutrients including serum retinol, beta carotene and zinc. The laboratory's successful measurement

of unknown samples was recently praised by a representative of the National Bureau of Standards at a national meeting of principle investigators of NCI-funded chemoprevention trials.

The methods used for separation and storage of the samples conform to those recommended by Olson (61) with the exception of his recommendation to flush the surface of the frozen serum with nitrogen prior to sealing the vials, so that oxygen is excluded. Instead, the vials were filled nearly to the top and sealed to exclude oxygen.

Atomic absorption spectrophotometry is highly specific and sensitive for determination of serum zinc concentration (90). The principle source of error to be avoided is contamination of the serum with zinc from red blood cells or from exogenous sources such as glass and rubber stoppers (91). Only plastic syringes and pipettes were used for storage and for all transfers, avoiding glass and rubber stoppers entirely. It is recognized however, that a degree of hemolysis may occur prior to separation of the serum, and this may introduce a source of error which cannot be readily controlled or detected. It is assumed that the hemolysis would occur equally in members of the polyp and control groups.

The dietary questionnaire used in this study is in

essence a food frequency checklist modified by the use of food models to estimate usual portion size. It seems reasonable to assume that this questionnaire is valid, given the long history of the use of dietary questionnaires including food frequency questionnaires in cancer research and other types of research. In addition a variety of dietary questionnaires have been demonstrated to be of sufficient validity for usefulness in epidemiologic reasearch of cancer (92, 93).

The most comprehensive data currently available (85-87) for the carotene content of foods do not distinguish beta carotene from other carotenoids such as alpha carotene and lycopene since the AOAC methods (4) used for Nutrient Food Table Analysis do not differentiate some of these carotenoids. Thus the lack of adequate food nutrient tables for the carotenoids is a problem which can not be overcome at present. When adequate tables are made available, the data collected for this study may be reanalyzed.

The dark adaptation test employed in this project is a rapid, simple test recently developed for use in situations where the standard hour-long test is impractical. Since the report by Vinton and Russell (88) concerning the correlation of the results of this test with serum retinol

levels, no independent confirmation of the validity of the test has appeared in the literature.

### 3.4 Statistics

Mean differences in cases and controls of all measured variables were assessed by t-tests using the TTEST procedure from SAS (94). Correlation coefficients between pairs of variables were calculated using the CORR procedure from SAS.

Logistic regression analysis was used to determine whether any of several measured variables (smoking status, age, serum levels of retinol or beta carotene, race, rank, present or past consumption of retinol or carotene or total vitamin A, or the same dietary consumption values divided by weight) correlated with the presence or absence of polyps. The regression was performed using the Logist Procedure from SAS with polyps status as the dependent variable. The regression was repeatedly run with various subsets of the independent variables to eliminate any confounding due to covariance among the variables.

The Logist Procedure showed that only smoking status was significantly associated with the presence or absence of polyps (see Results section for more details). In order

to define nonsignificant trends more precisely, the Mantel-Haenszel Procedure was employed using the TFREQ Procedure from SAS. This procedure provided a value for the relative risk of developing the dependent variable (polyps) for various strata of each independent variable. Smoking status was controlled for in all calculations involving the Mantel-Haenszel Procedure.

## Chapter IV

### RESULTS

#### 4.1 Description of the Study Population

One hundred subjects participated in the study. The raw data collected as stated in the methods section are shown in Appendix 4. The subjects had a mean age of 58.8 years ranging from 26 to 87 years. The mean height was 70.3  $\pm$  2.7 inches with a range of 64 to 76 inches. The mean weight was 184.5  $\pm$  32.4 pounds with a range of 112 to 291 pounds. Seventy-six subjects were white, and 50 were smokers of cigarettes. Most were retired senior officers or senior enlisted men. Many were engaged in or had retired from second careers. Their mean consumption of total vitamin A (retinol plus carotene) as assessed by the dietary questionnaire was 2248  $\pm$  1092 RE, well in excess of the RDA. The range was 686 to 6041 RE. Vitamin pills and fortified foods contributed means of 442 and 179 RE,

respectively, to this total so that the mean total vitamin A consumption from food alone was 1636 RE. This is still approximately 63% above the RDA of 1000 RE/day. However, it should be noted that a USDA food consumption survey (1977-78) found that males age 51-64 consumed a mean of 7087 IU/day (approximately 1417 RE/day) of vitamin A (95). In the South region which included the District of Columbia, Maryland, and Virginia, consumption was even higher, 7300 IU/day (approximately 1460 RE/day) for males of the same age (96). These values are approximately 40-50 percent above the RDA of 5000 IU/day whereas the values reported by the study population are 63% above the RDA. The relatively high consumptions reported by the study population may be due to their moderately high socioeconomic status or may be due to an unidentified but hopefully systematic bias in the questionnaire or the interview technique. Importantly, only 11 subjects reported current vitamin A consumption below the RDA, and only eight subjects reported past vitamin A consumption below this limit. If the reported consumption of retinol and carotene is a valid indicator, the nutritional status of the study population was good.

The serum retinol values reported for these subjects (mean  $\pm$  SD of  $74.5 \pm 18.0$  micrograms/dl) are higher than

those most frequently encountered in the literature (61), while the coefficient of variation of 24% for these subjects is well within the acceptable range of 20-30%, according to Olson (61). The reason for the higher than expected value is not totally clear. Perhaps it is due to more complete extraction or perhaps it is due to the very high vitamin A consumption reported by these subjects. Although serum levels of vitamin A are not good indicators of nutritional status, there is a slight positive correlation between serum levels and liver reserves (i.e. nutritional status) (61). The lowest level of serum retinol for any subject was 41.0 micrograms/dl, more than double the level of 20 micrograms/dl usually chosen to indicate a risk of deficiency of this vitamin (82).

The serum beta carotene levels reported here (mean  $16.3 \pm 11.9$  micrograms/dl) are much lower than the total carotene levels in papers reviewed in Chapter 2 and included in Table 2. This is because beta carotene accounts for only about 20 to 25% of the total serum carotenoids (97), most of which were measured as total carotene by analytical methods which existed prior to the development of HPLC.

It had been planned to exclude subjects with low serum zinc levels from the serum retinol analysis because low

serum zinc would result in depression of serum retinol in a manner independent of retinol nutritional status (89). However, our laboratory has no values for serum zinc from an independent series of normal subjects. For this reason normal values were obtained from the literature (57). No serum zinc value reported in this study falls below the lower limit for serum zinc established at another laboratory which also used atomic absorption spectrophotometry (57). Therefore, no subject was excluded because of low serum zinc levels.

#### 4.2 Demographic Data for Cases and Controls

Fifty-seven subjects were shown by colonoscopy and histologic examination to have at least one adenomatous colonic polyp. These are referred to as cases. Forty-three subjects had no adenoma and were considered controls. Table 3 compares the demographic features of cases and controls. As can be seen, cases and controls were well matched for age, weight, height, military rank (an indicator of socioeconomic status), and race. However 58% of cases were smokers of cigarettes, compared to only 39% of controls. This difference is statistically significant ( $p < 0.05$ ) and will be further discussed below.

Table 3. Demographic Features of Cases and Controls

	CASES (N=57) Mean±SD	CONTROLS (N=43) Mean±SD
Age (years)	59.4± 10.6	58.0± 10.1
Weight (pounds)	187.9± 34.8	180.0± 28.7
Height (inches)	70.6± 2.8	69.8± 2.6
Rank*	14.5± 6.6	13.3± 6.3
% Smokers	58.0	39.0**
% White	77.0	74.0

\* Rank is expressed on an ordinal scale from 1 to 24.  
(1 = recruit, 24 = four star general)

\*\* p < 0.05

#### 4.3 Comparison of Study Parameters for Cases and Controls

Table 4 compares the serologic and dietary data and the dark adaptation tests for cases and controls. Subjects with polyps had slightly lower serum levels of retinol, beta carotene, retinol binding protein, and zinc, but none of these differences approached statistical significance. Similarly, the patients with polyps reported lower consumption of both retinol (present and past) and carotene (past only). Again, none of these differences approached statistical significance. Correction of the nutrient consumption data for weight to obtain the dose of nutrient per pound present body weight resulted in more apparent differences between cases and control subjects. When this was done, there appeared to be a clear trend toward a lower dose of retinol and, to a lesser degree, carotene among patients with adenomas, but statistical significance was not achieved.

Table 4. Comparison of Serologic and Dietary Data for 57 Cases and 43 Controls.

	CASES		CONTROLS		p#
	Mean+SD		Mean+SD		
Serum Retinol*	73.3	± 16.7	76.1	± 19.7	0.44
Serum Beta Carotene*	15.6	± 10.9	17.2	± 13.4	0.52
Serum Zinc*	96.7	± 13.6	99.7	± 16.4	0.35
Retinol Binding Protein@	5.6	± 1.3	5.9	± 1.6	0.24
Past Retinol Consumption**	1313.0	± 1019.0	1462.0	± 860.0	0.29
Past Carotene Consumption**	869.0	± 466.0	881.0	± 389.0	0.89
Past Carotene+Retinol Consumption**	2183.0	± 1183.0	2344.0	± 1045.0	0.48
Present Retinol Consumption**	1246.0	± 878.0	1432.0	± 862.0	0.29
Present Carotene Consumption**	931.0	± 493.0	909.0	± 432.0	0.82
Present Carotene+Retinol Consumption**	2177.0	± 1079.0	2343.0	± 1114.0	0.46
Past Retinol Consumption/Wt***	7.8	± 5.35	8.63	± 5.6	0.19
Past Carotene Consumption/Wt***	4.48	± 2.82	5.08	± 2.49	0.65
Past Carotene+Retinol Consumption/Wt***	12.02	± 6.6	13.71	± 7.06	0.22
Present Retinol Consumption/Wt***	6.92	± 4.95	8.39	± 5.54	0.17
Present Carotene Consumption/Wt***	5.19	± 3.10	5.23	± 2.9	0.95
Present Carotene+Retinol Consumption/Wt***	12.11	± 6.51	13.62	± 7.32	0.28
First Dark Adaptation Test ##	362.00	± 134.00	322.00	± 98.00	0.16
Second Dark Adaptation Test ##	265.00	± 112.00	240.00	± 89.00	0.29

\* micrograms/dl

@ milligrams/dl

# p = level of significance

\*\* retinol equivalents/day

\*\*\* retinol equivalents/pound body weight/day

## seconds

#### 4.4 Correlations of Study Parameters with Each Other

Simple correlation coefficients for each pair of variables were calculated using the CORR Procedure from SAS. The results are voluminous, and the entire matrix is too large to print in tabular form in this paper. Therefore only the important correlations will be mentioned in narrative or tabular form. First, age was negatively associated with weight ( $p=0.015$ ) but also with height ( $p<0.05$ ), probably reflecting the increasing height of the population in recent years. Age was also slightly correlated with serum retinol level ( $r=0.24$ ), so that older subjects had higher serum retinol but not beta carotene ( $r=0.10$ ) values. Despite higher serum retinol levels, older subjects took longer to perform the dark adaptation tests ( $r=0.41$  and  $0.45$  for the two tests).

Correlation coefficients between selected nutritional parameters are shown in Tables 5 and 6. As can be seen, the present dietary consumption of carotene is only minimally correlated ( $r=0.28$ ) with the serum beta carotene, so that most of the variance in serum beta carotene can not be explained by variance in consumption of carotene. This may be due to the fact that the nutrient data base used for analysis of the questionnaire does not distinguish beta carotene from most other carotenoids (Section 1.4).

Table 5. Correlation Coefficients for Selected Dietary Consumption and Serologic Parameters.

	Serum Retinol	Serum Beta Carotene	Present Retinol	Present Carotene	Present Carotene + Retinol	Past Retinol	Past Carotene
Serum Beta Carotene	0.033* 0.743						
Present Retinol Consumption	0.164 0.103	0.083 0.409					
Present Carotene Consumption	0.253 0.011	0.276 0.006	0.250 0.012				
Present Carotene + Retinol Consumption	0.241 0.016	0.186 0.063	0.907 0.0001	0.635 0.0001			
Past Retinol Consumption	0.008 0.937	0.053 0.598	0.511 0.0001	0.151 0.135	0.473 0.0001		
Past Carotene Consumption	0.177 0.078	0.226 0.024	0.281 0.005	0.758 0.0001	0.554 0.0001	0.151 0.135	
Past Carotene + Retinol Consumption	0.061 0.544	0.132 0.189	0.541 0.0001	0.420 0.0001	0.614 0.0001	0.473 0.0001	0.554 0.0001

\*The first number in each pair is the correlation coefficient.  
The second number in each pair is the level of significance (p).

Table 6. Correlation Coefficients and Levels of Significance for Selected Parameters.

	SERUM ZINC	SERUM RBP	RETINOL	FIRST DARK ADAPTATION TEST
SERUM ZINC				
SERUM RETINOL BINDING PROTEIN (RBP)	0.04* 0.67			
SERUM RETINOL	0.06 0.54	0.87 0.0001		
FIRST DARK ADAPTATION TEST	0.18 0.13	0.09 0.43	0.11 0.33	
SECOND DARK ADAPTATION TEST	0.23 0.06	-0.05 0.66	-0.01 0.92	0.77 0.0001

\* The top number in each pair is the correlation coefficient. The bottom number is the level of significance (p).

Interestingly, this correlation is almost identical to the correlation reported by Willett et al. ( $r=0.29$ ) when they compared carotene consumption assessed by a dietary questionnaire with plasma total carotenoids (92). The present consumption of retinol appears to be independent of the serum retinol level. This is also expected since the serum retinol level is largely controlled by factors other than dietary consumption except at the extremes of consumption (61).

The present and past consumption levels for each individual nutrient category were highly correlated, suggesting that diet had not changed markedly for most subjects. The present and past carotene consumption were most highly correlated ( $r=0.76$ ). In addition, the present consumption levels of the various nutrients all show varying degrees of correlation with each other, as do the past consumption levels.

Table 6 shows several interesting correlations. First, the correlation between serum retinol and retinol binding protein is very high ( $r=0.87$ ). A very close correlation between retinol and retinol binding protein has been previously documented (98). This is to be expected since it is the availability of retinol binding protein which controls the release of retinol from the storage

site, the liver (97).

There is essentially no correlation between the serum retinol level and the dark adaptation tests. This is in contrast to the findings of Vinton and Russell (88) who studied a younger population (age range 20-60), and included vitamin A deficient subjects in their population. Vinton and Russell noted lower retinol levels in their older subjects, a trend not observed in this study. The population studied by Vinton and Russell was also screened to exclude individuals with defective vision whereas this colon polyp study included a number of subjects with poor eyesight. These factors may explain the lack of correlation of serum retinol and the dark adaptation test in this study.

Finally there is essentially no correlation between serum zinc and either serum retinol or retinol binding protein. This probably is due to the fact that none of the subjects had a low value for serum zinc.

#### 4.5 Comparison of Smokers and Nonsmokers

Since cigarette smokers were found to be more likely to have polyps than nonsmokers, cigarette smokers and nonsmokers were compared by means of the t-test for unpaired

variables. Table 7 shows the mean values for cigarette smokers and nonsmokers for several of the variables, as well as the p values for significant differences in the means.

In addition to serum beta carotene levels, age was the only difference between cigarette smokers and nonsmokers, with the cigarette smokers being slightly younger than the nonsmokers. The table shows that the mean serum level of beta carotene is much lower in smokers than in nonsmokers ( $p=0.001$ ). However, serum beta carotene was not strongly associated with the presence of polyps among smokers ( $p=0.43$ ) or among nonsmokers ( $p=0.72$ ).

The finding (Table 7) that smokers had much lower values for serum beta carotene than nonsmokers is not new, as a previous study (99) found a mean serum carotene level of 104.9 micrograms per dl in smokers and 125.5 micrograms per dl in nonsmokers ( $p<0.013$ ). The results in the previous study are presumably reported as total carotenoids as they are much too high to be beta carotene. The current study found the mean serum beta carotene level to be almost 40% lower in smokers (12.5 micrograms per dl) than in nonsmokers (20.3 micrograms per dl,  $p<0.001$ ). The smokers and nonsmokers were well matched for age, weight, and height. The previous report (99) did not investigate

Table 7: Comparison of Smokers and Nonsmokers.

Variable	Smokers Mean+SD	Nonsmokers Mean+SD	p****
Age (years)	56.6+10.9	61.1+9.4	0.03
Weight (pounds)	182.7+32.2	186.3+32.9	0.58
Height (inches)	70.2+2.8	70.3+2.6	0.75
Serum Retinol Binding Protein@	5.58+1.37	5.84+1.48	0.36
Serum Zinc*	97.8+14.8	98.1+14.9	0.93
Serum Retinol*	72.8+18.5	76.3+17.5	0.32
Serum Beta Carotene*	12.5+6.5	20.3+14.7	0.001
Present Retinol Consumption**	1389+924	1261+819	0.46
Present Carotene Consumption**	906+485	938+466	0.73
Present Retinol+Carotene Consumption**	2295+1142	2199+1047	0.66
Past Retinol Consumption**	1447+1125	1306+735	0.46
Past Carotene Consumption**	854+442	895+426	0.64
Past Retinol+Carotene Consumption**	2301+1249	2201+985	0.66
Present Retinol/Wt***	8.04+5.58	7.04+4.86	0.34
Present Carotene/Wt***	5.2+3.16	5.21+2.86	0.98
Present Retinol+Carotene/Wt***	13.23+7.27	12.26+6.48	0.48
Past Retinol/Wt ***	8.25+6.3	7.33+4.49	0.40
Past Carotene/Wt***	4.9+2.8	4.99+2.57	0.86
Past Retinol+Carotene/Wt***	13.15+7.43	12.32+6.17	0.55

@ milligrams/dl

\* micrograms/dl

\*\* retinol equivalents/day

\*\*\* retinol equivalents/pound body weight/day

\*\*\*\* p = level of significance

dietary consumption in the same individuals. The current study suggests (Table 7) that differences in dietary consumption of carotene do not fully explain the differences in serum levels of beta carotene between smokers and nonsmokers. In fact, the body weight-adjusted carotene consumptions for smokers and nonsmokers are virtually identical, suggesting that smokers may absorb, store, or metabolize beta carotene differently than nonsmokers.

#### 4.6 Logistic Regression

Because of the close correlation between the various dietary parameters in this study, the logistic regression was run repeatedly with various subsets of the data. Regardless of what combination of independent variables was used, the logistic regression procedure showed only one statistically significant correlation. This was the correlation between cigarette smoking status and the presence of polyps. Smokers were statistically significantly more likely to have polyps ( $p=0.018$ ). The odds ratio calculated from the logistic regression procedure was 2.26. That is, smokers were 2.26 times as likely to have polyps as nonsmokers. No other independent variable correlated significantly with polyps.

#### 4.7 Odds Ratios for Parameters Other than Smoking

The Mantel-Haenszel statistics were computed for each independent variable, controlling in each case for smoking status. Table 8 shows the comparison of subjects above the mean for each independent variable against subjects below the mean for the same variable. For each variable a relative risk greater than 1.0 means that subjects with a value for the variable greater than the mean value were more likely to have polyps than subjects with a value below the mean value. A relative risk less than 1.0 thus indicates that subjects with relatively high levels for the variable were less likely to have polyps. Thus, a relative risk less than 1.0 may signify a protective effect for the variable. The confidence limits for the relative risk are also shown in Table 8 for each variable. As can be seen from Table 8, no independent variable showed a statistically significant association with the presence of polyps. The relative risk for high versus low values of several (but not all) dietary and serologic variables were considerably less than 1.0, however. Among these were serum retinol, retinol binding protein, zinc, and present retinol consumption. When present and past dietary consumptions were expressed on the basis of body weight,

TABLE 8. Mantel-Haenszel Statistics Comparing Patients with Values Above and Below the Mean, Controlling for Smoking.

Variable	Relative Risk	Confidence Limits	p*
Age	1.104	0.495, 2.462	0.808
Body Mass Index	0.978	0.437, 2.192	0.958
First Dark Adaptation Test	1.693	0.690, 4.153	0.250
Second Dark Adaptation Test	1.004	0.427, 2.359	0.993
Serum Beta Carotene	1.056	0.436, 2.556	0.904
Serum Retinol	0.669	0.295, 1.517	0.336
Serum Retinol Binding Protein	0.581	0.260, 1.302	0.220
Serum Zinc	0.620	0.268, 1.439	0.266
Present Retinol Consumption	0.551	0.238, 1.277	0.165
Present Carotene Consumption	1.026	0.444, 2.372	0.952
Present Carotene+Retinol Consumption	1.060	0.468, 2.402	0.889
Past Retinol Consumption	0.724	0.317, 1.652	0.443
Past Carotene Consumption	1.342	0.573, 3.141	0.498
Past Carotene+Retinol Consumption	1.012	0.445, 2.299	0.978
Present Retinol Consumption/Wt	0.584	0.251, 1.359	0.212
Present Carotene Consumption/Wt	0.650	0.281, 1.505	0.315
Present Carotene+Retinol Consumption/Wt	0.623	0.273, 1.422	0.261
Past Retinol Consumption/Wt	0.669	0.294, 1.521	0.337
Past Carotene Consumption/Wt	0.911	0.396, 2.099	0.827
Past Carotene+Retinol Consumption/Wt	0.569	0.253, 1.280	0.173

\* p = level of significance

the relative risks associated with higher doses of these nutrients were found to range from 0.569 to 0.911. However, for each of these parameters the confidence limits overlapped 1.0. Although none of the dietary parameters reached statistical significance, it is possibly important to note that all four parameters of retinol consumption show stronger negative associations with polyps than the respective parameters of carotene consumption. The unadjusted dietary parameters seem to show a slightly stronger trend for present rather than past consumption levels, and this is more apparent after adjustment of data for body weight. Individuals who had higher than the mean score on the first dark adaptation test (that is, those who failed to adapt readily to darkness) were more likely to have polyps (relative risk=1.693). Again, however, the confidence limits overlapped 1.0, and the results did not approach statistical significance.

The Mantel-Haenszel statistics comparing the highest quartile with the lowest quartile for each independent variable are shown in Table 9. Once again, no relative risk approached statistical significance, but trends were apparent even in these small groups (approximately 25 subjects per quartile). It is felt that the groups are too small to permit meaningful discussion of the nonsignificant

TABLE 9. Mantel-Haenszel Statistics for Highest and Lowest Quartiles Controlling for Smoking.

Variable	Relative Risk	Confidence Limits	p*
Serum Retinol	0.756	0.239, 2.389	0.633
Serum Beta Carotene	1.233	0.362, 4.200	0.737
Past Retinol Consumption	0.585	0.183, 1.870	0.366
Past Carotene Consumption	0.722	0.216, 2.406	0.595
Past Retinol+Carotene Consumption	0.467	0.142, 1.535	0.210
Present Retinol Consumption	0.523	0.149, 1.832	0.311
Present Carotene Consumption	1.058	0.314, 3.566	0.927
Present Retinol+Carotene Consumption	0.543	0.164, 1.798	0.318
Past Retinol Consumption/Wt**	0.706	0.218, 2.288	0.562
Past Carotene Consumption/Wt	0.595	0.178, 1.986	0.399
Past Retinol+Carotene Consumption/ Wt	0.600	0.186, 1.932	0.392
Present Retinol Consumption/Wt	0.557	0.159, 1.952	0.361
Present Carotene Consumption/Wt	1.187	0.371, 3.798	0.773
Present Retinol+Carotene Consumption/ Wt	0.514	0.151, 1.743	0.285

\* p = levels of significance

\*\* Body Weight

trends noted in comparison of the highest and lowest quartiles.

## Chapter V

### DISCUSSION

The finding that smoking status correlated with the presence of colonic adenomatous polyps was unexpected and has not been previously reported. It is not too surprising, however, since smoking has been associated with cancer in such sites as the urinary bladder and the pancreas which do not come into direct contact with tobacco smoke.

It is interesting to speculate on the reasons that smoking has not been previously associated with colonic polyps. First, no previous case control study of risk factors for colonic polyps has been reported. Previous studies have dealt with colon cancer, not colonic polyps. Colonic polyps are fairly common in the general population. Therefore, a case control study of colon cancer may include patients with colonic polyps among the controls unless potential control subjects are carefully studied with barium enemas or colonoscopies. Since colonic polyps and colon cancer may be different stages of the same disease,

the effect might be to obscure significant causal factors such as smoking. Second, if a single risk factor such as smoking predisposes to two diseases, e.g. both coronary artery disease and colon cancer, and if one of these diseases (coronary artery disease) is common and lethal and occurs at an earlier age than the other, then a longitudinal case control study of the disease which occurs later in life (colon cancer) may not demonstrate the risk factor unless the early deaths from other smoking-related illness(es) are somehow controlled for. Since these possible problems have not been adequately controlled in previous studies of colon cancer, it is not surprising that this relationship between smoking and colonic polyps is, until now, unpublished.

There are many possible reasons for a lack of association of the dietary and serologic parameters with colonic polyps in this study. Obviously, it is possible that vitamin A does not protect against polyps, but it is also possible that this study has so far failed to detect a true relationship. Indeed, this is suggested by the fact that all six of the body weight-adjusted dietary parameters show (nonsignificant) trends in the direction of a lower relative risk for colonic polyps for high consumers. If a correlation actually exists, the most likely reason for

missing it is the relatively small number of subjects studied. This study to date includes only 43 control subjects, relatively few for a case-control study of this type. It is possible that the trends will become statistically significant as more subjects are studied.

It is also possible that the high nutrient consumption of the study population prevented the detection of a relationship. Only 11 subjects reported consumption of total vitamin A below the RDA, and several of these were borderline. Similarly the serum retinol analysis failed to detect even borderline low values.

The possibility that no statistically significant trends were observed for the dietary and serologic variables due to data unreliability should be addressed. As stated earlier, the procedures used for analysis of the serum samples for retinol, retinol binding protein, and beta carotene are state-of-the-art. It is unlikely that significant errors have occurred. On the other hand, no dietary recall of food frequencies is entirely accurate or reliable. The accuracy of this instrument is highly dependent upon the published nutrient data base which was derived from USDA Handbook 456 and Handbooks 8-9 and 8-11. This data base is relatively good for retinol but relatively poor for carotenoids. Furthermore, the data

base systematically de-emphasizes carotenoid content of foods since the carotenoid content is reported as retinol equivalents which are 1/6 or less of the actual gram value. Also, some carotenoids such as lutein that may have significant ability to trap free radicals are not counted at all or are underestimated because of the AOAC separation techniques. Thus it is possible that the data base is grossly inaccurate for the truly protective food factor(s), whatever they may be. When better food composition data are made available, the data accumulated in this study can be reanalyzed.

It is interesting that the dark adaptation test was associated more closely with the presence of polyps than with serum retinol levels (Tables 6 and 8). Since neither trend is statistically significant at this point, one should not make too much of this observation. On the other hand, the dark adaptation test is a test of end-organ responsiveness to retinol. Polyp occurrence, if it is related to retinol, is an end-organ response also. Perhaps abnormal delivery of retinol to cells predisposes to the development of colonic polyps even in subjects with normal circulating levels of retinol. This is highly speculative, of course, but it will be interesting to observe these parameters as more cases and controls are accrued.

## Chapter VI

### SUMMARY AND CONCLUSIONS

Colon cancer is a leading cause of cancer deaths in this country. Treatment for advanced colon cancer is usually unsuccessful. Currently available screening procedures lack sensitivity and frequently miss the disease when it is in a curable or precancerous phase. Clearly, if dietary alterations could be proven to be beneficial in preventing colon cancer, this would be a welcome finding.

Previous studies have suggested but have not proven that dietary beta carotene or retinol may inhibit the development of a variety of human cancers. All previous retrospective dietary studies have shared a common problem in that they interviewed sick patients who may already have changed their eating habits because of the cancer.

This study used as its endpoint colonic adenomatous polyps rather than colon cancer. These polyps are precursors of cancer. The study was designed to determine whether subjects with relatively lower consumption of

retinol or carotene or lower serum levels of retinol or beta carotene were more likely to have colonic adenomas. This is the only study of cancer or a cancer precursor which has examined both diet and serum nutrient levels.

One hundred subjects received colonoscopy, submitted to a dietary and demographic questionnaire, and submitted blood for analysis for retinol and beta carotene. The study failed to find a statistically significant relationship between diet or serum nutrient levels and colon polyps, but cigarette smokers were found to be more likely to have polyps than nonsmokers.

As previously discussed, the reasons for the failure to show significant associations for the dietary parameters are unclear. The nonsignificant trends apparent in the data suggest that a real association may have been missed. The two most obvious reasons for this would be that the number of subjects is too small or that the dietary nutrient data base is too inaccurate since it fails to distinguish dietary beta carotene from other carotenoids.

This study will continue, funded by a grant from the National Cancer Institute. It is hoped that approximately 300 subjects will eventually be studied. If a more adequate nutrient data base becomes available, the results can be analyzed for each individual carotenoid as well as

for total carotenoids. The serological data also can be reanalyzed for carotenoids other than beta carotene to determine whether relatively low levels of these carotenoids are associated with increased risk of polyps.

## LITERATURE CITED

1. Winawer SJ, Enker WE, Lightdale CJ. Malignant Tumors of the Colon and Rectum. In: Berk JE, ed. Bockus Gastroenterology. Philadelphia: WB Saunders, 1985: 2531-74.
2. Muto T, Bussey HJ, Morson BC. The evolution of cancer of the colon and rectum. *Cancer* 1975;36:2251-70.
3. Simpson KL. Relative value of carotenoids as precursors of vitamin A. *Proc Nutr Soc* 1983;42:7-17.
4. Horwitz W., ed. Official methods of analysis of the Association of Official Analytical Chemists. Washington, D.C.: AOAC, 1980:230-1,734-40.
5. Beecher GR, Khachik F. Evaluation of vitamin A and carotenoid data in food composition tables. *J Natl Cancer Inst* 1984;73:1397-1404.
6. National Research Council. Recommended Dietary Allowances. 9th ed. Washington, D.C.: National Academy of Sciences, 1980:55-60.
7. Stich HF, Rosin MP, Vallejera MO. Reduction with vitamin A and beta-carotene administration of proportion of micronucleated buccal mucosal cells in Asian betel nut and tobacco chewers. *Lancet* 1984;1: 1204-6.
8. Wolbach SB, Howe PR. Tissue changes following deprivation of fat-soluble A vitamin. *J Exp Med* 1925;42:753-77.
9. Fujimake Y. Formation of cancer in albino rats fed on deficient diets. *J Cancer Res* 1926;10:469-477.
10. Saffiotti U, Montesano R, Sellakumar AR, Borg SA. Experimental cancer of the lung. *Cancer* 1967;20:857-64.

11. Fleiszer DM, Murray D, Richards GK, Brown RA. Effects of diet on chemically induced bowel cancer. *Can J Surg* 1980;23:67-73.
12. Newberne PM, Rogers AE. Rat colon carcinomas associated with aflatoxin and marginal vitamin A. *J Natl Cancer Inst* 1973;50:439-48.
13. Rogers AE, Herndon BJ, Newberne PM. Induction by dimethylhydrazine of intestinal carcinoma in normal rats and rats fed high or low levels of vitamin A. *Cancer Res* 1973;33:1003-9.
14. Grubbs CJ, Moon RC, Sporn MB, Newton DL. Inhibition of mammary cancer by retinyl methyl ether. *Cancer Res* 1977;37:599-602.
15. Newberne PM, Suphakarn V. Preventive role of vitamin A in colon carcinogenesis in rats. *Cancer* 1977;40:2553-6.
16. Narisawa T, Reddy BS, Wong CQ, Weisburger JH. Effect of vitamin A deficiency on rat colon carcinogenesis by N-Methyl-N'-nitro-N-nitrosoguanidine. *Cancer Res* 1976;36:1379-83.
17. Port CD, Sporn MB, Kaufman DG. Prevention of lung cancer in hamsters by 13-cis-retinoic acid. *Proc Am Assoc Cancer Res* 1975;16:21.
18. Becci PJ, Thompson HJ, Grubbs CJ, Brown CC, Moon RC. Effect of delay in administration of 13-cis-retinoic acid on the inhibition of urinary bladder carcinogenesis in the rat. *Cancer Res* 1979;39:3141-4.
19. Longnecker DS, Kuhlmann ET, Curphey TJ. Effects of four retinoids in N-nitrosobis(2-oxopropyl)amine-treated hamsters. *Cancer Res* 1983;43:3226-30.
20. Longnecker DS, Kuhlmann ET, Curphey TJ. Divergent effects of retinoids on pancreatic and liver carcinogenesis in azaserine-treated rats. *Cancer Res* 1983;43:3219-25.

21. Becci PJ, Thompson HJ, Grubbs CJ, et al. Inhibitory effect of 13-cis-retinoic acid on urinary bladder carcinogenesis induced in C57BL/6 mice by N-butyl-N-(4-hydroxybutyl)-nitrosamine. *Cancer Res* 1978;38:4463-5.
22. Smith DM, Rogers AE, Herndon BJ, Newberne PM. Vitamin A (retinyl acetate) and benzo(a)pyrene-induced respiratory tract carcinogenesis in hamsters fed a commercial diet. *Cancer Res* 1975;35:11-6.
23. Rettura G, Stratford F, Levenson SM, Seifter E. Prophylactic and therapeutic actions of supplemental beta carotene in mice inoculated with C3HBA adenocarcinoma cells: lack of therapeutic action of supplemental ascorbic acid. *J Clin Invest* 1982;69:73-7.
24. Seifter E, Rettura G, Padawer J, Stratford F, Goodwin P, Levenson SM. Regression of C3HBA mouse tumor due to x-ray therapy combined with supplemental beta carotene or vitamin A. *J Clin Invest* 1983;71:409-17.
25. Bjelke E. Epidemiologic studies of cancer of the stomach, colon, and rectum with special emphasis on the diet. Thesis, University of Minnesota. Ann Arbor, Michigan: University Microfilms, 1973.
26. Bjelke E. Dietary vitamin A and human lung cancer. *Int J Cancer* 1975;15:561-5.
27. Kvale G, Bjelke E, Gart JJ. Dietary habits and lung cancer risk. *Int J Cancer* 1983;31:397-405.
28. Hirayama T. Diet and cancer. *Nutr Cancer* 1979;1:67-80.
29. Shekelle RB, Liu S, Raynor WJ, Lepper M, Maliza C, Rossof AH, Paul O, Shryock AM, Stamler J. Dietary vitamin A and risk of cancer in the Western Electric study. *Lancet* 1981;2:1185-90.
30. Nomura A, Yamakawa H, Ishidate T, Kamiyama S, Masuda H, Stemmermann GN, Heilbrun LK, Hankin JH. Intestinal metaplasia in Japan: association with diet. *J Natl Cancer Inst* 1982;68:401-5.

31. Romney SL, Palan PR, Duttagupta C, Wassertheil-Smoller S, Wylie J, Miller G, Slagle NS, Lucido D. Retinoids and the prevention of cervical dysplasias. *Am J Obstet Gynecol* 1981;141:890-4.
32. Graham S. Epidemiology of retinoids and cancer. *J Natl Cancer Inst* 1984;73:1423-8.
33. Mettlin C, Graham S, Swanson M. Vitamin A and lung cancer. *J Natl Cancer Inst* 1979;62:1435-8.
34. Mettlin C, Graham S. Dietary risk factors in human bladder cancer. *Am J Epidemiol* 1979;110:255-63.
35. Marshall JR, Graham S, Mettlin C, Sheed D, Swanson M. Diet in the epidemiology of oral cancer. *Nutr Cancer* 1982;3:145-9.
36. Graham S, Mettlin C, Mashall JR, Priore R, Rzepka T, Shedd D. Dietary factors in the epidemiology of cancer of the larynx. *Am J Epidemiol* 1981;113:675-80.
37. Marshall JR, Graham S, Byers T, Swanson M, Brasure, J. Diet and smoking in the epidemiology of cancer of the cervix. *J Natl Cancer Inst* 1983;70:847-51.
38. Byers T, Marshall J, Graham S, Mettlin C, Swanson M. A case-control study of dietary and nondietary factors in ovarian cancer. *J Natl Cancer Inst* 1983;71:681-6.
39. Graham S, Marshall J, Mettlin C, Rzepka T, Nemoto T. Diet in the epidemiology of breast cancer. *Am J Epidemiol* 1982;116:68-75.
40. Graham S, Dayal H, Swanson M, Mittelman A, Wilkinson G. Diet in the epidemiology of cancer of the colon and rectum. *J Natl Cancer Inst* 1978;61:709-14.
41. Mettlin C, Graham S, Priore R, Marshall J, Swanson M. Diet and cancer of the esophagus. *Nutr Cancer* 1981;2:143-7.
42. Graham S. Results of case-control studies of diet and cancer in Buffalo, New York. *Cancer Res* 1983;43:2409s-13s.

43. Kolonel LN, Nomura AM, Hinds MW, Hirohata T, Hankin JH, Lee J. Role of diet in cancer incidence in Hawaii. *Cancer Res* 1983;43:2397s-2402s.
44. MacLennan R, Da Costa J, Day NE, Law CH, Ng YK, Shanmugaratnam K. Risk factors for lung cancer in Singapore Chinese, a population with high female incidence rates. *Int J Cancer* 1977;20:854-60.
45. Gregor A, Lee PN, Roe FJ, Wilson MJ, Melton A. Comparison of dietary histories in lung cancer cases and controls with special reference to vitamin A. *Nutr Cancer* 1980;2:93-7.
46. Samet JM, Skipper BJ, Humble CG, Pathak DR. Lung cancer risk and vitamin A consumption in New Mexico. *Am Rev Respir Dis* 1985;131:198-202.
47. Ziegler RG, Mason TJ, Stemhagen A, Hoover R, Schoenberg JB, Gridley G, Virgo PW, Altman R, Fraument JF, Jr. Dietary carotene and vitamin A and risk of lung cancer among white men in New Jersey. *J Natl Cancer Inst* 1984;73:1429-35.
48. Ershow AG, Ziegler RG, Pickle LW, Mason TW, Buffler PA. Dietary retinol and carotene and risk of human lung cancer. *Fed Proc* 1985;44:1338.
49. Winn DM, Ziegler RG, Pickle LW, Gridley G, Blot WJ, Hoover RN. Diet in the etiology of oral and pharyngeal cancer among women from the southern United States. *Cancer Res* 1984;44:1216-22.
50. Tuyns AJ, Pequignot G, Jensen OM. Nutrition, alcohol et cancer de l'oesophage. *Bull Cancer* 1978;65:59-64.
51. Cook-Mozaffari PJ, Azordegan F, Day NE, Ressicaud A, Sabai C, Aramesh B. Oesophageal cancer studies in the Caspian Littoral of Iran: results of a case-control study. *Br J Cancer* 1979;39:293-309.
52. Modan B, Cuckle H, Lubin F. A note on the role of dietary retinol and carotene in human gastrointestinal cancer. *Int J Cancer* 1981;28:421-4.
53. Wattenberg LW, Loub WD. Inhibition of polycyclic aromatic hydrocarbon-induced neoplasia by naturally occurring indoles. *Cancer Res* 1978;38:1410-3.

54. Wattenberg LW. Studies of polycyclic hydrocarbon hydroxylases of the intestine possibly related to cancer. *Cancer* 1971;28:99-102.
55. Ibrahim K, Jafarey NA, Zuberi SJ. Plasma vitamin A and carotene levels in squamous cell carcinoma of oral cavity and oro-pharynx. *Clin Oncol* 1977;3:203-7.
56. Atukorala S, Basu TK, Dickerson JW, Donaldson D, Sakula A. Vitamin A, zinc and lung cancer. *Br J Cancer* 1979;40:927-31.
57. Mellow MH, Layne EA, Lipman TO, Kaushik M, Hostetler C, Smith JC. Plasma zinc and vitamin A in human squamous carcinoma of the esophagus. *Cancer* 1983;51:1615-20.
58. Basu TK, Donaldson D, Jenner M, Williams DC, Sakula A. Plasma vitamin A in patients with bronchial carcinoma. *Br J Cancer* 1976;33:119-21.
59. Wahi PN, Bodkhe RR, Arora S, Srivastava MC. Serum vitamin A studies in leukoplakia and carcinoma of the oral cavity. *Indian J Pathik Bacteriol* 1962;5:10-6.
60. Bichler E. Plasma levels of retinol and retinol-binding protein in patients with squamous cell carcinomas of the head and neck region. *Arch Otolaryngol* 1982;236:115-21.
61. Olson JA. Serum levels of vitamin A and carotenoids as reflectors of nutritional status. *J Natl Cancer Inst* 1984;73:1439-44.
62. Wald N, Idle M, Boreham J, Bailey A. Low serum-vitamin A and subsequent risk of cancer. *Lancet* 1980;2:813-5.
63. Wald NJ, Boreham J, Hayward JL, Bulbrook RD. Plasma retinol, beta carotene and vitamin E levels in relation to the future risk of breast cancer. *Br J Cancer* 1984;49:321-4.
64. Willet WC, Polk BF, Underwood BA, Stampfer MJ, Pressel S, Rosner B, Taylor JO, Schneider K, Hames CG. Relation of serum vitamins A and E and carotenoids to the risk of cancer. *N Engl J Med* 1984;310:430-4.

65. Willett WC, Polk BF, Underwood BA, Hames CG. Hypertension Detection and Follow-up Program study of serum retinol, retinol-binding protein, total carotenoids, and cancer risk: a summary. *J Natl Cancer Inst* 1984;73:1459-62.
66. Stahelin HB, Rosel F, Buess E, Brubacher G. Cancer, vitamins, and plasma lipids: prospective Basel study. *J Natl Cancer Inst* 1984;73:1463-8.
67. Kark JD, Smith AH, Switzer BR, Hames CG. Serum vitamin A (retinol) and cancer incidence in Evans County, Georgia. *J Natl Cancer Inst* 1981;66:7-16.
68. Peleg I, Heyden S, Knowles M, Hames CG. Serum retinol and risk of subsequent cancer: extension of the Evans County, Georgia, study. *J Natl Cancer Inst* 1984;73:1455-8.
69. Neeld JB, Pearson WN. Macro- and micromethods for the determination of serum vitamin A using trifluoroacetic acid. *J Nutr* 1963;79:454-62.
70. Wolf G, Kiorpes TC, Masushige S, Schreiber JB, Smith MJ, Anderson RS. Recent evidence for the participation of vitamin A in glycoprotein synthesis. *Fed Proc* 1979;38:2540-3.
71. Ganguly J, Rao MR, Murthy SK, Sarada K. Systemic mode of action of vitamin A. *Vitam Horm* 1980;38:1-54.
72. DeLuca LM, Bhat PV, Sasak W, Adamo S. Biosynthesis of phosphoryl and glycosyl phosphoryl derivatives of vitamin A in biological membranes. *Fed Proc* 1979;38:2535-9.
73. Weber F. Biochemical mechanisms of vitamin A action. *Fed Proc* 1983;42:31-41.
74. Olson JA, Bridges CD, Packer L, Chytil F, Wolf G. The function of vitamin A. *Fed Proc* 1983;42:2740-6.
75. Chytil F, Ong DE. Cellular retinol- and retinoic acid-binding proteins in vitamin A action. *Fed Proc* 1979;38:2510-4.
76. Omori M, Chytil F. Mechanism of vitamin A action. *J Biol Chem* 1982;257:14370-4.

77. Cerutti PA. Prooxidant states and tumor promotion. *Science* 1985;227:375-81.
78. Krinsky NI, Deneke SM. Interaction of oxygen and oxy-radicals with carotenoids. *J Natl Cancer Inst* 1982; 69:205-10.
79. Winawer SJ, Andrews M, Flehinger B, Sherlock P, Schottenfeld D, Miller DG. Progress report on controlled trial of fecal occult blood testing for detection of colorectal neoplasia. *Cancer* 1980;45: 2959-64.
80. Ram MM, Bamji MS. Serum vitamin A and retinol-binding protein in malnourished women treated with oral contraceptives: effects of estrogen dose and duration of treatment. *Am J Obstet Gynecol* 1979;135:470-2.
81. Morson BC. Genesis of colorectal cancer. *Clin Gastroenterol* 1976;5:505-25.
82. Sauberlich HE, Dowdy RP, Skala JH. Vitamin A. In: *Laboratory tests for the assessment of nutritional status*. Boca Raton: CRC Press, 1974:4-12.
83. Rodriguez MS, Irwin MI. A conspectus of research on vitamin A requirements of man. *J Nutr* 1972;102:909-68.
84. Lui NST, Roels OA. Vitamin A and carotene. In: Goodhart RS, Shils ME, eds. *Modern nutrition in health and disease*. 6th ed. Philadelphia: Lea & Febiger, 1980:142-59.
85. Adams CF. *Nutritive Value of American Foods in Common Units*. US Dept of Agriculture Handbook No. 456. Washington, D.C., 1975.
86. USDA. *Composition of Foods: Fruits and Fruit Juices Raw, Processed, Prepared*. US Dept of Agriculture Handbook No. 8-9, Washington, D.C., 1982.
87. USDA. *Composition of Foods: Vegetables and Vegetable Products*. US Dept of Agriculture Handbook No. 8-11, Washington, D.C., 1984.
88. Vinton NE, Russell RM. Evaluation of a rapid test of dark adaptation. *Am J Clin Nutr* 1981;34:1961-6.

89. Smith JC, Jr, Brown ED, McDaniel EG, Chan W. Alterations in vitamin A metabolism during zinc deficiency and food and growth restriction. *J Nutr* 1976;106:569-74.
90. Stika KM, Morrison GH. Analytical methods for the mineral content of human tissues. *Fed Proc* 1981;40:2115-25.
91. Williams DM. Trace metal determinations in blood obtained in evacuated collection tubes. *Clin Chim Acta* 1979;99:23-9.
92. Willett WC, Stampfer MJ, Underwood BA, Speizer FE, Rosner B, Hennekens CH. Validation of a dietary questionnaire with plasma carotenoid and alpha-tocopherol levels. *Am J Clin Nutr* 1983;38:631-9.
93. Block G. A review of validations of dietary assessment methods. *Am J Epidemiol* 1982;115:492-505.
94. SAS Institute Inc. *SAS User's Guide*, 1982 Edition. Cary, NC: SAS Institute Inc., 1982.
95. USDA, Human Nutrition Information Service. *Nutrient Intakes: Individuals in 48 States, Year 1977-78. Nationwide Food Consumption Survey 1977-78*, USDA, NFCS Rep. No. I-2, 1984.
96. USDA, Human Nutrition Information Service. *Food and Nutrient Intakes: Individuals in Four Regions, Year 1977-78. Nationwide Food Consumption Survey 1977-78*, USDA, NFCS Rep. No. I-3, 1985.
97. Goodman DS. Overview of current knowledge of metabolism of vitamin A and carotenoids. *J Natl Cancer Inst* 1984;73:1375-9.
98. Baker H, Frank O, Hutner SH. Vitamin analyses in medicine. In: Goodhart RS, Shils ME., eds. *Modern Nutrition in Health and Disease*. 6th ed. Philadelphia: Lea & Febiger, 1980:611-40.
99. Witter FR, Blake DA, Baumgardner R, Mellits ED, Niebyl JR. Folate, carotene, and smoking. *Am J Obstet Gynecol* 1982;144:857.

SS# \_\_\_\_\_

STUDY # A- \_\_\_\_\_

PROTOCOL #1450  
CASE CONTROL STUDY

ADENOMATOUS COLONIC POLYPS  
CHECKLIST

NAME: \_\_\_\_\_ DATE: \_\_\_\_\_

ADDRESS: \_\_\_\_\_ PHONE:(home) \_\_\_\_\_  
\_\_\_\_\_ (work) \_\_\_\_\_

Friend or relative that would know of forwarding address:

NAME: \_\_\_\_\_ PHONE: \_\_\_\_\_

ADDRESS: \_\_\_\_\_

DOCTOR'S NAME (GI CLINIC): \_\_\_\_\_

REFERRING PHYSICIAN'S NAME: \_\_\_\_\_

PHONE: \_\_\_\_\_

LOCATION: \_\_\_\_\_

CRITERIA FOR ELIGIBILITY:	YES	NO
1. Healthy adult male or postmenopausal female	—	—
2. Scheduled to undergo diagnostic colonoscopy for the following indications:		
(a) Guaiac positive stools that remain unexplained after evaluation short of colonoscopy; or	—	—
(b) Barium enema showing possible colonic polyp	—	—

CRITERIA FOR EXCLUSION:	YES	NO
1. Colonoscopy for other than stated indications	—	—
2. Prior diagnosis of adenocarcinoma of colon or adenomatous colonic polyps	—	—
3. Familial polyps of colon	—	—
4. Gardner's Syndrome	—	—
5. Inflammatory bowel disease	—	—
6. Fever	—	—
7. Acute and chronic infections	—	—
8. Active tuberculosis	—	—
9. Liver disease	—	—
10. Thyroid disease	—	—
11. Kidney disease	—	—
12. Alcoholism	—	—
13. Fat malabsorption syndromes	—	—
14. Weight loss of > 15 pounds/6 months	—	—
15. Mineral oil use	—	—
16. Contraindications to colonoscopy	—	—

ADDITIONAL COMMENTS: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

APPENDIX 2  
VOLUNTEER AGREEMENT

WORK UNIT # \_\_\_\_\_

I, \_\_\_\_\_, HAVING ATTAINED MY EIGHTEENTH (18TH) BIRTHDAY, AND OTHERWISE HAVING FULL CAPACITY TO CONSENT, DO HEREBY VOLUNTEER TO PARTICIPATE IN AN:

INVESTIGATIONAL STUDY ENTITLED:

Investigation of the Relationship of Patient Vitamin A Status to the Presence or Absence of Adenomatous Colonic Polyps

UNDER THE DIRECTION OF James W. Kikendall OF THE DEPARTMENT/SERVICE/INSTITUTE OF Gastroenterology Service, WALTER REED ARMY MEDICAL CENTER, WASHINGTON, D.C.

THE IMPLICATIONS OF MY VOLUNTARY PARTICIPATION, THE NATURE, DURATION AND PURPOSE OF THE STUDY; THE METHODS BY WHICH THE STUDY IS TO BE CONDUCTED; AND THE KNOWN INCONVENIENCES AND HAZARDS HAVE BEEN THOROUGHLY EXPLAINED TO ME BY THE PRINCIPAL INVESTIGATOR OR BY ONE OF THE COINVESTIGATORS AND SUCH INCONVENIENCES AND HAZARDS ARE SET FORTH IN DETAIL ON THE ATTACHED PAGE OF THIS AGREEMENT, ALONG WITH MY INITIALS OR SIGNATURE. I HAVE BEEN GIVEN AN OPPORTUNITY TO ASK QUESTIONS CONCERNING THIS INVESTIGATIONAL STUDY AND MY PARTICIPATION IN THE STUDY, AND ANY SUCH QUESTIONS HAVE BEEN ANSWERED TO MY FULL AND COMPLETE SATISFACTION.

DURING THE COURSE OF MY TREATMENT AS A PATIENT AT WALTER REED ARMY MEDICAL CENTER, I HAVE BEEN PROVIDED WITH A COPY OF A PRIVACY ACT STATEMENT (DD FORM 2005) WHICH HAS MADE ME AWARE OF THE SAFEGUARDS AVAILABLE TO ME BECAUSE OF THE PRIVACY ACT OF 1974. I HAVE BEEN GIVEN THE OPPORTUNITY TO REVIEW THE DD FORM 2005, ASK QUESTIONS AND RETAIN A PERSONAL COPY. I HAVE BEEN MADE AWARE THAT THE INFORMATION GAINED ABOUT ME, BECAUSE OF MY PARTICIPATION IN THIS INVESTIGATIONAL STUDY, MAY BE PUBLICIZED IN MEDICAL LITERATURE, DISCUSSED AS AN EDUCATIONAL MODEL, AND USED GENERALLY IN THE FURTHERANCE OF MEDICAL SCIENCE. I FREELY CONSENT TO PROVIDE SUCH PERSONAL INFORMATION AS IS REQUESTED OF ME FOR THIS INVESTIGATIONAL STUDY AND FREELY CONSENT TO THE DISCLOSURE OF PERTINENT PERSONAL INFORMATION DERIVED FROM MY PARTICIPATION IN THIS INVESTIGATIONAL STUDY FOR REASONS OF PUBLICATION IN MEDICAL LITERATURE, DISCUSSED AS AN EDUCATIONAL MODEL AND FOR THOSE ADDITIONAL REASONS WHICH SPECIFICALLY RELATE TO THE FURTHERANCE OF MEDICAL SCIENCE.

I UNDERSTAND THAT IN THE EVENT OF PHYSICAL INJURY RESULTING FROM THE RESEARCH PROCEDURES, MEDICAL TREATMENT FOR INJURIES OR ILLNESS IS AVAILABLE AND THAT COMPENSATION MAY BE AVAILABLE THROUGH JUDICIAL AVERUES. INFORMATION REGARDING JUDICIAL AVERUES OF COMPENSATION IS AVAILABLE FROM THE CENTER JUDGE ADVOCATE.

I AM AWARE THAT AT ANY TIME DURING THE COURSE OF THIS INVESTIGATIONAL STUDY I MAY REVOKE MY CONSENT AND WITHDRAW FROM THIS STUDY, WITHOUT PREJUDICE; HOWEVER, I MAY BE REQUESTED FOR MEDICAL REASONS TO UNDERGO FURTHER EXAMINATIONS IF IN THE OPINION OF MY ATTENDING PHYSICIAN SUCH EXAMINATIONS ARE NECESSARY FOR MY HEALTH OR WELL BEING.

IF THERE IS ANY PORTION OF THIS EXPLANATION THAT YOU DON'T UNDERSTAND, ASK YOUR DOCTOR BEFORE SIGNING.

\_\_\_\_\_  
SIGNATURE

\_\_\_\_\_  
DATE

\_\_\_\_\_  
PRINTED NAME

\_\_\_\_\_  
DATE

\_\_\_\_\_  
ADDRESS (PERMANENT)

I WAS PRESENT DURING THE EXPLANATION REFERRED TO ABOVE, AS WELL AS DURING THE VOLUNTEER'S OPPORTUNITY TO ASK QUESTIONS. I HEREBY WITNESS THE VOLUNTEER'S SIGNATURE.

\_\_\_\_\_  
WITNESS SIGNATURE

\_\_\_\_\_  
PRINCIPAL INVESTIGATOR'S SIGNATURE

RAMC FL 49  
JAN 82

\_\_\_\_\_  
DATE

PATIENT EXPLANATION CONSENT SHEET

**INSTITUTE:** Walter Reed Army Medical Center, Washington, D.C.

**TITLE:** Investigation of the Relationship of Patient Vitamin A Status to the Presence or Absence of Adenomatous Colonic Polyps

**PRINCIPAL INVESTIGATOR:** James Walter Kikendall, MAJ, MC  
Assistant Chief, Gastroenterology Service  
Walter Reed Army Medical Center  
576-1765

**PARTICIPATION INFORMATION:** You have been asked to participate in a research study conducted at the Walter Reed Army Medical Center. It is very important that you read and understand the following general principles which apply to all participants in our studies, whether normal or patient volunteers:

- a) Your participation is entirely voluntary.
- b) You may withdraw from participation in this study or any part of the study at any time. Refusal to participate will involve no penalty or loss of medical benefits to which you are entitled.
- c) After you read the explanation, please feel free to ask any questions that will allow you to clearly understand the nature of the study.

**NATURE OF STUDY:** This study is designed to show whether Vitamin A is protective against the development of a certain type of colon tumor. Animal data suggest that Vitamin A may be protective against the development of a variety of benign and malignant tumors of various parts of the body. Data in humans suggest that adequate intake of Vitamin A may protect against the development of lung cancer, colon cancer, and stomach cancer. However, these studies were not designed solely with the intent to assess Vitamin A consumption, and thus failed to assess Vitamin A consumption as accurately as might have been desired. It is also possible that another substance in vegetables (an indole substance) may also protect against the development of these various tumors. The study which we are asking you to participate in is designed not only to assess your Vitamin A status, but also, to assess the intake of this indole-containing substance. If you agree to participate in this study, we will ask you to donate a sample of your blood (approximately 2 tablespoonsful) and a sample of your urine for testing. We will also administer a questionnaire concerning your intake of foods, and we will perform a test designed to determine the ability of your eyes to adjust to darkness. This final test will take about 15 minutes and will involve your choosing discs of a particular color from a group of discs on a table in a room lit with a dim light. All of these studies will be performed on the day of your colonoscopy while you are undergoing preparation for that study. You will not be asked to return to the hospital on any other day for completion of these studies.

**BENEFIT:** You will not be compensated for your participation in this project. It is not anticipated that you, yourself, will receive any direct medical benefit from this study. However, if this study does show that Vitamin A protects against the development of colon polyps, then we will be able to determine the level of consumption which is protective. This will allow us to make recommendations to the general public concerning adequacies of Vitamin A consumption. We may also be able to recommend that the diet of patients in high risk groups for the development of colon polyps or cancer should be supplemented with Vitamin A or beta-carotene in the form of pills.

**DURATION OF STUDY:** Your participation in this study will be limited to the studies outlined above which will all be completed on the day of your colonoscopy. It is not anticipated that your participation in this study will increase the time that you spend in the hospital on the day of your colonoscopy.

**RISKS, INCONVENIENCES AND DISCOMFORTS:** Because your participation in this study will require only that you answer a questionnaire and provide samples of your blood and urine, your participation will involve no substantial risk. It is of course possible that you will develop a bruise at the site from which we draw blood.

**CONFIDENTIALITY OF RESEARCH RECORDS:** We will keep records of the tests performed above. We will also attempt to correlate the results of these test with the results of your colonoscopy. However, we will not publish data which would allow for you to be identified as a participant in this study.

**SAFEGUARDS:** Your participation in this study involves no substantial risk. Even if you agree to participate at this time, you may change your mind and withdraw from participation at any time prior to the completion of these studies.

**ALTERNATIVES TO PARTICIPATION IN THIS STUDY:** If you do not wish to participate in this study, your decision will not in any way affect the care which you receive for your medical problem.

**CIRCUMSTANCES UNDER WHICH YOUR PARTICIPATION MAY BE TERMINATED WITHOUT YOUR CONSENT:**

- a) Health conditions under which your participation possibly would be dangerous.
- b) Other conditions which might occur that would make your participation detrimental to you or your own health.

**COSTS TO YOU FROM PARTICIPATION:** NONE

**SIGNIFICANT NEW FINDINGS:** Any significant new information regarding new findings that develop during the study will be made available to you.

**NUMBER OF SUBJECTS IN THE STUDY:** 150

**UNFORSEEN RISKS FROM PARTICIPATION:** NONE

**FOR FURTHER INFORMATION:** Please contact the Principal Investigator:  
JAMES W. KIKENDALL, MAJ, MC  
GASTROENTEROLOGY SERVICE, 576-1765

For information regarding the rights of research subjects, or in the event there is a research related injury, please contact the Center Judge Advocate, 576-4096/4097.

**SIGNATURES:**

\_\_\_\_\_  
PATIENT/VOLUNTEER SIGNATURE

OR \_\_\_\_\_  
SIGNATURE OF NEXT OF KIN/GUARDIAN

\_\_\_\_\_  
INVESTIGATOR SIGNATURE

\_\_\_\_\_  
WITNESS SIGNATURE

DATE & TIME \_\_\_\_\_

## Appendix 3: VITAMIN A QUESTIONNAIRE

1. Name: \_\_\_\_\_ Date: \_\_\_\_\_
2. Social Security Number: \_\_\_\_\_ Sex: \_\_\_\_\_
3. Race (circle one): BLACK WHITE ORIENTAL  
AMERICAN INDIAN HISPANIC OTHER
4. Year of birth: \_\_\_\_\_
- 5a. Reported weight: \_\_\_\_\_ 5b. Reported height: \_\_\_\_\_
- 6a. Who does the cooking at your house? \_\_\_\_\_  
 b. Are you on a special diet? yes \_\_\_\_\_ no \_\_\_\_\_ if yes explain \_\_\_\_\_  
 c. How often do you eat out? \_\_\_\_\_ d. Do you have a garden? \_\_\_\_\_  
 e. Do you eat a lot differently on weekends? \_\_\_\_\_
- 7a. Have you had anything to eat yet today? \_\_\_\_\_  
 b. Did you eat differently last week because of your test today? \_\_\_\_\_
8. How many average size cups of coffee do you drink daily? \_\_\_\_\_
9. Have you ever smoked cigarettes? \_\_\_\_\_  
 age started \_\_\_\_\_ year started \_\_\_\_\_  
 age ended \_\_\_\_\_ year ended \_\_\_\_\_ avg # /day \_\_\_\_\_
10. Have you taken a vitamin or zinc preparation in the last year?  
 yes \_\_\_\_\_ no \_\_\_\_\_ if yes, please list:  
 BRAND YEARS DOSAGE
- 
- 
11. Have you ever used cod liver oil? yes \_\_\_\_\_ no \_\_\_\_\_  
 if yes, please indicate when it was used. \_\_\_\_\_
12. On the average how many times per week, per month or per year do you eat the following foods? Serving size is standard unless otherwise stated.

<u>FOOD ITEM</u>	<u>Times/wk</u>	<u>Times/mo</u>	<u>Times/yr</u>
<u>DAIRY PRODUCTS</u>			
Milk:			
skim	_____	_____	_____
2%	_____	_____	_____
whole	_____	_____	_____
other	_____	_____	_____

Milkshakes	_____	_____	_____
Instant breakfast	_____	_____	_____
Ice cream	_____	_____	_____
Puddings,etc w/milk	_____	_____	_____
Yogurt	_____	_____	_____
<u>Vegetables-</u>	_____	_____	_____
Asparagus 1/2 c	_____	_____	_____
Broccoli 1/2 c	_____	_____	_____
Brussel sprouts 1/4 c	_____	_____	_____
Cabbage 1/2 c	_____	_____	_____
Carrots 1/2 c	_____	_____	_____
Carrots 1 stick	_____	_____	_____
Carrot juice 1/2 c	_____	_____	_____
Cauliflower 1/2 c	_____	_____	_____
Chard 1/2 c	_____	_____	_____
Endive 1/2 c	_____	_____	_____
Greens 1/2 c	_____	_____	_____
Collards	_____	_____	_____
Kale	_____	_____	_____
Mustard greens	_____	_____	_____
Turnip greens	_____	_____	_____
Green beans 1/2 c	_____	_____	_____
Green peas 1/2 c	_____	_____	_____
Mixed veg. 1/2 c	_____	_____	_____
Pepper,green 1/2 c	_____	_____	_____
pepper, green 1 pod	_____	_____	_____
pepper, red, 1/2 c	_____	_____	_____
Pumpkin	_____	_____	_____
Pie 1 sector	_____	_____	_____
Bread 1 slice	_____	_____	_____
Spinach 1/2 c	_____	_____	_____
spinach raw, 1 c	_____	_____	_____
Squash, acorn 1/2 ea	_____	_____	_____
Sweet potato 1/4 c	_____	_____	_____
sweet potato, 1 ea	_____	_____	_____
Pie 1 sector	_____	_____	_____
Bread 1 slice	_____	_____	_____
Tomato 1 slice	_____	_____	_____
Tomato 1 whole	_____	_____	_____
Tomato 1/2 c	_____	_____	_____
Tomato juice 1/2 c	_____	_____	_____
V-8 juice 1/2 c	_____	_____	_____
<u>Soups</u>	_____	_____	_____
Cream type 1 c	_____	_____	_____
Vegetable 1 c	_____	_____	_____
Minestrone 1 c	_____	_____	_____
Tomato 1 c	_____	_____	_____

<u>FOOD ITEM</u>	<u>Times/wk</u>	<u>Times/mo</u>	<u>Times/yr</u>
<u>Meat/Fish</u>			
Beef liver 2 oz	_____	_____	_____
Calf liver 2 oz	_____	_____	_____
Chicken liver 1 each	_____	_____	_____
Bratwurst 1 link	_____	_____	_____
Liverwurst 1 oz	_____	_____	_____
Liver pate 1 Tb	_____	_____	_____
Crabmeat 1/2 c	_____	_____	_____
Sword fish 3 oz	_____	_____	_____
<u>Cheeses</u>			
Cottage cheese 1/2 c	_____	_____	_____
Swiss cheese 1 oz	_____	_____	_____
Cheddar cheese 1 oz	_____	_____	_____
American cheese 1 oz	_____	_____	_____
Cream cheese 1 oz.	_____	_____	_____
Cheese cake 1 sl	_____	_____	_____
other (specify brand)	_____	_____	_____
<u>Casserole Dishes</u>			
Mac and cheese 1 c	_____	_____	_____
Tomato Sauce 1/4 c	_____	_____	_____
Spaghetti 1/2c sauce	_____	_____	_____
Pizza 1 sector	_____	_____	_____
Lasagna 3"x4"	_____	_____	_____
Ravioli 1 c	_____	_____	_____
Chili 1 c	_____	_____	_____
Meat Stew&carrots 1 c	_____	_____	_____
<u>Eggs</u>			
Egg/Whole 1 ea	_____	_____	_____
<u>Fruits</u> fresh, canned and dried			
Apricots 1 ea	_____	_____	_____
Apricots, dried 1/2 ea	_____	_____	_____
Apricot Nectar 1/2 c	_____	_____	_____
Cantaloupe 1/4 ea	_____	_____	_____
Peaches 1 ea	_____	_____	_____
Peaches 1/2 c	_____	_____	_____
Peach pie 1 sector	_____	_____	_____
Peach nectar 1/2 c	_____	_____	_____
Mangos 1 ea	_____	_____	_____
Nectarines 1 ea	_____	_____	_____
Orange 1 ea	_____	_____	_____
Orange juice 1/2 c	_____	_____	_____
Prunes 2 sm	_____	_____	_____
Prune juice 1/2 c	_____	_____	_____
Tangerine 1 ea	_____	_____	_____

<u>FOOD ITEM</u>	<u>Times/wk</u>	<u>Times/mo</u>	<u>Times/yr</u>
Tang 1 c	_____	_____	_____
Watermellon 1 c	_____	_____	_____
<u>Fats</u>			
Margarine 1 tsp	_____	_____	_____
Butter 1 tsp	_____	_____	_____
<u>Ready to eat cereals</u>			
Bran 1 tb	_____	_____	_____
Specify brand: 1/2 c	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

13. Think back to the years 1974 through 1979. Were you in the armed forces? yes \_\_\_\_\_ no \_\_\_\_\_

14. Where were you living or stationed during those years? \_\_\_\_\_

15. Who cooked your meals during those years?  
 spouse \_\_\_\_\_ mess hall/cafeteria \_\_\_\_\_  
 self \_\_\_\_\_ other \_\_\_\_\_

16. What was your rank during the years 1974-1979? \_\_\_\_\_

17. During the years 1974-1979 how many times per week, per month, or per year did you eat the following foods? Please consider each item carefully. If you cannot remember any difference from your present intake, answer the same as before.

<u>FOOD ITEM</u>	<u>Times/wk</u>	<u>Times/mo</u>	<u>Times/yr</u>
<u>DAIRY PRODUCTS</u>			
Milk:	_____	_____	_____
skim	_____	_____	_____
2%	_____	_____	_____
whole	_____	_____	_____
other	_____	_____	_____
Milkshakes	_____	_____	_____
Instant breakfast	_____	_____	_____
Ice cream	_____	_____	_____
Puddings, etc w/milk	_____	_____	_____
Yogurt	_____	_____	_____

<u>FOOD ITEM</u>	<u>Times/wk</u>	<u>Times/mo</u>	<u>Times/yr</u>
<u>Vegetables-</u>			
Asparagus 1/2 c	_____	_____	_____
Broccoli 1/2 c	_____	_____	_____
Brussel sprouts 1/4 c	_____	_____	_____
Cabbage 1/2 c	_____	_____	_____
Carrots 1/2 c	_____	_____	_____
Carrots 1 stick	_____	_____	_____
Carrot juice 1/2 c	_____	_____	_____
Cauliflower 1/2 c	_____	_____	_____
Chard 1/2 c	_____	_____	_____
Endive 1/2 c	_____	_____	_____
Greens 1/2 c	_____	_____	_____
Collards	_____	_____	_____
Kale	_____	_____	_____
Mustard greens	_____	_____	_____
Turnip greens	_____	_____	_____
Green beans 1/2 c	_____	_____	_____
Green peas 1/2 c	_____	_____	_____
Mixed veg. 1/2 c	_____	_____	_____
Pepper, green 1/2 c	_____	_____	_____
pepper, green 1 pod	_____	_____	_____
pepper, red, 1/2 c	_____	_____	_____
Pumpkin	_____	_____	_____
Pie 1 sector	_____	_____	_____
Bread 1 slice	_____	_____	_____
Spinach 1/2 c	_____	_____	_____
spinach raw, 1 c	_____	_____	_____
Squash, acorn 1/2 ea	_____	_____	_____
Sweet potato 1/4 c	_____	_____	_____
sweet potato, 1 ea	_____	_____	_____
Pie 1 sector	_____	_____	_____
Bread 1 slice	_____	_____	_____
Tomato 1 slice	_____	_____	_____
Tomato 1 whole	_____	_____	_____
Tomato 1/2 c	_____	_____	_____
Tomato juice 1/2 c	_____	_____	_____
V-8 juice 1/2 c	_____	_____	_____
<u>Soups</u>			
Cream type 1 c	_____	_____	_____
Vegetable 1 c	_____	_____	_____
Minestrone 1 c	_____	_____	_____
Tomato 1 c	_____	_____	_____
<u>Meat/Fish</u>			
Beef liver 2 oz	_____	_____	_____
Calf liver 2 oz	_____	_____	_____

<u>FOOD ITEM</u>	<u>Times/wk</u>	<u>Times/mo</u>	<u>Times/yr</u>
Chicken liver 1 each	_____	_____	_____
Bratwurst 1 link	_____	_____	_____
Liverwurst 1 oz	_____	_____	_____
Liver pate 1 Tb	_____	_____	_____
Crabmeat 1/2 c	_____	_____	_____
Sword fish 3 oz	_____	_____	_____

Cheeses

Cottage cheese 1/2 c	_____	_____	_____
Swiss cheese 1 oz	_____	_____	_____
Cheddar cheese 1 oz	_____	_____	_____
American cheese 1 oz	_____	_____	_____
Cream cheese 1 oz.	_____	_____	_____
Cheese cake 1 sl	_____	_____	_____
other (specify brand)	_____	_____	_____

Casserole Dishes

Mac and cheese 1 c	_____	_____	_____
Tomato Sauce 1/4 c	_____	_____	_____
Spaghetti 1/2c sauce	_____	_____	_____
Pizza 1 sector	_____	_____	_____
Lasagna 3"x4"	_____	_____	_____
Ravioli 1 c	_____	_____	_____
Chili 1 c	_____	_____	_____
Meat Stew&carrots 1 c	_____	_____	_____

Eggs

Egg/Whole 1 ea	_____	_____	_____
----------------	-------	-------	-------

Fruits fresh, canned and dried

Apricots 1 ea	_____	_____	_____
Apricots, dried 1/2 ea	_____	_____	_____
Apricot Nectar 1/2 c	_____	_____	_____
Cantaloupe 1/4 ea	_____	_____	_____
Peaches 1 ea	_____	_____	_____
Peaches 1/2 c	_____	_____	_____
Peach pie 1 sector	_____	_____	_____
Peach nectar 1/2 c	_____	_____	_____
Mangos 1 ea	_____	_____	_____
Nectarines 1 ea	_____	_____	_____
Orange 1 ea	_____	_____	_____
Orange juice 1/2 c	_____	_____	_____
Prunes 2 sm	_____	_____	_____
Prune juice 1/2 c	_____	_____	_____
Tangerine 1 ea	_____	_____	_____
Tang 1 c	_____	_____	_____
Watermellon 1 c	_____	_____	_____

Fats

Margarine 1 tsp

Butter 1 tsp

_____	_____	_____
_____	_____	_____

Ready to eat cereals

Bran 1 tb

Specify brand: 1/2 c

_____	_____	_____
_____	_____	_____

18. Did you take vitamin or zinc supplements during the years 1974-1979?

yes \_\_\_\_\_ no \_\_\_\_\_

If yes, what brand and what dose?

BRAND

YEARS

DOSAGE

---



---

Appendix 4

RAW DATA

## Legend for Appendix 4

PT#: Patient number

Race: 1 = black, 2 = white, 5 = other

Age: Years

Wt: Weight in pounds

Ht: Height in inches

PLP: Polyp status (1 = present, 2 = absent)

Ser-A: Serum retinol (micrograms/dl)

RK: Rank (1 = recruit, 24 = four star general)

BC: Serum beta carotene (micrograms/dl)

SMK: Smoking status (1 = current cigarette smoker,  
2 = nonsmoker, 3 = cigar smoker)

pres ret: present retinol consumption in RE/day

pres car: present carotene consumption in RE/day

pres RE: present retinol plus carotene consumption in RE/day

past ret: past retinol consumption in RE/day

past car: past carotene consumption in RE/day

past RE: past retinol plus carotene consumption in RE/day

RBP: Serum retinol binding protein (mg/dl)

ZINC: Serum zinc (micrograms/dl)

dkad1: first dark adaptation test (seconds)

dkad2: second dark adaptation test (seconds)

PT#	RACE	Age	Wt	Ht	PLP	Ser-A	RK	BC	SMK
1	2	55	175	70	2	90.0	19	20.0	1
4	2	47	163	72	2	70.5	18	23.5	1
5	2	65	195	70	1	97.0	19	9.0	1
6	2	50	204	76	1	76.0	19	6.0	1
7	2	46	196	71	1	77.0	6	29.5	2
8	1	50	210	68	2	54.0	19	34.0	2
9	2	41	173	72	2	93.0	17	19.0	1
10	2	55	166	69.8	1	88.5	19	45.5	2
11	2	61	238	75	1	79.0	20	11.0	1
12	2	54	220	70	2	63.0	6	19.5	3
14	1	50	218	73	1	91.0	7	13.3	1
15	2	59	165	65	2	69.0	5	6.0	1
17	1	46	220	72	1	42.0	7	7.8	1
18	5	26	206	72	2	43.0	5	9.3	1
21	1	42	170	68	2	46.0	6	9.5	2
22	2	70	112	64	2	58.5	17	2.5	2
23	2	71	182	70.5	2	43.5	20	16.3	3
24	2	71	180	67.5	2	85.0	19	22.8	2
25	2	54	235	73	1	77.5	7	10.0	1
27	2	75	178	69.5	1	88.5	24	21.0	2
29	2	53	193	69	1	107.0	7	10.5	2
31	1	63	175	68.5	2	76.5	8	27.0	2
32	1	34	165	67	1	77.5	6	13.0	1
34	2	50	177	70	1	69.0	20	8.3	1
35	2	69	160	68.5	1	74.5	23	10.0	2
38	1	53	215	73.5	1	65.5	20	15.8	1
40	1	57	187	69	1	49.0	7	18.3	1
42	2	72	183	71	1	63.0	19	33.3	2
43	1	51	222	74	2	100.5	8	16.0	1
44	2	75	175	70.5	1	117.5	20	7.3	1
46	1	66	134	67	1	60.0	5	11.0	1
47	1	56	290	75.5	1	69.5	7	7.0	2
48	2	47	165	71	1	104.0	19	16.0	1
49	1	52	160	70	2	104.0	6	16.0	1

PT#	RACE	Age	Wt	Ht	PLP	Ser-A	RK	BC	SMK
52	2	42	190	69	2	89.5	6	4.5	1
53	2	68	172	71	1	79.0	19	18.3	2
54	2	55	123	66	1	45.0	5	24.8	1
55	2	65	144	68	2	90.5	5	42.8	2
57	2	55	214	71.5	2	86.0	17	8.5	2
58	2	41	291	71.5	1	92.0	8	3.0	2
59	2	46	160	70	2	73.5	8	14.5	1
60	2	54	213	75	1	51.0	18	37.8	2
61	2	58	140	67	2	76.0	8	23.5	2
63	2	55	171	68.5	1	74.0	20	34.5	2
64	1	51	163	68	2	52.5	8	5.0	1
65	2	59	+250a	73	2	54.5	5	13.5	1
66	1	34	178	71	1	41.0	6	10.5	1
67	2	49	185	71	1	84.5	20	33.5	2
68	1	51	210	69.5	2	79.0	6	14.5	1
69	2	64	170	65	2	81.0	18	7.3	2
70	1	77	207	75.5	1	87.0	7	3.0	1
71	2	64	163	72	1	66.5	24	30.3	2
72	2	68	230	72	1	68.5	19	25.3	3
74	2	67	183	70.5	1	84.0	18	11.5	1
76	2	67	197	67	1	70.0	6	21.8	2
81	1	63	220	71.5	1	60.0	19	9.3	2
82	2	48	168	72	1	55.5	19	8.8	1
83	2	71	129	73	2	64.0	5	23.0	2
86	2	63	194	69.5	1	81.0	24	10.0	2
88	2	70	146	64	1	51.5	8	16.5	1
89	1	50	195	72	1	55.5	19	11.3	1
90	2	66	185	69	2	81.0	19	9.3	1
91	2	66	239	72	1	82.0	20	12.8	1
93	2	64	185	70	1	68.5	20	29.8	1
94	2	56	174	72	1	93.5	13	10.0	2
95	2	87	135	67	1	98.5	19	51.5	2
96	2	64	201	69.5	2	49.5	20	7.0	2

PT#	RACE	Age	Wt	Ht	PLP	Ser-A	RK	BC	SMK
97	2	51	180	70	2	63.0	22	65.5	2
98	1	60	207	67.5	1	61.5	6	9.8	1
100	2	62	192	66	2	55.0	22	57.5	2
101	2	64	148	66	1	88.5	8	30.0	1
102	1	62	188	72.5	2	101.0	6	25.5	3
108	2	62	165	70	1	59.0	12	7.0	1
109	2	52	162	66.5	1	59.5	20	7.8	1
110	2	60	165	70	2	93.5	20	10.5	2
111	2	70	172	67	2	107.5	20	10.5	1
112	1	55	135	66.5	2	92.0	6	30.0	1
113	1	48	220	74	2	58.0	19	8.0	2
116	2	57	195	72	1	83.0	20	5.8	2
117	2	66	139	64	1	90.0	20	16.0	1
118	2	64	205	72	2	81.5	18	2.0	2
119	2	74	140	69	1	63.5	19	12.0	1
120	2	60	162	71.5	2	92.5	19	14.0	2
121	2	56	213	71	1	66.0	18	6.0	1
122	2	67	162	73.5	1	72.0	8	11.0	1
123	2	59	227	75	1	58.5	5	17.0	2
124	2	62	215	68.5	2	51.0	20	11.0	1
125	2	44	172	72	1	53.5	18	7.0	2
126	2	58	135	72	2	57.0	9	8.5	1
127	2	57	192	74	2	69.0	14	17.0	2
130	2	77	175	71	2	76.5	19	11.0	2
132	2	69	138	70	1	72.5	22	10.0	1
133	2	68	174	68	1	94.5	19	12.5	2
134	2	66	188	68	2	96.0	8	8.0	2
135	2	53	220	71	1	70.5	7	6.0	1
136	2	66	164	70	1	60.0	6	2.0	1
138	2	70	170	70	2	127.5	19	21.5	3
140	1	69	220	68	1	65.0	7	15.0	1
142	2	65	188	73	2	84.5	20	13.0	2
143	2	64	180	68	2	93.5	14	11.0	2

PT #	pres ret	pres car	pres RE	past ret	past car	past RE
1	2117.9	977.8	3095.7	549.8	1301.7	1851.5
4	1405.2	1703.3	3108.5	792.5	1408.4	2200.9
5	4580.3	1460.4	6040.6	3645.0	1500.2	5145.2
6	843.9	1706.9	2550.8	871.0	1707.2	2578.2
7	719.3	880.8	1600.1	1613.0	1072.2	2685.2
8	504.3	656.2	1160.5	902.1	853.0	1755.1
9	2575.9	1216.1	3792.0	477.4	702.2	1179.7
10	542.4	1731.1	2273.4	1462.5	1721.2	3183.7
11	472.3	825.2	1297.5	680.7	832.6	1513.4
12	1821.5	1152.6	2974.1	509.1	825.3	1334.4
14	442.6	457.3	899.9	442.6	458.3	900.8
15	1793.4	629.9	2423.2	2466.5	498.5	2964.9
17	722.7	787.2	1509.9	5983.5	159.6	6143.1
18	1398.7	527.8	1926.5	1348.2	691.1	2039.3
21	358.9	480.1	839.0	425.9	435.4	861.3
22	868.4	467.3	1335.7	1297.1	843.2	2140.3
23	743.5	647.4	1390.9	1063.1	627.8	1690.9
24	688.5	937.5	1626.0	1122.2	836.1	1958.4
25	2852.4	932.2	3784.7	1077.5	586.7	1664.2
27	2209.6	965.2	3174.8	725.3	922.1	1647.4
29	1377.2	479.3	1856.5	1531.2	422.2	1953.4
31	2067.4	1669.2	3736.6	1714.6	2008.2	3722.8
32	1305.5	880.8	2186.3	208.4	423.3	631.7
34	331.1	840.7	1171.8	1756.9	1264.7	3021.6
35	600.6	436.4	1037.1	662.3	541.2	1203.4
38	158.4	840.5	998.8	273.9	869.4	1143.3
40	1403.6	652.2	2055.8	655.4	450.7	1106.2
42	2599.7	1071.3	3671.1	860.5	952.2	1812.8
43	567.3	694.4	1261.7	710.2	706.0	1416.2
44	776.1	687.1	1463.2	776.1	687.1	1463.2
46	1093.5	1203.3	2296.8	1416.5	1380.8	2797.3
47	2391.2	1022.3	3413.5	2148.2	1627.2	3775.4
48	2376.1	1574.2	3950.3	3533.9	1610.3	5144.2
49	1609.0	2082.7	3691.8	1889.6	1584.9	3474.6

PT #	pres ret	pres car	pres RE	past ret	past car	past RE
52	1805.7	937.6	2743.2	2965.5	576.4	3541.9
53	1675.7	1207.2	2882.9	1837.3	1290.4	3127.7
54	760.3	447.4	1207.7	760.3	447.4	1207.7
55	1050.5	489.4	1539.9	1703.4	443.3	2146.8
57	620.2	657.4	1277.6	673.8	657.4	1331.2
58	1191.2	615.3	1806.5	859.2	369.5	1228.7
59	1119.0	888.9	2007.9	2565.7	703.0	3268.7
60	1590.6	779.2	2369.7	1763.7	779.2	2542.9
61	2436.8	2037.6	4474.4	2637.6	1367.7	4005.3
63	817.9	2006.3	2824.2	867.7	1516.4	2384.2
64	3381.8	371.3	3753.1	2833.5	1204.1	4037.5
65	695.3	795.2	1490.5	688.6	832.1	1520.7
66	309.6	522.5	832.1	1870.9	417.8	2288.7
67	1149.2	895.1	2044.4	1087.3	714.8	1802.1
68	567.7	600.2	1167.8	567.7	600.2	1167.8
69	2896.3	1155.6	4051.9	2896.3	1155.6	4051.9
70	415.6	429.5	845.1	528.6	639.7	1168.4
71	3014.7	452.6	3467.3	2723.7	451.9	3175.6
72	705.0	1120.0	1825.0	1183.1	1113.5	2296.6
74	1179.6	762.2	1941.8	1379.0	751.7	2130.7
76	364.8	1960.5	2325.3	180.8	42.5	223.3
81	743.3	302.0	1045.3	1972.9	576.1	2549.0
82	425.6	409.7	835.4	290.7	387.3	678.0
83	1485.4	754.8	2240.3	1620.8	1014.3	2635.1
86	794.9	765.8	1560.7	623.4	756.9	1380.3
88	2077.6	490.9	2568.5	1473.8	588.0	2061.8
89	449.5	236.5	686.0	1367.8	273.2	1641.0
90	694.8	1098.7	1793.5	737.5	1098.7	1836.2
91	370.4	508.0	878.4	450.5	511.5	962.0
93	2059.8	1975.6	4035.4	2918.3	1925.6	4843.9
94	571.8	843.8	1415.7	393.5	770.4	1163.9
95	1208.3	1067.5	2275.8	1237.2	1067.5	2304.7
96	452.4	714.2	1166.6	414.0	638.9	1052.9

PT #	pres ret	pres car	pres RE	past ret	past car	past RE
97	2983.9	1496.8	4480.7	2795.4	1508.6	4304.0
98	478.2	999.9	1478.1	641.5	1003.2	1644.7
100	140.6	557.3	698.0	1965.0	759.0	2724.0
101	2264.5	2113.7	4378.2	230.0	1060.3	1290.3
102	466.5	699.9	1166.4	473.0	699.1	1172.1
108	2420.6	314.1	2734.7	2348.3	181.1	2529.4
109	856.7	747.6	1604.3	826.2	1350.2	2176.4
110	2705.8	782.7	3488.4	1610.6	822.7	2433.4
111	925.9	886.3	1812.2	1834.7	545.0	2379.7
112	3205.6	860.8	4066.3	3202.4	849.1	4051.5
113	874.9	548.6	1423.4	1380.4	472.1	1852.5
116	816.9	1656.9	2473.7	816.9	1650.6	2467.5
117	1409.1	1566.8	2975.9	1505.8	1125.1	2630.8
118	859.3	226.4	1085.6	458.8	215.0	673.7
119	1207.6	1571.1	2778.7	799.4	1485.2	2284.6
120	823.5	659.0	1482.5	823.5	659.0	1482.5
121	1176.5	1527.6	2704.1	2535.3	1417.6	3952.9
122	1272.2	1381.4	2653.6	1306.2	1412.5	2718.7
123	266.7	632.1	898.8	259.4	679.2	938.6
124	1551.9	352.3	1904.2	614.8	371.8	986.6
125	356.8	481.2	837.9	699.1	458.0	1157.1
126	1043.9	454.0	1497.9	998.7	451.1	1449.9
127	1259.0	1248.0	2507.1	1220.2	1257.7	2477.9
130	1211.3	1752.1	2963.3	1372.0	1716.9	3088.9
132	1605.5	604.9	2210.3	1605.5	838.6	2444.0
133	916.0	584.5	1500.4	828.4	612.6	1441.0
134	2686.7	1369.8	4056.6	2879.9	1066.4	3946.3
135	1482.7	420.0	1902.7	1326.2	400.5	1726.7
136	2226.1	568.7	2794.8	2224.6	631.5	2856.0
138	1603.8	582.2	2186.0	1603.8	767.6	2371.4
140	2566.5	689.2	3255.7	820.9	675.8	1496.7
142	2383.7	1081.6	3465.3	2557.7	1064.1	3621.8
143	1150.3	1225.4	2375.7	1523.4	1063.7	2587.1

PT#	RBP	ZINC	dkad1	dkad2
1	6.10	86.0	NA	NA
4	5.80	104.0	233	154
5	7.30	122.0	508	383
6	5.80	96.0	345	257
7	6.50	86.0	290	150
8	3.80	95.0	275	216
9	6.20	77.5	273	162
10	7.10	118.0	344	333
11	5.80	137.0	442	369
12	5.00	139.0	356	476
14	6.80	87.0	NA	NA
15	5.20	103.0	432	287
17	3.50	114.5	300	240
18	3.50	105.0	219	186
21	3.30	92.0	298	286
22	5.80	90.0	417	321
23	3.00	102.0	436	360
24	6.90	100.5	290	206
25	5.10	124.0	401	242
27	6.30	106.0	NA	NA
29	8.00	115.0	252	198
31	4.90	120.5	311	265
32	5.40	100.0	235	126
34	5.50	97.0	282	321
35	6.10	98.0	413	246
38	5.50	102.0	259	118
40	3.20	85.0	183	176
42	4.60	90.5	340	244
43	7.00	89.0	287	124
44	8.30	110.5	282	236
46	4.20	82.0	NA	NA
47	5.00	98.0	287	240
48	7.60	88.0	406	110
49	7.40	106.0	249	185

PT#	RBP	ZINC	dkad1	dkad2
52	6.40	117.0	306	152
53	5.20	80.0	245	190
54	3.00	102.0	351	284
55	6.50	79.0	NA	NA
57	7.30	85.0	NA	NA
58	8.10	95.0	NA	NA
59	6.50	103.0	180	156
60	4.20	94.5	318	149
61	6.50	98.0	390	326
63	6.00	97.0	225	140
64	5.20	121.0	459	186
65	5.50	102.0	235	154
66	4.00	107.0	139	96
67	6.30	86.0	202	137
68	6.30	84.0	392	182
69	6.60	133.0	504	255
70	6.80	95.0	NA	NA
71	5.10	82.0	363	NA
72	4.60	107.0	855	577
74	5.90	101.0	297	368
76	5.20	94.0	493	306
81	4.10	88.5	513	426
82	4.50	83.0	404	327
83	5.10	90.5	501	432
86	6.60	82.0	NA	NA
88	4.20	72.0	NA	NA
89	4.75	93.0	152	127
90	5.70	111.0	205	198
91	7.10	86.0	441	337
93	5.70	100.0	NA	NA
94	5.20	89.0	235	195
95	8.40	89.0	444	NA
96	4.30	118.0	285	223

PT#	RBP	ZINC	dkad1	dkad2
97	5.00	106.5	315	279
98	5.00	116.0	461	NA
100	3.70	94.0	237	147
101	6.90	87.0	409	414
102	6.50	107.0	321	317
108	4.30	113.0	341	212
109	5.30	89.0	320	263
110	7.30	92.0	NA	NA
111	8.80	97.0	194	135
112	8.60	74.0	NA	NA
113	4.60	77.0	288	195
116	6.80	108.0	630	423
117	6.90	91.0	504	439
118	6.40	97.0	NA	NA
119	4.80	74.0	NA	NA
120	6.80	133.0	303	258
121	4.40	101.0	NA	NA
122	5.60	93.0	NA	NA
123	4.70	91.0	294	202
124	3.40	90.0	396	344
125	4.30	82.0	287	241
126	4.25	75.0	217	170
127	5.20	91.0	NA	NA
130	5.50	89.0	258	329
132	5.10	83.0	417	346
133	5.80	115.0	583	NA
134	6.80	127.0	561	NA
135	5.30	103.0	420	428
136	4.40	76.0	NA	NA
138	8.40	104.0	NA	NA
140	4.60	90.5	NA	NA
142	5.90	99.0	NA	NA
143	10.70	114.5	NA	NA

**The vita has been removed from  
the scanned document**