

## SERUM RETINOL AND RETINOL-BINDING PROTEIN LEVELS DO NOT PREDICT SUBSEQUENT LUNG CANCER

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Retinol and retinol-binding protein levels were measured in sera previously obtained, and stored in the frozen state, at multiphasic health checkups from 151 persons subsequently found to have lung cancer (cases) and 302 persons who remained free of cancer (controls). Two controls were matched to each case for sex, skin color, age, date of multiphasic health checkup, and aspects of the smoking habit. Mean levels in cases and controls were, respectively, retinol: 82.17 and 82.37  $\mu\text{g}/\text{dl}$  ( $p = 0.93$ ), and retinol-binding protein: 6.04 and 6.00  $\text{mg}/\text{dl}$  ( $p = 0.81$ ). Mean differences between cases and controls were, retinol: 0.195  $\mu\text{g}/\text{dl}$  with 95% confidence limits, -3.91 and 4.30  $\mu\text{g}/\text{dl}$ ; retinol-binding protein: -0.033  $\text{mg}/\text{dl}$  with 95% confidence limits, -0.31 and 0.24  $\text{mg}/\text{dl}$ . No significant trend in relative risk of lung cancer was observed when the retinol or retinol-binding protein distribution was divided into quintiles. No significant associations were observed in subgroups based on age, sex, histologic type of cancer, cigarette consumption, or interval between blood drawing and cancer diagnosis. In this large study, retinol and retinol-binding protein levels were not useful in predicting the subsequent development of lung cancer.

lung neoplasms; retinol; retinol binding proteins; vitamin A

Several lines of evidence suggest that a person's retinoid (vitamin A) status may be a determinant of the risk of developing cancer. First of all, retinoids have powerful effects on cell differentiation and prolifer-

ation (1, 2). These effects have been demonstrated with both normal and neoplastic cells. Since carcinogenesis is fundamentally a disorder of cell differentiation, it is possible that the retinoid status of a cell sig-

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nificantly influences its potential for cancer development. Second, synthetic retinoids have been shown to be useful and effective in the prevention of carcinogenesis in experimental animals (3, 4). Third, a number of epidemiologic studies have suggested that the dietary intake, or the plasma level, of vitamin A and/or beta-carotene may be inversely associated with the incidence or risk of cancer (5, 6).

Two epidemiologic studies reported that low serum retinol levels were associated with an increased risk of cancer, particularly lung cancer (7, 8). However, in two recent studies, no association was found between the level of serum retinol and the subsequent development of cancer (9, 10).

We now report a study designed to test the hypothesis that a low level of serum retinol is associated with an increased risk of lung cancer in human beings. The levels of retinol and of retinol-binding protein in frozen serum specimens obtained from 151 persons who later developed lung cancer were compared with those from 302 matched subjects who remained free of the disease.

## METHODS

### *Study subjects*

Between 1964 and 1971, 263,000 serum specimens were obtained routinely from persons receiving multiphasic health checkups (11) at the Oakland and San Francisco facilities of the Kaiser Permanente Medical Care Program in Northern California. These were kept in frozen storage ( $-23^{\circ}\text{C}$  or colder) in Oakland until 1980, when they were packed in dry ice and shipped to a frozen storage facility of the Orentreich Foundation for the Advancement of Science, Inc., in New York. In Oakland the specimens were maintained at  $-30^{\circ}\text{C}$  until June 1969, and at  $-25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  thereafter. It is unlikely that sample temperatures ever exceeded  $-23^{\circ}\text{C}$  except for a single short episode in December 1968 when a 22-hour power outage led to a rise in air temperature to  $-15^{\circ}\text{C}$  and an estimated rise in specimen temperature to  $-21^{\circ}\text{C}$ .

In New York, the 263,000 specimens have been kept at approximately  $-40^{\circ}\text{C}$ , and a computer-stored catalog was prepared and maintained by the Orentreich Foundation.

The subjects for the present study were persons from whom at least one of these frozen serum specimens had been drawn and who also belonged to a special study cohort of 143,574 persons who had received at least one prescription from the Kaiser Permanente pharmacy in San Francisco between 1969 and 1973. This study cohort has been carefully followed up to determine which of its members developed cancer (12). We aimed to select 156 cases and two matched controls per case, a sample-size that provided 95 per cent power to detect a case-control difference of  $6\ \mu\text{g}/\text{dl}$  (0.35 standard deviation units), the smaller of the two differences found previously (7, 8) (two-sided  $\alpha = 0.05$ ). There were 233 persons who developed lung cancer by the end of 1978 who had serum frozen at least two years before diagnosis and whose chest x-rays at the multiphasic health checkup at that time showed no suspicious density. To be selected, a control subject had to be of the same sex and skin color, and to match the case for age within one year and date of multiphasic health checkup within three months. Also, we attempted to match for cigarette smoking status and, among smokers, for quantity of cigarettes per day (less than one pack, one to two packs, two or more packs), duration of smoking (less than 10 years, 10 to 20 years, 20 or more years), and inhalation (yes or no). Controls also had to be free of suspicious densities on chest x-ray in the multiphasic health checkup and they must not have developed any cancer through 1978.

Of the 233 cases who met the above criteria, we were able to find two matched controls each for 160. Failures in matching were due mostly to (a) peculiarities in the case's reported smoking habits, e.g., unknown inhalation or quantity of cigarettes, or short duration of smoking for age, and to (b) cases being of minority races or in

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older age groups containing few potential controls. To obtain the planned 156 sets, we omitted the last four of the 160 case-control triplets (arranged by the case's medical record number). Lists of the triplets were prepared so that the three specimens in each would be thawed and analyzed at about the same time. The case's position in the triplet—first, second, or third—was assigned using a table of random numbers. Laboratory personnel and investigators at both Columbia University and the Orentreich Foundation were kept blind to case-control status. All analyses were performed between August 22 and December 5, 1983.

After the laboratory work was completed, five case-control triplets were excluded, leaving 151. One was dropped because one control subject had insufficient serum for analysis. In each of the other four there was one subject (two cases and two controls) who had measurable quantities of retinyl esters (16–148  $\mu\text{g}$  retinol equivalents/dl). The presence of retinyl esters in the serum reflects either recent dietary intake of vitamin A (chylomicron retinyl esters) or represents a manifestation of hypervitaminosis A (13). Since it was not possible to distinguish between these two alternatives, the four case-control triplets were dropped to ensure that no subjects with abnormal vitamin A status were included in the study.

The subjects ranged in age from 26 to 78 years at time of multiphasic health checkup; mean age was 53.9 years; 65.6 per cent were men and 34.4 per cent were women; 92.1 per cent were white, 6.0 per cent were black, 2.0 per cent were of other and unknown race. The respective percentages of cases and controls in the main cigarette smoking categories were as follows: nonsmokers—7.3 per cent and 7.6 per cent, exsmokers—13.9 per cent and 21.5 per cent; smokers of less than one pack—8.6 per cent and 8.9 per cent; smokers of one to two packs—45.7 per cent and 41.4 per cent; smokers of two or more packs—21.9 per cent and 17.5 per cent; and unknown—2.6

per cent and 3.0 per cent. There were 94 case-control triplets that were perfectly matched in these main categories. (The mismatches, discovered after the laboratory work was completed, were due to a failure in communication between one of us (G. F.) and a programmer, who assumed that all subjects were smokers and ignored a question about current smoking status.)

Of the 151 cases, 105 (70 per cent) had tissue samples examined by a pathologist, 21 (14 per cent) were confirmed by sputum or pleural fluid cytology without additional tissue examination, and 25 (17 per cent) did not have information in their records indicating a pathologic examination. The histologic classification used in this study was based on the pathology reports in the records. The cases without pathologic examination are included in the subgroup labeled as "unknown."

#### *Assay of retinol and retinol-binding protein levels*

Serum samples were packed in dry ice and shipped to Columbia University where they were stored at  $-20\text{ C}$  until assayed. Sera were thawed in the dark at room temperature on the day they were assayed for retinol. After thawing, two aliquots of 0.1 ml each were removed from each serum sample for duplicate retinol determinations. Four additional aliquots of each serum sample were also removed and frozen at  $-20\text{ C}$  for later duplicate retinol-binding protein analyses and sodium determinations. The remaining serum was then refrozen and kept at  $-20\text{ C}$  until all assays were completed.

Serum retinol levels were determined by high performance liquid chromatography using modifications of the procedure described by Bieri et al. (14). High performance liquid chromatography was conducted with a  $\mu\text{Bondapak C}_{18}$  column (Waters Associates, Milford, MA), eluted at a constant flow rate of 2.0 ml/minute by the following solvents: 0–5 minutes, methanol: $\text{H}_2\text{O}$  (90 per cent/10 per cent); 5–15 minutes, linear gradient to 100 per cent methanol; 15–25

minutes, 100 per cent methanol. Retinol and retinyl acetate were detected by absorbance at 325 nm; a second absorbance detector set at 436 nm was used to monitor for the possible presence of carotenoids. Coefficients of variation for within- and between-assay precision of retinol determinations were 4.4 per cent and 7.8 per cent, respectively. A quality control serum specimen of known retinol content was included with each assay, and the measured value for this specimen was always found to deviate by less than two standard deviations from its known retinol value.

The high performance liquid chromatography chromatograms obtained for the retinol extracts from all case-control triplets were qualitatively identical to those obtained from fresh serum. No unusual or additional high performance liquid chromatography peaks were observed in any of these chromatograms. (In our experience, additional ultraviolet-absorbing peaks, presumably representing modified or degraded retinol, are often observed in extracts of serum samples which were either poorly collected or poorly stored.)

Beta-carotene was found in only 16.6 per cent (75 samples) of the sera assayed in this study. In those samples where beta-carotene was detected, the levels were substantially lower than those observed consistently in fresh serum samples. These observations strongly suggest that beta-carotene was not stable under the storage conditions used for these sera. Hence, the level of beta-carotene could not be included as an additional measured parameter in this study.

Radioimmunoassay for retinol-binding protein was carried out as described for the assay of rat retinol-binding protein (15). The coefficients of variation for within- and between-assay precision were, respectively, 6.8 per cent and 8.4 per cent. A quality control serum specimen of known retinol-binding protein content was measured as part of each assay and always gave a value which varied by less than two standard deviations from its known retinol-binding protein value.

All serum retinol and retinol-binding protein assays were carried out in duplicate, and the mean values were used for the final data analysis. Duplicate values differed from their mean by less than 8 per cent in the great majority of instances. When the agreement between duplicate assays was less good (less than 10 per cent of samples) another assay was carried out in duplicate on that particular serum sample. The mean so obtained was then used for the data analysis instead of the mean obtained from the first duplicate assay.

To check for dessication, sodium concentration was measured in each specimen at the Orentreich Foundation laboratory by a Beckman model E2A electrolyte analyzer (Beckman Instruments, Inc., Irvine, CA) and at Columbia University on pooled specimens by flame photometry. Mean levels on pooled sera were 148-149 mM, with expected mean values of 140-142 mM for fresh normal serum.

#### Statistical methods

Statistical significance of case-control differences in mean levels was determined by the one-sample *t* test in which the unit for each triplet was the difference between the value for the case and the mean of the values for the two controls. For contingency table analysis by quintiles, relative risks were estimated both by simple odds ratios with matching ignored and in a matched fashion using conditional logistic regression for matched sets. Multivariate analyses with control for cholesterol level and smoking status also employed logistic regression for matched sets.

#### RESULTS

There was virtually no difference between the cases and the controls in the mean level of either retinol or retinol-binding protein. The mean concentrations for the cases and controls were, respectively, 82.17 and 82.37  $\mu\text{g}/\text{dl}$  for retinol ( $p = 0.93$ ), and 6.04 and 6.00  $\text{mg}/\text{dl}$  for retinol-binding protein ( $p = 0.81$ ). The mean case-control difference for retinol was 0.195  $\mu\text{g}/\text{dl}$  with 95 per cent confidence limits, -3.91 and

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and retinol-binding protein were determined in duplicate, and the mean values were used for the final analysis. The mean difference between the duplicate assays was less than 8 per cent in most instances. When the duplicate assays were performed on the same sample, the mean difference was less than 5 per cent. The mean values obtained from the duplicate assays were used for the data analysis.

For sodium concentration, sodium concentration was determined in each specimen at the laboratory by a spectrophotometer (Beckman DU-40, Irvine, CA) using a sodium chloride standard solution. Mean levels were 148-149 mM, with a range of 140-142 mM for the controls.

#### Methods

The design of the case-control study was determined by the unit in which the unit of analysis was the mean of the quintiles. For contingency tables, relative risks and simple odds ratios were calculated and in a matched case-control study, multivariate analysis of logistic regression was employed. Logistic regression was also employed for the analysis of cholesterol level and other variables.

There was no difference between the controls in the mean retinol or retinol-binding protein concentrations for the cases, respectively, 4.30  $\mu\text{g/dl}$  for retinol ( $p = 0.93$ ) and 4.30  $\mu\text{g/dl}$  for retinol-binding protein ( $p = 0.93$ ).

4.30  $\mu\text{g/dl}$ ; for retinol-binding protein the mean difference was  $-0.033 \text{ mg/dl}$  with 95 per cent confidence limits,  $-0.31$  and  $0.24 \text{ mg/dl}$ . Progressing by quintiles from quintile V (highest) to quintile I (lowest) there was no clear trend of relative risk for lung cancer associated with either retinol ( $\chi^2$  for trend = 0.004,  $p = 0.94$ ) or retinol-binding protein ( $\chi^2$  for trend = 0.109,  $p = 0.74$ ) (table 1). In the matched analysis the relative risk was estimated to be 1.0 for a decrease in retinol concentration of 20  $\mu\text{g/dl}$  with 95 per cent confidence interval, 0.8-1.2. The relative risk associated with a decrease in retinol-binding protein of 1  $\text{mg/dl}$  was 1.0 with 95 per cent confidence interval, 0.8-1.1.

As expected from the results for retinol and retinol-binding protein, the retinol-binding protein/retinol molar ratio (defined as retinol-binding protein/retinol  $\times 13.45$ ) also showed no association with lung cancer. Mean levels were 1.0039 for the 151 cases and 1.0059 for the 302 controls. The relative risks by quintiles going from lowest to highest were 1.15, 1.13, 1.09, 1.50, and 1.00 (reference) with  $\chi^2$  for trend = 0.002,  $p = 0.96$ .

The study group was also subdivided by age and sex, histologic type of lung cancer, interval from multiphasic health checkup

to lung cancer diagnosis, and smoking category. No statistically significant case-control difference in mean retinol or retinol-binding protein was found in any subgroup. One difference with a  $p$  value of 0.06 is quite compatible with chance variation since 46 comparisons were made (table 2). Because of our failure to match fully for smoking—particularly current vs. former smoking—as we had intended, we also conducted a multivariate analysis of the relation of retinol and of retinol-binding protein to lung cancer controlling for smoking habit. As noted for all 94 smoking-matched triplets (table 2), no association was evident.

Both retinol and retinol-binding protein showed small ( $r = 0.20$ ) but statistically significant ( $p < 0.001$ ) correlations with serum cholesterol level measured by an autoanalyzer at time of multiphasic health checkup. (Cholesterol values were recorded for 125 cases and 257 controls.) Among the 98 triplets with no missing values the mean cholesterol level for lung cancer cases was 233.3  $\text{mg/dl}$ ; for controls it was 241.0  $\text{mg/dl}$ ,  $p = 0.09$ . The relative risks for lung cancer in these 98 triplets for the quintiles of cholesterol were as follows: Quintile V (highest and reference category), 1.0; Quintile IV, 0.9; Quintile III, 1.3; Quintile II, 1.3;

TABLE 1  
Estimated relative risk of developing lung cancer according to quintile of retinol and retinol-binding protein.  
Quintile V is the reference category

Quintile		No. of cases	No. of controls	Relative risk (odds ratio)	
				Unmatched analysis	Matched analysis
	Retinol ( $\mu\text{g/dl}$ )				
V (high)	98.7-173.3	28	62	1.0	1.0
IV	85.0- 98.6	35	57	1.4	1.3
III	75.0- 84.9	30	60	1.1	1.1
II	65.6- 74.9	27	66	0.9	0.9
I (low)	38.1- 65.5	31	57	1.2	1.2
	Retinol-binding protein (mg/dl)				
V (high)	6.80-15.12	32	59	1.0	1.0
IV	6.12- 6.79	24	67	0.7	0.6
III	5.55- 6.11	34	58	1.1	1.1
II	4.99- 5.54	31	59	1.0	1.0
I (low)	2.91- 4.98	30	59	0.9	0.9

TABLE 2  
Comparison of mean retinol and retinol-binding protein in cases vs. controls in various subgroups

Subgroup	No. of triplets	Mean retinol ( $\mu\text{g}/\text{dl}$ )			Mean retinol-binding protein ( $\text{mg}/\text{dl}$ )		
		Case	Control	<i>p</i>	Case	Control	<i>p</i>
Age-sex group							
Men	45	86.7	87.3	0.88	6.1	6.1	0.59
<55 years old							
55+ years old	54	79.6	84.4	0.13	5.8	6.1	0.28
Women							
<55 years old	36	76.6	73.0	0.42	5.9	5.7	0.58
55+ years old	16	90.6	82.8	0.37	6.6	6.1	0.52
Histology							
Squamous/epidermoid	33	79.3	81.7	0.61	5.9	5.9	0.81
Small cell anaplastic	18	80.9	87.8	0.21	5.6	6.2	0.12
Adenocarcinoma	50	80.7	84.6	0.52	6.2	6.0	0.66
Large cell undifferentiated	11	78.0	73.8	0.53	6.2	5.6	0.25
Bronchial/alveolar cell	13	85.8	85.1	0.90	6.1	6.2	0.70
Other (e.g., carcinoid)	5	102.7	84.5	0.06	6.8	7.1	0.49
Unknown	34	83.3	82.5	0.87	6.2	6.1	0.75
Interval (years): multiphasic health checkup to diagnosis							
2.1-3.5	25	82.6	76.6	0.13	5.8	5.8	0.84
3.6-5.5	25	87.3	86.5	0.89	6.5	6.1	0.34
5.6-6.8	25	80.8	82.6	0.71	6.3	6.1	0.70
6.9-8.6	25	80.6	81.3	0.91	5.8	6.0	0.61
8.7-10.4	25	82.2	83.8	0.75	6.0	5.9	0.56
10.5-13.8	26	79.6	83.4	0.46	5.8	6.2	0.12
Smoking category (matched triplets)							
Nonsmoker	10	78.1	84.8	0.50	6.5	6.2	0.79
Ex-smoker	6	95.7	90.2	0.77	6.4	6.8	0.28
<1 pack/day	7	75.8	75.7	0.98	6.1	6.0	0.80
1-2 packs/day	50	84.9	80.9	0.32	6.2	5.9	0.34
2+ packs/day	21	80.1	83.8	0.44	5.8	6.1	0.32
All smoking-matched	94	83.1	82.2	0.73	6.1	6.1	0.73

Quintile I, 1.4 ( $\chi^2$  for trend = 1.106,  $p = 0.29$ ). Control for cholesterol in multivariate analysis did not change our finding that retinol and retinol-binding protein levels were not predictive of lung cancer.

#### DISCUSSION

Much has been learned in the past two decades about the metabolism of retinoids. Dietary provitamin A carotenoids are largely converted to retinol during intestinal absorption in the mucosal cell. In humans, a limited amount of carotenoids can also be absorbed intact. Intestinal vitamin A (either derived from the diet or newly synthesized from carotene) is absorbed as retinyl esters in association with

lymph chylomicrons. Newly-absorbed vitamin A is stored in the liver as retinyl esters. From the liver, vitamin A is mobilized and transported in plasma as retinol bound to its specific transport protein, retinol-binding protein. Retinol-binding protein thus serves to deliver retinol to peripheral target tissues. For detailed recent reviews of retinol and retinol-binding protein metabolism see references 16-18.

Under normal conditions, the plasma levels of retinol and retinol-binding protein are highly correlated, and the molar ratio of retinol-binding protein to retinol is close to 1.1 (17). Moreover, in any single individual, the plasma level of retinol is normally quite constant, even with fairly large vari-

ations in dietary vitamin A. Retinol-binding protein levels, because of their high binding capacity, reflect the hepatic synthesis of this protein. In the case of lung cancer, both retinol and retinol-binding protein levels, because of their high binding capacity, reflect the hepatic synthesis of this protein. Hence, we would expect that measurement of these two factors would serve as a useful measure of liver function. It is conceivable that these factors might also have some utility in the diagnosis of lung cancer. Under certain conditions, retinol-binding protein levels also reflect the hepatic synthesis of this protein.

Wolbach and his colleagues (3) reported that vitamin A deficiency in rats led to the replacement of retinol in many tissues. In many tissues, then, extended treatment with retinoids might be expected to lead to differentiation of these tissues through their effect on the replacement of retinol in many tissues. In experimental animals, retinoids have been shown to be effective in the treatment of bladder cancer. Carcinogenesis in the lung (3), and in vitro, retinoids have been shown to be effective in the treatment of plastic cells in culture. Moreover, retinoids have been shown to be effective in the treatment of plastic cells in culture.

The effect of retinoids on cancer risk is and continues to be reviewed. Retinol and retinol-binding protein levels were not predictive of lung cancer. The effect of retinoids on cancer risk is and continues to be reviewed. Retinol and retinol-binding protein levels were not predictive of lung cancer.

Various subgroups	
in retinol-binding protein (mg/dl)	
Control	<i>p</i>
6.1	0.59
6.1	0.28
5.7	0.58
6.1	0.52
5.9	0.81
6.2	0.12
6.0	0.66
5.6	0.25
6.2	0.70
7.1	0.49
6.1	0.75
5.8	0.84
6.1	0.34
6.1	0.70
6.0	0.61
5.9	0.56
6.2	0.12
6.2	0.79
6.8	0.28
6.0	0.80
5.9	0.34
6.1	0.32
6.1	0.73

ations in dietary intake or in liver stores of vitamin A. This constancy appears to reflect the high degree with which retinol-binding protein production rate is regulated. In the present study we measured both retinol and retinol-binding protein levels, because it was known that retinol-binding protein is very stable in stored sera. Hence, we hypothesized that the measurement of retinol-binding protein levels would serve as a good proxy, confirmatory measure of retinol. Moreover, it was conceivable that retinol-binding protein might have some independent relationship with lung cancer risk, although under normal conditions retinol-binding protein circulates almost exclusively as the retinol + retinol-binding protein complex.

Wolbach and Howe reported in 1925 (19) that vitamin A deficiency in the rat led to the replacement of differentiated epithelia in many tissues with squamous cells. Since then, extensive studies have shown that retinoids profoundly influence cell differentiation and proliferation (1, 2). It is through this effect that low levels of retinol in a tissue are thought possibly to encourage the development of epithelial cancer. In experimental animals given carcinogens, retinoids can prevent cancer of the urinary bladder, mammary gland, and skin (3, 4). Carcinogenesis at other sites, including the lung (3), is also inhibited by retinoids. *In vitro*, retinoids can act directly on nonneoplastic cells in culture to suppress the process of malignant transformation (1, 4, 18). Moreover, retinoids can convert neoplastic cells in culture to differentiated, nonneoplastic ones (2, 20, 21).

The epidemiologic evidence suggesting an inverse relation between vitamin A and cancer risk comes mainly from serum studies and dietary recall studies. Peto et al. (5) reviewed eight studies in which blood retinol and/or beta-carotene levels were measured in patients with cancer of a variety of sites and in persons free of cancer. In all these studies retinol and beta-carotene levels were lower in cases than in comparison subjects. Unfortunately, these after-

the-fact measurements could be biased by metabolic effects, anorexia, or difficulty in eating (as with oral and gastrointestinal sites) associated with the presence of cancer, particularly if advanced.

Four other serum studies were prospective like ours, in that serum in which vitamin A levels were measured was obtained before cancer was clinically evident. Wald et al. (7) analyzed frozen sera collected from men who received health examinations in London between 1975 and 1978. There were 86 specimens from men who later developed cancer of various sites and 172 from matched control subjects. Relative risks of cancer at any site progressed from 1.0 for those in the highest quintile of retinol levels to 2.2 for those in the lowest quintile. This negative association was even stronger for lung cancer (14 cases) and was independent of cigarette smoking habit and serum cholesterol level. Kark et al. (8) compared sera previously drawn from 85 cases of cancer and 174 matched control subjects in Evans County, Georgia. Here, a negative association was also observed for all cancers combined, with relative risks ranging from 1.0 in the highest quintile of retinol to 5.7 in the lowest. This finding was independent of age, sex, race, obesity, social class, and cigarette smoking. Almost all individual cancer sites and histologic types showed a negative association with retinol level. These investigators also found higher risks of cancer, particularly of the colon, in association with low cholesterol level and suggested that this may have been due to cholesterol's association with retinol. Willet et al. (9) performed a case-control study of frozen sera obtained from 111 participants in the Hypertension Detection and Follow-up Program who later developed cancer and 210 participants who remained cancer-free. No relationship was found between retinol, retinol-binding protein, or carotenoids, and cancer of all sites combined. For lung cancer, retinol was slightly higher in the 17 cases than in their 28 controls. Wald et al. (10), in a case-control study using previously stored frozen plasma, found no

newly-absorbed vitamin A is mobilized in plasma as retinol-binding protein. Retinol-binding protein delivers retinol to cells. For detailed references 16-18. In these studies, the plasma retinol-binding protein and the molar ratio of retinol to retinol-binding protein is close to that in any single individual. Retinol is normally found in a fairly large vari-

relation of retinol level to risk of breast cancer; carotene was somewhat lower in the 39 women who developed breast cancer than in the 78 comparison subjects, but the difference was not statistically significant. In a recent monograph on vitamin A and cancer (22), two new serum studies were reported. In one, Peleg et al. (23) conducted a new case-control study on the Evans County population followed through 1981 and did not confirm in 135 cases and 237 controls the predictive value of retinol reported earlier by Kark et al. (8). In the Basel study, Stähelin et al. (24) found no statistically significant case-control differences in plasma vitamin A level, but did find a significantly lower mean level of beta-carotene in 35 lung cancer cases than in their 102 controls. More recently, Nomura et al. (25), in a study of frozen sera collected from Hawaiian men of Japanese ancestry, found no predictive value of retinol for any cancer. However, the 74 men who later developed lung cancer had a significantly lower mean beta-carotene level than did 302 control subjects.

In three large prospective studies, dietary intake assessed by interview, rather than levels of substances in the blood, was studied in relation to cancer development. In a study in Japan (26), decreased risk of cancer of the lung, stomach, and bladder was noted among those who more frequently consumed yellow-green vegetables rich in beta-carotene. A study of Norwegian men (27) yielded similar results for lung cancer and consumption of foods rich in beta-carotene. In a study of Western Electric employees in Chicago (28), intake of beta-carotene but not of retinol was inversely related to the incidence of lung cancer, independent of cigarette smoking.

In the study by Willett et al. (9), persons who were in both the highest tertile of retinol-binding protein and lowest tertile of retinol showed a relative risk of 8.0 based on only seven cases and two controls. Our analysis of the retinol-binding protein/retinol molar ratio did not confirm that persons with relatively high retinol-binding

protein and low retinol levels were at increased risk. We also performed a tertile analysis analogous to that of Willett et al. and the relative risk in the high retinol-binding protein low retinol tertile was only 0.19 based on one case and nine controls. Apparently, persons with both high retinol-binding protein and low retinol have been few and subject to considerable sampling variability in studies to date.

In our subjects, serum cholesterol level was weakly correlated with retinol and retinol-binding protein and it also showed an inverse relationship with lung cancer that was weak and not statistically significant. Others have found a correlation between retinol and cholesterol levels in serum (8, 9, 29), but findings regarding cholesterol and lung cancer have been inconsistent (30). Analytic control for cholesterol level did not change our finding of no predictive value for lung cancer of retinol and retinol-binding protein.

Both the mean sodium and mean retinol values were somewhat higher than are ordinarily observed on fresh specimens and were therefore consistent with some slight dessication of the frozen serum specimens. Since this should have applied equally to those of the cases and the controls, matched as they were for date of multiphasic health checkup, no bias should have resulted. It is possible, however, that different amounts of dessication in the individual samples could have increased the person-to-person variability of retinol and retinol-binding protein levels, making it more difficult to discern overall case-control differences. We strongly doubt that this accounts for our negative findings.

The present study is, statistically, the most powerful study of the relationship between serum retinol level and subsequent lung cancer that has been done, and it covers latency periods between retinol measurement and lung cancer diagnosis of from two to almost 14 years. It failed to demonstrate any predictive value for retinol or retinol-binding protein levels. However, the possibility remains that can-

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ers of other sites are related to retinol level. Also, because dietary intake of retinoids and, especially, of carotenoids has little effect on retinol levels in the blood (6), this study does not rule out a possible preventive effect, for lung cancer, of eating relatively large amounts of retinoids or carotenoids. If beta-carotene and other carotenoids have cancer-preventive properties, these may be based on characteristics that these substances do not share with retinol, such as beta-carotene's high efficiency in quenching singlet oxygen (5). Although these other relationships are still possible, our data strongly suggest that retinol level cannot be used to help identify persons at high risk of developing lung cancer.

## REFERENCES

1. Sporn MB, Roberts AB. The role of retinoids in differentiation and carcinogenesis. *Cancer Res* 1983;43:3034-40.
2. Roberts AB, Sporn MB. Cellular biology and biochemistry of the retinoids. In: Sporn MB, Roberts AB, Goodman DS, eds. *The retinoids*. Vol. 2. New York: Academic Press, 1984:209-86.
3. Moon RC, Itri LK. Retinoids and cancer. In: Sporn MB, Roberts AB, Goodman DS, eds. *The retinoids*. Vol. 2. New York: Academic Press, 1984:327-71.
4. Sporn MB, Newton DA. Chemoprevention of cancer with retinoids. *Fed Proc* 1979;38:2528-34.
5. Peto R, Doll R, Buckley JD, et al. Can dietary beta-carotene materially reduce human cancer rates? *Nature* 1981;290:201-8.
6. Willett WC, MacMahon B. Diet and cancer—an overview. *N Engl J Med* 1984;310:633-8.
7. Wald N, Idle J, Boreham J, et al. Low serum vitamin A and subsequent risk of cancer. Preliminary results of a prospective study. *Lancet* 1980;2:813-15.
8. Kark JD, Smith AH, Switzer BR, et al. Serum vitamin A (retinol) and cancer incidence in Evans County, Georgia. *JNCI* 1981;66:7-16.
9. Willett WC, Polk BF, Underwood BA, et al. Relation of serum vitamins A and E and carotenoids to the risk of cancer. *N Engl J Med* 1984;310:430-4.
10. Wald NJ, Boreham J, Hayward JL, et al. Plasma retinol,  $\beta$ -carotene and vitamin E levels in relation to the future risk of breast cancer. *Br J Cancer* 1984;49:321-4.
11. Collen MF, Davis LF. The multitest laboratory in health care. *J Occup Med* 1969;11:355-60.
12. Friedman GD, Ury HK. Screening for possible drug carcinogenesis: second report of findings. *JNCI* 1983;71:1165-75.
13. Smith FR, Goodman DS. Vitamin A transport in human vitamin A toxicity. *N Engl J Med* 1976;294:805-8.
14. Bieri JG, Tolliver TJ, Catignani GL. Simultaneous determination of  $\alpha$ -tocopherol and retinol in plasma of red cells by high pressure liquid chromatography. *Am J Clin Nutr* 1979;32:2500-7.
15. Smith JE, Deen DD Jr, Sklan D, et al. Colchicine inhibition of retinol binding protein secretion by rat liver. *J Lipid Res* 1980;21:229-37.
16. Goodman DS, Blaner WS. Biosynthesis, absorption, and hepatic metabolism of retinol. In: Sporn MB, Roberts AB, Goodman DS, eds. *The retinoids*. Vol. 2. New York: Academic Press, 1984:1-39.
17. Goodman DS. Plasma retinol-binding protein. In: Sporn MB, Roberts AB, Goodman DS, eds. *The retinoids*. Vol. 2. New York: Academic Press, 1984:41-88.
18. Goodman DS. Vitamin A and retinoids in health and disease. *N Engl J Med* 1984;310:1023-31.
19. Wolbach SB, Howe PR. Tissue changes following deprivation of fat soluble A vitamin. *J Exp Med* 1925;42:753-77.
20. Strickland S, Mahdavi V. The induction of differentiation in teratocarcinoma stem cells by retinoic acid. *Cell* 1978;15:393-403.
21. Breitman TR, Selonick E, Collins SJ. Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc Natl Acad Sci USA* 1980;77:2936-40.
22. Underwood BA, ed. Vitamin A and cancer prevention conference. *JNCI* 1984;73:1365-1489.
23. Peleg I, Heyden S, Knowles M, et al. Serum retinol and risk of subsequent cancer: extension of the Evans County, Georgia, Study. *JNCI* 1984;73:1455-8.
24. Stähelin HB, Rosel F, Buess E, et al. Cancer, vitamins, and plasma lipids: prospective Basel study. *JNCI* 1984;73:1463-8.
25. Nomura AMY, Stemmerman GN, Heilbrun LK, et al. Serum vitamin levels and the risk of cancer of specific sites in men of Japanese ancestry in Hawaii. *Cancer Res* 1985;45:2369-72.
26. Hirayama T. Diet and cancer. *Nutr Cancer* 1979;1:67-81.
27. Bjelke E. Dietary vitamin A and human lung cancer. *Int J Cancer* 1975;15:561-9.
28. Shekelle R, Lepper M, Lin S, et al. Dietary vitamin A and risk of cancer in the Western Electric study. *Lancet* 1981;2:1185-90.
29. Kark JD, Smith AH, Hames CG. Serum retinol and the inverse relationship between serum cholesterol and cancer. *Br Med J* 1982;284:152-4.
30. Sidney S, Farquhar JW. Cholesterol, cancer, and public health policy. *Am J Med* 1983;75:494-508.