Exhibit 307

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VITAMIN A SUPPLEMENTS, FRIED FOODS, FAT AND UROTHELIAL CANCER. A CASE-REFERENT STUDY IN STOCKHOLM IN 1985–87

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In a population-based case-referent study of urothelial cancer in Stockholm during 1985–87, information was obtained from 78% of 418 identified cases and 77% of 511 selected referents. The relative risk (with 95% confidence interval) for intake of vitamin A supplements was 0.5 (0.2–1.0), with a dose-response relationship with increasing frequency of consumption. Increased risks of urothelial cancer were seen for several fried foods, for example fried meat [relative risk 1.4 (1.0–1.8) for weekly intake] and fried potatoes [relative risk 1.6 (1.1–2.6) for weekly intake]. Subjects with a high intake of fried foods, as defined by a collapsed variable, had a relative risk of 2.4 (1.4–4.2). A dose-response relationship was also seen with an increasing average daily intake of fat [relative risk 1.7 (1.0–2.8) in the highest quintile], but adjusting for fried foods decreased the relative risk, and it is uncertain whether the adjustment allowed for residual confounding. No association was noted for meat other than fried, but the analysis was based on small numbers.

In contrast to information on tobacco smoking and certain industry-related chemicals (IARC, 1985; Steineck *et al.*, 1990), epidemiological data on the association between dietary factors and urothelial cancer are sparse. We found increased risks of urothelial cancer associated with high intake of beef and pork in a cohort study (Steineck *et al.*, 1988), and hypothesized that this could be due to the practice of frying meat. If so, other fried foods could also be of interest with regard to urothelial cancer. Data on mutagens formed during cooking and of mutagenic activity in the urine following meals of fried meat are rapidly accumulating (Lijinsky and Shubik, 1964; Sugimura and Sato, 1983; Dolara *et al.*, 1984; Baker *et al.*, 1986; Felton *et al.*, 1986; Övervik, 1989).

It has been suggested that carotene decreases the risk of urinary bladder cancer (Mettlin and Graham, 1979; Hirayama, 1986), and small decreases in risk have also been associated with vitamin supplements (Kolonel *et al.*, 1985, Paganini-Hill *et al.*, 1987). Retinoids, analogues of vitamin A, are used to treat superficial cancer of the urinary bladder (Alfthan *et al.*, 1983; Studer *et al.*, 1984). Animal data support an inhibitory effect of retinoids on carcinogenesis in the urinary bladder (Sporn *et al.*, 1977; Miyata *et al.*, 1978; Kanamuru *et al.*, 1988).

In the industrialized world some 130,000 cases of urinary bladder cancer occurred in 1980 as estimated by Parkin *et al.* (1988). In Sweden, the registered age-adjusted incidence rate of urinary bladder cancer has increased during the last 20 years and was (× 100,000 years) 33.2 among men and 10.4 among women 1985 (National Board of Health and Welfare, 1989). Cancer of the urinary bladder comprises almost all cases of cancer in the lower urinary tract—the incidence of cancer of the renal pelvis, ureter and urethra is usually in the vicinity of 1/10 of the incidence of cancer of the urinary bladder. The vast majority of cancers in the lower urinary tract are urothelial cancers, less than 3% are pure squamous-cell carcinomas and less than 2% are adenocarcinomas (Kantor *et al.*, 1988). Urothelial cancer may share some, if not all, risk factors with squamous-cell carcinomas, but probably not with adenocarcinoma (Kantor *et al.*, 1988).

The purpose of the present study was to investigate the association between urothelial cancer and dietary factors, with special reference to vitamin supplements, dietary vitamins and fried foods.

STUDY BASE AND METHODS

A population-based case-referent study of urothelial cancer was carried out in Stockholm, Sweden, in 1985–87. The study base was generated by all subjects living in the County of Stockholm during part, or all, of the observation period 15 September 1985 to 30 November 1987. Only subjects born in Sweden during 1911–45 were included and those with a history of neoplastic disease of the urothelium before the observation period were excluded. We attempted to include as cases all subjects with histologically or cytologically confirmed urothelial cancer and/or squamous-cell carcinoma in the lower urinary tract (renal pelvis, ureter, urinary bladder and urethra) diagnosed in the study base. Prevalent cases found incidentally at autopsy were not included. Information on the new cases was obtained from the regional Cancer Registry as well as from the urological departments in the County of Stockholm, which receive all referrals of patients with urothelial cancer in the study population. Referents were selected, by stratified (gender and year of birth in 6 categories) random sampling, 4 times during the observation period from a computerized register covering the study population. The register is updated every month.

Information on exposure was collected by means of a questionnaire. This was mailed to the cases (and the corresponding referents) as soon as the cases had been identified. Thus, all subjects were contacted in their homes and the procedures for the collection of data were identical for cases and referents. The questionnaire was presented as a general health survey and included questions on previous diseases and intake of drugs, occupation, smoking, and life events in addition to dietary information. For almost all cases and referents there was some missing information in the delivered questionnaire. This information was supplemented, if possible, by a telephone interview of the subject by a nurse. We did not collect information from relatives when the subjects could not participate, *e.g.* due to death or disablement.

Separate questions were asked about vitamin supplements mainly containing vitamins A, B and C and about "other kinds of vitamin supplements and tonics". Frequency of consumption ("almost daily", "more seldom" and "never") and consumption periods were specified. In the analysis, any consumption of vitamin supplements after 1981 was ignored, thus allowing for a latency period.

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The subjects were asked to recall their dietary habits 3 years prior to interview. Frequency of consumption was scored for 56 food items, chosen primarily to cover the major contributors of active carotene, retinol, vitamin C and fried foods in the Swedish diet.

Five categories of consumption frequency were used: at least 5 days a week (termed below "almost daily"), 1–4 days a week ("weekly"), 1–3 days a month ("monthly"), more seldom and never. In some analyses, certain categories were collapsed. For meat, vegetables and fish the usual size of an ingested portion was estimated from a photograph (included in the questionnaire) to be "small", "moderate" or "large". The thickness of fat on a sandwich was estimated as "none", "thin", "moderate" or "thick". Information on intake of "fried meat" was obtained by questions on fried and oven-cooked meat/pork/sausages, smoked ham and bacon.

The answers obtained by the questionnaire were translated into an average daily intake of certain nutrients, using a data base at the Swedish National Food Administration and a standard ("small"/"moderate"/"large", as determined from the above-mentioned photograph, when appropriate) portion size. For each subject and nutrient, the total average daily intake was estimated by summing up the food items. A categorization into quintiles, according to the distribution among the referents, was carried out for each nutrient. In some analyses, quintiles 2, 3 and 4 were collapsed ("moderate average daily intake"). As mentioned below, a different categorization was done for fat in a few analyses; quintiles 1 and 2 ("low average daily intake") and quintiles 3 and 4 ("moderate average daily intake) are collapsed. Information on nutrients is lacking from 4 subjects.

The subjects were categorized into those who had ever regularly smoked at least 5 cigarettes or a corresponding amount of tobacco per week and those who had not (never smoked). Current smokers (smoking after 1981) supplied information about the number of cigarettes smoked per week, or whether they smoked a pipe/cigar/cigarillos only. All subjects were asked how much time they spent answering the questionnaire and whether they had used any written documentation, *e.g.* a diary, when pondering upon the questions.

An estimate of the relative risk, together with a 95% confidence interval, was calculated using the logistic regression analysis method described by Breslow and Day (1980). No interaction terms were used. If not stated otherwise, adjustment was made for gender, year of birth (3 categories) and smoking (current smoker/former smoker/never smoked). In some analyses, we included year of birth in 6 categories and smoking in 7 (never smoked/former smoker/1–34, 35–69, 70–139, 140+ cigarettes per week and pipe/cigar/cigarillos only). Also, in a few analyses an exact crude confidence interval was calculated according to Thomas (1975). The EPILOG (1987) computer program from Epicenter Software processed the data.

RESULTS

Information was obtained from 78% of the identified cases and 77% of the selected referents (Table I). Among women, 69/98 (69%) of the identified cases and 105/148 (71%) of the selected referents participated in the study. The median year of birth was 1919 for the cases and 1920 for the referents. As primary localization, 11 cases had their tumour(s) in the renal pelvis, 6 in the ureter, 305 in the urinary bladder, and 3 at multiple sites; none had the primary in the urethra.

The mean time spent on the questionnaire was 2.6 hrs for cases and 2.8 hrs for referents. Four hours or more were spent by 51/319 (16%) of the cases and 68/382 (18%) of the referents (missing information from 16 subjects). Consultation of written documention for questions about diet and tobacco was reported by 71/325 (22%) of cases and 94/392 (24%) of referents.

TABLE I – NON-RESPONSE AMONG CASES AND REFERENTS IN THE POPULATION-BASED CASE-REFERENT STUDY OF UROTHELIAL CANCER IN STOCKHOLM, SWEDEN 1985–87

	Cases		Referents	
Total number (%) of subjects selected/identified	418	(100%)	511	(100%)
Number (%) of subjects not participating (non-response)				
—refused	40	(10%)	86	(17%)
—disabled	18	(4%)	21	(4%)
—dead	32	(8%)	6	(1%)
—not located	1	(0%)	2	(0%)
—other reason	2	(1%)	4	(1%)
Number (%) of subjects participating	325	(78%)	392	(77%)
Number of subjects significantly exposed to aromatic amines	2			
Number of subjects analysed	323		392	

Two cases but no referents were significantly exposed to aromatic amines and they were omitted from all analyses. The relative risk (with 95% confidence interval) for current smokers was 3.0 (2.1–4.5) and for former smokers 1.7 (1.1–2.6).

A dose-response relationship was found with increasing frequency of consumption of supplements, mainly containing vitamin A (Table II). The crude relative risk for any intake of vitamin A supplements was 0.4, and the relative risk (with 95% confidence interval) after adjusting for gender, year of birth and smoking, was 0.5 (0.2–1.0). Among men the relative risk was 0.4 (0.2–1.0), and among women it was 0.7 (0.2–2.9). Lower risks were seen with longer duration of intake and an early year of starting the intake of vitamin A supplements (Table III).

Only 2 cases and 4 referents reported intake of vitamin A supplements but not vitamin C supplements. Including gender, year of birth and smoking in a regression analysis, together with certain vitamin supplements, gave the following relative risks (with 95% confidence interval): 0.5 (0.2–1.1) for any intake of vitamin A supplements; 0.8 (0.4–1.5) for an almost daily intake and 1.0 (0.7–1.5) for more infrequent intake of

TABLE II – RELATIVE RISK OF UROTHELIAL CANCER ASSOCIATED WITH VITAMIN SUPPLEMENTS. THE STOCKHOLM CASE-REFERENT STUDY 1985–87[1]

	Number of cases	Number of referents	RR	(95% CI)
Vitamin A mainly				
Almost daily	0	8	0.0	(0.0–0.7)[2]
More seldom	10	21	0.6	(0.3–1.2)
Never	310	359	1.0	—
Vitamin B mainly				
Almost daily	18	19	1.2	(0.6–2.4)
More seldom	29	44	0.9	(0.5–1.4)
Never	274	324	1.0	—
Vitamin C mainly				
Almost daily	17	32	0.7	(0.4–1.3)
More seldom	79	102	1.0	(0.7–1.4)
Never	225	253	1.0	—
Other vitamin supplements				
Almost daily	22	39	0.7	(0.4–1.3)
More seldom	48	55	1.2	(0.8–1.9)
Never	250	295	1.0	—

[1]Relative risk (RR) with a 95% confidence interval (95% CI). Missing information: 7 subjects for vitamin A, 7 subjects for vitamin B, 7 subjects for vitamin C, 6 subjects for other vitamin supplements.–[2]Crude relative risk and exact 95% confidence interval. The remaining relative risks and confidence limits are all adjusted for gender, year of birth and smoking.

TABLE III – RELATIVE RISK OF UROTHELIAL CANCER ASSOCIATED WITH DETAILS IN THE INTAKE OF VITAMIN A SUPPLEMENTS. THE STOCKHOLM CASE-REFERENT STUDY 1985–87[1]

	Number of cases	Number of referents	RR	(95% CI)
Duration				
1–9 years	5	5	1.5	(0.4–5.5)
10+ years	4	16	0.3	(0.1–1.0)
Year of starting				
Before 1962	2	12	0.2	(0.0–0.9)
1962–1971	2	6	0.5	(0.1–2.7)
1972–1981	5	3	2.5	(0.6–11.2)

[1]Relative risk (RR) with a 95% confidence interval (95% CI). Adjusted for gender, year of birth and smoking. Information missing on duration and year of starting from 1 case and 8 referents.

vitamin C supplements; 0.8 (0.4–1.5) for almost daily intake and 1.3 (0.8–2.0) for more infrequent intake of other vitamin supplements.

Regarding *dietary* vitamins, a moderate average daily intake of active carotene gave a relative risk (with 95% confidence interval) of 1.2 (0.8–1.7) and high daily intake a relative risk of 0.9 (0.6–1.5). For retinol, moderate intake gave a relative risk of 1.3 (0.8–1.9) and high daily intake a relative risk of 1.2 (0.7–2.0). Vitamin C gave a relative risk of 1.1 (0.8–1.7) for moderate daily intake and 0.9 (0.5–1.5) for high daily intake. Adjusting retinol (a fat-soluble vitamin) for fat (5 categories), in addition to gender, year of birth and smoking, gave a relative risk of 1.0 (0.6–1.6) for moderate and 0.8 (0.5–1.5) for high intake.

An increased relative risk was seen for certain fried foods (Table IV). Fried eggs, fried potatoes and gravy (at least monthly intake/more seldom) as well as fried meat (at least weekly intake/more seldom) were collapsed into a single variable for fried foods. In the unexposed group, we allowed exposure to one of the mentioned fried foods to avoid small numbers. The relative risk (with 95% confidence interval) for subjects exposed to all 4 fried foods was 2.4 (1.4–4.2) and for subjects exposed to 2 or 3 of the fried foods 1.6 (0.9–2.9). The crude relative risks were 2.4 and 1.7, respectively. Adjustment for fat (5 categories), in addition to gender, year of birth and smoking, gave relative risks of 2.2 (1.2–4.1) and 1.7 (0.9–3.0), respectively. The relative risk among men was 2.9 (1.4–6.0) and among women 1.5 (0.6–3.9) for high intake of fried foods.

A dose-response relationship was seen with an increasing average daily intake of fat (Table V). The crude relative risks were 1.1, 1.3, 1.4 and 1.8 in quintiles 2, 3, 4 and 5, respectively. Among men, the relative risk (with 95% confidence interval) was 1.5 (0.9–2.5) for moderate daily intake and 1.8 (1.0–3.3) for high daily intake. Among women, the corresponding figures were 1.0 (0.5–2.1) and 2.4 (0.6–10.7).

A regression model, including gender, year of birth and smoking, gave the following relative risks (with 95% confidence interval): 1.1 (0.6–2.1) for moderate and 1.8 (0.8–4.1) for high average daily intake of fat; 0.8 (0.5–1.4) for moderate and 1.2 (0.6–2.5) for high intake of protein; 1.1 (0.6–2.9) for moderate and 0.8 (1.3–1.8) for high intake of carbohydrate; 1.3 (0.6–3.1) for moderate and 0.9 (0.3–2.9) for high intake of energy.

Adjusting for the above-mentioned collapsed variable for fried foods, in addition to gender, year of birth and smoking,

TABLE IV – RELATIVE RISK OF UROTHELIAL CANCER ASSOCIATED WITH CERTAIN FOOD ITEMS. THE STOCKHOLM CASE-REFERENT STUDY 1985–87[1]

	Number of cases	Number of referents	RR	(95% CI)
Eggs, boiled				
Weekly	171	192	1.0	(0.6–1.6)
Monthly	109	149	0.9	(0.5–1.4)
More seldom	43	51	1.0	—
Eggs, fried[2]				
Weekly	151	167	1.8	(1.0–3.1)
Monthly	146	178	1.6	(0.9–2.8)
More seldom	26	46	1.0	—
Fish, fried				
Weekly	91	103	1.1	(0.7–1.8)
Monthly	161	195	1.0	(0.7–1.5)
More seldom	71	94	1.0	—
Gravy, brown sauce				
Weekly	111	123	1.6	(1.0–2.4)
Monthly	149	163	1.5	(1.0–2.2)
More seldom	63	105	1.0	—
Grilled foods				
Weekly	10	11	1.6	(0.7–4.2)
Monthly	31	47	0.8	(0.5–1.4)
More seldom	282	334	1.0	—
Meat, pork, sausages				
Fried, weekly	224	245	1.3	(0.9–1.8)
Not fried, weekly	8	14	0.8	(0.3–1.9)
More seldom	91	132	1.0	—
Meat, pork, sausages; fried				
Almost daily	13	13	1.4	(0.6–3.3)
Weekly	211	232	1.4	(1.0–1.8)
More seldom	99	146	1.0	—
Potatoes, fried				
Weekly	111	123	1.6	(1.1–2.6)
Monthly	158	168	1.8	(1.2–2.7)
More seldom	54	100	1.0	—

[1]Relative risk (RR) with a 95% confidence interval (95% CI). Adjusted for gender, year of birth and smoking. Missing information: 1 subject for fried eggs, 1 subject for gravy and brown sauce, 1 subject for meat, pork and sausages and 1 subject for fried potatoes.–[2]Including omelette and pancakes.

TABLE V – RELATIVE RISK OF UROTHELIAL CANCER ASSOCIATED WITH AVERAGE DAILY INTAKE OF FAT, PROTEIN, CARBOHYDRATE AND TOTAL ENERGY. THE STOCKHOLM CASE-REFERENT STUDY 1985–87[1]

	Number of cases	Number of referents	RR	(95% CI)
Fat				
Percentile 5	87	79	1.7	(1.0–2.8)
4	69	77	1.4	(0.8–2.3)
3	63	78	1.4	(0.8–2.3)
2	53	78	1.0	(0.6–1.7)
1	49	78	1.0	—
Protein				
Percentile 5	81	78	1.4	(0.9–2.3)
4	70	79	1.2	(0.8–2.0)
3	61	78	1.0	(0.6–1.7)
2	50	78	0.9	(0.5–1.5)
1	59	77	1.0	—
Carbohydrate				
Percentile 5	62	78	1.2	(0.7–2.0)
4	80	78	1.6	(1.0–2.7)
3	64	77	1.2	(0.8–2.2)
2	66	79	1.2	(0.7–2.0)
1	49	78	1.0	—
Total energy				
Percentile 5	70	78	1.4	(0.9–2.4)
4	81	78	1.7	(1.0–2.8)
3	69	77	1.5	(0.9–2.5)
2	52	79	1.1	(0.6–1.8)
1	49	78	1.0	—

[1]Relative risk (RR) with a 95% confidence interval (95% CI). Categorization into percentiles according to distribution among referents. Adjusted for gender, year of birth and smoking. Information missing from 4 subjects.

TABLE VI – RELATIVE RISK OF UROTHELIAL CANCER ASSOCIATED WITH INTAKE OF VITAMIN A SUPPLEMENTS, INDEPENDENTLY OF AND TOGETHER WITH CERTAIN OTHER EXPOSURES. THE STOCKHOLM CASE-REFERENT STUDY 1985–87

		History of smoking[1]		
		No	Former	Current
Intake of vitamin A supplements	Yes	1.0	1.5	1.2
	No	1.5	2.5	4.8
		Intake of fried meat[2]		
		No	Yes	
Intake of vitamin A supplements	Yes	1.0	0.7	
	No	1.5	2.1	
		Intake of fat[3]		
		Low	Moderate	High
Intake of vitamin A supplements	Yes	1.0	1.1	0.8
	No	1.6	2.2	2.9
		Intake of β-carotene		
		Low	Moderate	High
Intake of vitamin A supplements	No	1.0	1.1	1.0
	Yes	0.6	0.8	0.1

[1]For explanation of categories, see Methods.–[2]Yes = at least weekly. No = more seldom.–[3]Low = quintiles 1 and 2. Moderate = quintiles 3 and 4.

diminished the relative risk to 1.0 (0.6–1.6) for moderate and 1.3 (0.7–2.2) for high average daily intake of fat. If instead quintiles 1 and 2, and 3 and 4, respectively, were collapsed for fat, adjustment for fried foods gave a relative risk of 1.2 (0.8–1.8) for moderate intake and 1.4 (0.9–2.2) for high daily intake. Adjusting protein for fried foods (collapsed variable) gave a relative risk of 1.0 (0.6–1.8) for high daily intake.

In an overall logistic regression analysis we included gender, year of birth in *six* categories, smoking in *seven* categories and the following variables, giving relative risks (with 95% confidence interval) of: 0.5 (0.2–1.2) for any intake of vitamin A supplements; 1.6 (0.9–2.9) for moderate and 2.2 (1.2–4.1) for high intake of fried foods (collapsed variable); 1.2 (0.8–1.8) for moderate (quintiles 3 and 4) and 1.4 (0.9–2.3) for high average daily intake of fat.

When analysing effect-modification, we categorized the subjects into 4, 6 or 9 categories, as determined by the 2 variables investigated. Subjects with a high intake of fried foods (collapsed variable) together with a high daily average intake of fat had a relative risk (with 95% confidence interval) of 3.2 (1.5–6.7), as compared to those unexposed to fried foods and in the two lowest quintiles for fat. The intake of vitamin A supplements modified the effect of fried meat, intake of fat, and smoking (Table VI). For example, current smokers who did not report an intake of vitamin A supplements had a relative risk of 4.8 (1.5–15.1). This 380% increase is more than the sum of the 20% and 50% increases for the exposures when studied separately.

DISCUSSION

Misclassification

A common criticism of case-referent studies with retrospective data collection is that patients (regardless of their disease) would differ from population referents in their way of answering questions about past exposure (Cole, 1979). There is, however, not much empirical information in support of this view. We find it improbable, for example, that the diagnosis of urothelial cancer would make the cases forget about an earlier intake of vitamin supplements. Moreover, the answering time was similar for the cases and the referents and a slightly higher proportion of the referents, compared with the cases, used an aid when providing information. This indicates that cases were *not* more likely to ruminate on their answers than the referents.

Differential exposure misclassification could also occur if the cases changed their dietary habits because of the disease under study, and if these changes influenced the information given (Byers *et al.*, 1987; Persson *et al.*, 1990). Again, we find it improbable that the disease would make the cases stop eating vitamin supplements, or that they would change their habits and eat more fried or fatty foods.

Because of small numbers, we did not study the sites of the lower urinary tract independently. Although the effect may vary at different locations, a carcinogen probably operates on the mucosa in the whole of the lower urinary tract, justifying the inclusion of all sites in one outcome.

Misrepresentation

An important threat to the validity of case-referent studies is a lack of representativeness in all cases and person-time in the study base among the subjects participating. In the present study, routine registers facilitated the identification of all the diagnosed cases of urothelial cancer in the study base, and made it possible to draw a random sample of the study population at different times during the observation period.

Non-response due to death was more frequent among the cases than among the referents (Table I). In order to explain the main findings, those who provided no information due to death would have to have taken more vitamin supplements and have been less exposed to fried or fatty foods than those participating. This is improbable.

Non-response due to refusal was more common among the referents than among the cases (Table I). Those refusing could

share some exposure characteristics, thus introducing a bias that spuriously increases or decreases the relative risk. We have no information on the non-participants that could be used as an estimate of misrepresentation; socio-economic status, for example, was not measured. The exposure distribution, however, would have to be markedly different among those not participating from those providing information, in order to explain the main findings of the study.

Confounding

Aromatic amines and cigarette smoking are important determinants of urothelial cancer (IARC, 1973; IARC, 1985). To avoid confounding, we excluded all subjects (2 cases) significantly exposed to aromatic amines from the analysis. The changes in estimates were small after adjustment for gender, year of birth and cigarette smoking. This indicates that confounding from these factors, if any, was negligible.

In several regression analyses we considered different exposures together. Such an adjustment involves validity problems. The questionnaire was not designed to measure fat, protein or the total energy intake and there was probably also some inaccuracy in the assessment of fried foods, thereby introducing a non-differential exposure misclassification that attenuated the relative risk when analysing these items *per se*. When adjusting for the mentioned factors, however, the misclassification may allow for residual confounding. Moreover, since, for example, the misclassificaton of total energy intake may be differential over the strata for fat, the adjustment of fat for total energy may spuriously change the relative risk (Greenland, 1980). In addition, multiple adjustments involve loss of precision. Thus, it remains uncertain whether the slight increase in the relative risk of fat after adjustment for protein, carbohydrates and total energy is due to true confounding. Moreover, the relative risk when adjusting fat for fried foods may allow for residual confounding, and the true relative risk for fat may be even lower than the adjusted estimate.

Vitamins

Before the study, we expected a significant reduction of the risk for active carotene but were uncertain regarding vitamin A supplements. The retinoids used in vitamin A supplements are chemically more closely related to retinol than to the plant-derived active carotene, and the evidence of a protective effect against cancer is stronger for active carotene than for retinol (Ziegler, 1989). The retinoids used in treating superficial cancer of the urinary bladder (Alfthan *et al.*, 1983; Studer *et al.*, 1984) probably have a stronger biological effect than the retinyl-esters used in vitamin supplements.

We found no association for *dietary* vitamins. Instead, we noted a significant decrease in risk after intake of vitamin A supplements with a dose-response relationship for frequency of consumption. Although based on small numbers, the results indicate that the consumption must start more than a decade before the observation period and/or last at least 10 years to have an effect. As mentioned above, we find it unlikely that validity problems would explain the results although, of course, statistical variability (''chance'') can never be ruled out as an explanation of a finding.

There is, nevertheless, some support for an effect of vitamin A supplements on urothelial cancer in the 2 studies we know of that have dealt with the association (Kolonel *et al.*, 1985; Paganini-Hill *et al.*, 1987). In addition, retinoids which are used in animal models to inhibit carcinogenesis in the urinary bladder (Sporn *et al.*, 1977; Miyata *et al.*, 1978; Kanamuru *et al.*, 1988) are chemically related to the vitamin A used in many vitamin supplements.

In the present study, nearly all subjects exposed to vitamin A supplements were also exposed to vitamin C supplements, so the effect of vitamin A supplements may have been modified by vitamin C supplements. No effect was seen for fried meat and fat among users of vitamin A supplements, in contrast to never-users (Table VI), and an effect modification was also seen between smoking and vitamin A supplements. Mechanisms for a biological interaction have been suggested: vitamin A may trap free radicals, thereby neutralizing the effect of a carcinogen (Krinsky and Deneke, 1982), and/or act prophylactically against chromosomal damage induced by carcinogens (Stich *et al.*, 1984).

Fried foods and fat

As mentioned in the opening paragraphs, we postulated in a previous study that mutagens formed during cooking increase the risk of urothelial cancer (Steineck *et al.*, 1988). An alternative explanation of the finding in our cohort study of an increased risk of urothelial cancer after ingestion of meat would be an effect of the fat or the protein in meat *per se*.

The present findings do support an increased risk of urothelial cancer after the intake of fried foods—a strong association which was largely unaltered by adjusting for fat, was found with a collapsed variable indicating fried foods.

However, we also found a dose-response relationship with intake of fat (Table V). The relative risk for fat changed towards 1.0 after adjusting for fried foods. As discussed above, the adjustment may involve validity problems such as residual confounding. Moreover, no separate questions were asked about the frying of fat *per se*, and we could not separate the effects of fried fat and fat other than fried in the analysis, or determine, for example, to what extent the increased risk associated with fried potatoes could be due to the use of fat during cooking.

Less or no effect was seen for protein *per se*—the relative risk for meat other than fried was below unity but based on small numbers, and only a weak association was found with protein which, furthermore, disappeared after adjusting for fried foods.

Claude *et al.* (1986) noted an association between urinary bladder cancer and a diet high in fat content, and Risch *et al.* (1988) observed a dose-response relationship between intake of cholesterol and urinary bladder cancer. Otherwise, we know of no epidemiological data for fat or fried foods and urothelial cancer. The present findings, however, warrant further attention to these associations.

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REFERENCES

ALFTHAN, O., TARKKANEN, J., GRÖHN, P., HEINONEN, E., PYRHÖNEN, S. and SÄILÄ, K., Tigason (etretinate) in prevention of recurrence of superficial bladder tumors. A double-blind clinical trial. *Europ. Urol.*, **9**, 6–9 (1983).

BAKER, R.S., DARNTON-HILL, I., BONIN, A.M., ARLAUSKAS, A., BRAITHWAITE, C., WOOTTON, M. and TRUSWELL, A.S., Urine mutagenicity as an indicator of exposure to dietary mutagens formed during cooking of foods. *Environ. Hlth. Perspect.*, **67**, 147–152 (1986).

BRESLOW, N.E. and DAY, N.E., Statistical methods in cancer research. Vol. 1. The analysis of case-control studies. *IARC Scientific Publication 32*, pp. 191–279, IARC, Lyon (1980).

BYERS, T., MARSHALL, J., ANTHONY, E., FIEDELER, R. and ZIELEZNY,

M., The reliability of dietary history from the distant past. *Amer. J. Epidemiol.*, **125,** 999–1011 (1987).

CLAUDE, J., KUNZE, E., FRENTZEL-BEYME, R., PACZKOWSKI, K., SCHNEIDER, J. and SCHUBERT, H., Life-style and occupational risk factors in cancer of the lower urinary tract. *Amer. J. Epidemiol.* **124,** 578–589 (1986).

COLE, P., The evolving case-control study. *J. chron. Dis.*, **32,** 15–27 (1979).

DOLARA, P., CADERNI, G., SALVADORI, M., TRINGALE, L. and LODOVICI, M., Urinary mutagens in humans after fried pork and bacon meals. *Cancer Lett.*, **22,** 275–280 (1984).

EPILOG, Epidemiology and clinical trials statistics package. Epicenter Software, P.O. Box 90073, Pasadena, CA 91109, USA (1987).

FELTON, J.S., KNIZE, M.G., SHEN, N.H., ANDRESEN, B.D., BJELDANES, L.F. and HATCH, F.T., Identification of the mutagens in cooked beef. *Environ. Hlth. Perspect.*, **67,** 17–24 (1986).

GREENLAND, S., The effect of misclassification in the presence of covariates. *Amer. J. Epidem.*, **112,** 564–569 (1980).

HIRAYAMA, T., Nutrition and cancer, a large scale cohort study. *Prog. clin. Biol. Res.*, **206,** 299–311 (1986).

IARC, Some aromatic amines, hydrazine and related substances, N-nitroso compounds and miscellaneous alkylating agents. *IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans*. Vol. **4,** IARC, Lyon (1973).

IARC, Tobacco smoking. *IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans,* Vol. **38,** 244–268, IARC, Lyon (1985).

KANAMARU, H., HASHIMURA, T. and YOSHIDA, O., Effects of retinoids and inhibitors of arachidonic acid metabolism on tumor-promoter-induced soft agar colony formation of mouse epidermal cells and rat urinary bladder cells. *Jap. J. Cancer Res.*, **79,** 1043–1047 (1988).

KANTOR, A.F., HARTGE, P., HOOVER, R.N. and FRAUMENI, F., Epidemiological characteristics of squamous cell carcinoma and adenocarcinoma of the bladder. *Cancer Res.*, **78,** 3853–3855 (1988).

KOLONEL, N.L., HINDS, M.W., NOMURA, M.Y., HANKIN, J.H. and LEE, J., Relationship of dietary vitamin A and ascorbic acid intake to the risk for cancers of the lung, bladder, and prostate in Hawaii. *Nat. Cancer Inst. Monogr.*, **69,** 137–142 (1985).

KRINSKY, N.I. and DENEKE, S.M., Interaction of oxygen and oxy-radicals with carotenoids. *J. nat. Cancer Inst.*, **69,** 205–210 (1982).

LIJINSKY, W. and SHUBIK, P., Benzo(a)pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. *Science,* **145,** 53–55 (1964).

METTLIN, C., and GRAHAM, S., Dietary risk factors in human bladder cancer. *Amer. J. Epidemiol.*, **110,** 255–263 (1979).

MIYATA, Y., TSUDA, H., MATAYOSHI-MIYASATO, K., FUKUSHIMA, S., MURASAKI, G., OGISO, T. and ITO, N., Effect of vitamin A acetate on urinary bladder carcinogenesis induced by N-butyl-N-4-hydroxybutyl)nitrosamine in rats. *Jap. J. Cancer Res.* **69,** 845–848 (1978).

NATIONAL BOARD OF HEALTH AND WELFARE. THE CANCER REGISTRY, *Cancer incidence in Sweden 1985*. National Board of Health and Welfare, Stockholm (1989).

ÖVERVIK, E., *Formation and biological effects of cooked-food mutagens*. Doctorial thesis, Karolinska Institute, Stockholm (1989).

PAGANINI-HILL, A., CHAO, A., ROSS, R.K. and HENDERSON, B.E., Vitamin A, β-carotene, and the risk of cancer: a prospective study. *J. nat. Cancer Inst.*, **79,** 443–448 (1987).

PARKIN, D.M., LÄÄRÄ, E. and MUIR, C.S., Estimates of the worldwide frequency of sixteen major cancers in 1980. *Int. J. Cancer,* **41,** 184–197 (1988).

PERSSON P.G., AHLBOM A. and NORELL S.E., Retrospective versus original information on diet. Implications for epidemiologic studies. *Int. J. Epidemiol.*, in press (1990).

RISCH H.A., BURCH J.D., MILLER A.B., HILL G.B., STEELE R. and HOWE G.R., Dietary factors and the incidence of cancer of the urinary bladder. *Amer. J. Epidemiol.*, **127,** 1179–1191 (1988).

SPORN, M.B., SQUIRE, R.A., BROWN, C.C., SMITH, J.M., WENK, M.L. and SPRINGER, S., 13-*cis*-Retinoic acid: inhibition of bladder carcinogenesis in the rat. *Science,* **195,** 487–489 (1977).

STEINECK, G., NORELL, S.E. and FEYCHTING, M., Diet, tobacco, and urothelial cancer; a 14-year follow-up of 16,477 subjects. *Acta Oncol.*, **77,** 323–327 (1988).

STEINECK, G., PLATO, N., NORELL, S.E. and HOGSTEDT, C., Urothelial cancer and some industry-related chemicals, an evaluation of the epidemiologic literature. *Amer. J. indust. Med.*, **17,** 371–391 (1990).

STICH, H.F., ROSIN, M.P. and VALLEIERA, M., Reduction of vitamin A and beta-carotene administration of the proportion of micronucleated buccal mucosal cells in Asian betel nut and tobacco chewers. *Lancet,* **I,** 1204–1205 (1984).

STUDER, U.E., BIEDERMANN, C., CHOLLET, D., KARRER, P., KRAFT, H., TOGGENBURT, H. and VONBANK, F., Prevention of recurrent superficial bladder tumors by oral etretinate: preliminary results of a randomized, double blind multicenter trial in Switzerland. *J. Urol.*, **131,** 47–49 (1984).

SUGIMURA, T. and SATO, S., Mutagens-carcinogens in foods. *Cancer Res.*, **43**(Suppl.), 2415–2421 (1983).

THOMAS, D.C., Exact and asymptotic methods for the combination of 2 × 2 tables. *Comput. Biomed. Res.*, **8,** 423–446 (1975).

ZIEGLER, R.G., A review of epidemiologic evidence that carotenoids reduce the risk of cancer. *J. Nutr.*, **119,** 116–122 (1989).