Nitrite studies in oesophageal cancer¹

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SUMMARY As nitrate consumption may have considerable importance for the *in vivo* formation of nitrites and potentially carcinogenic N-Nitroso compounds, we have studied salivary nitrite levels in patients with oesophageal cancer and adult volunteers before and after administering 100 ml beet juice containing 160 mg nitrate. Initial salivary nitrite levels were slightly lower in the cancer patients, perhaps because of previous malnutrition. In both groups there was a marked increase in salivary nitrite levels 90 minutes after ingesting beet juice and the attained levels in the two groups were similar. The results imply that formation of salivary nitrite is highly dependent on exogenous dietary nitrate and that there is no difference in the capacity to form nitrites between oesophageal cancer patients and healthy adult subjects.

Available evidence strongly suggests that excessive drinking and smoking are risk factors for oesophageal cancer (Wynder and Bross, 1961, Tuyns, 1970); yet, despite intensive epidemiological study, the exact relation of these habits to subsequent tumour formation remains unclear. Moreover, as there are areas of the world, such as the Caspian Littoral, where very high rates of oesophageal cancer have been observed in the absence of either smoking or drinking, there is good reason to search for additional causative factors.

Nitrosamines readily induce experimental oesophageal cancer (Magee, 1971; Shank, 1975) and one intriguing possibility is that these powerful carcinogens might be formed *in vivo* by the interaction of nitrites and nitrosatable amines. Amines are widely distributed substances and nitrites could either be ingested or formed from nitrates by bacterial reduction in the mouth.

Investigations of nitrite levels seem especially worthwhile because the rate of formation of many nitrosamines follows the general equation: rate = k (concentration amine)ⁿ × (concentration nitrite)ⁿ, where n = 2 or more (Sander *et al.*, 1975). Thus, an increase in nitrite concentration would significantly increase the rate of formation of nitrosamines.

Recently, Ruddell and co-workers (1976) have suggested that gastric juice nitrite might well be a

risk factor for gastric cancer. The purpose of this study was to investigate salivary and gastric nitrite levels in patients with oesophageal cancer and in normal subjects.

Methods

The study was carried out under the auspices of the International Agency for Research on Cancer, in the Centre Régional François Baclesse, Caen, Normandy, a region of France where the incidence of oesophageal cancer is unusually high.

Twenty male patients ranging in age from 39-78 years with biopsy-proven oesophageal cancer were available for study. All but two patients either had received or were being treated with radiotherapy for control of their tumour. All patients gave a previous history of smoking and drinking; three patients were still smoking at the time of the study.

The control subjects consisted of six male and nine female hospital employees ranging in age from 20-50 years who were in good general health at the time of the study. Three were smokers; none was a heavy drinker.

SALIVARY STUDIES

Salivary nitrite determinations were performed in 13 of the oesophageal cancer patients and all of the control subjects. An initial 5 ml basal sample of saliva was collected in the morning after a light breakfast consisting of approximately 250 ml coffee and milk. The saliva was obtained over a period of

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approximately five minutes by having the subjects expectorate into a sterile container.

After obtaining the initial sample, both control subjects and cancer patients ingested 100 ml beet juice containing 160 mg nitrate. The beet juice was a commercial brand obtained from a local health food shop.

Ninety minutes after ingesting the beet juice a second sample of saliva was collected in the same fashion as the first sample.

Nitrite analysis was performed by spectrophotometric analysis using a Bausch and Lomb Mini-20 spectrophotometer and the nitrite determination procedure was based on the Griess reaction with sulphanilic acid and naphthylethylenediamine in an acidic medium. The determinations were performed promptly after obtaining the specimens and, where it was necessary to dilute the specimens, nitrite-free distilled water was used. Repeat determinations showed agreement to within 15%.

In addition to measuring nitrite levels, we determined the pH of the samples of saliva with a glass electrode.

Student's *t* test was used to calculate the significance of observed differences between groups of measurements.

GASTRIC JUICE STUDIES

The pH and nitrite content of fasting gastric juice was studied in seven patients with oesophageal obstruction and a feeding gastrostomy using the same methods as for the salivary samples. In these patients saliva did not reach the stomach.

DENTAL STATUS

Oral formation of nitrites has been related to dental hygiene and this factor was estimated by counting the number of teeth, noting the presence or absence of decayed or loose teeth, and by observing the condition of the gingival margins. The dental status of each subject was then graded as follows: excellent, good, bad, edentulous.

Results

SALIVARY STUDIES (Table)

Basal nitrite levels were $115 \pm 93 \mu\text{M}$ and $48 \pm 59 \mu\text{M}$ (mean $\pm$ SD) in control and cancer groups respectively and this difference was significant ($P < 0.05$). After administration of beet juice the attained levels of salivary nitrite in the control and cancer groups reached $913 \pm 348 \mu\text{M}$ and $674 \pm 435 \mu\text{M}$ respectively and the increase over basal levels was highly significant ($P < 0.001$). After administration of beet juice the difference between

Table Salivary nitrite and pH concentrations

	Nitrite concentration (μM) (mean $\pm$ SD)		pH (mean $\pm$ SD)	
	Control	Cancer	Control	Cancer
Basal	115 ± 93	$48 \pm 59^*$	$7.1 \pm .3$	$6.9 \pm .2$
After nitrate	$913 \pm 348^\dagger$	$674 \pm 435^\dagger$	$7.4 \pm .2$	$7.2 \pm .7$

*Differs significantly from control basal level ($P = < 0.05$).

†Differs significantly from corresponding basal level ($P = < 0.001$).

the cancer and the control groups observed initially disappeared.

There was no significant difference in the pH of the saliva between the control subjects and the cancer patients either before or after administration of beet juice.

GASTRIC JUICE STUDIES

The mean pH of gastric juice in the seven oesophageal cancer patients with a gastrostomy was 3.5 ± 1.3 . The mean gastric juice nitrite concentration was $1.3 \pm 2.2 \mu\text{M}$.

DENTAL STATUS

There was a marked difference observed between the control subjects and the cancer patients. The average number of teeth in the control group was 30 (range 28-32) while for the cancer patients the average number of teeth was only eight (range 0-16). The dental status of 13 of the control subjects was graded as excellent and two good, whereas 10 of the cancer patients were graded as bad and three were edentulous.

Discussion

Oral microorganisms are believed to be responsible for the conversion of salivary nitrate to nitrite (Tannenbaum *et al.*, 1974) and patients with poor oral hygiene generally have higher levels of salivary nitrite than patients with good oral hygiene (Spiegelhalter *et al.*, 1976). Therefore we were surprised to find that cancer patients, with markedly inferior oral hygiene, had lower initial nitrite levels than the control subjects. One reason could be the reduced number of teeth in the cancer patients, but another explanation is that the poorly nourished cancer patients had consumed less dietary nitrate in the period before the study. In both groups, however, the initial salivary nitrite levels were within the range recorded by others (Spiegelhalter *et al.*, 1976; Walters *et al.*, 1976; Ruddell *et al.*, 1977).

The oesophageal cancer patients had normal levels of gastric acidity and the nitrite levels were comparable to levels found by Ruddell and co-workers (1976) in normal patients. The observed

gastric pH range in the cancer patients would be conducive to the formation of N-nitroso compounds.

In all individuals (including the three edentulous cancer patients) ingestion of beet juice induced a marked rise in salivary nitrites. Although the control subjects and the cancer patients differed with respect to sex, history of smoking and alcoholism, age, and dental status, we were unable to detect any significant difference in ability to convert nitrate to nitrite in the saliva and therefore it would appear that these factors are less important for the *in vivo* formation of nitrite than the amount of nitrate in the diet.

Nitrate excess has been suggested as one possible risk factor for gastric cancer (Correa *et al.*, 1975; Hill *et al.*, 1973; Tannenbaum *et al.*, 1976) and it is possible that nitrates are also related to oesophageal cancer. (If so, then one important nitrate source could be the large dairy herds concentrated in north western France, since animal manure is a major source of nitrates found in drinking water (Owens, 1970). Further studies dealing with environmental nitrate levels in high and low incidence areas of France are contemplated.

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