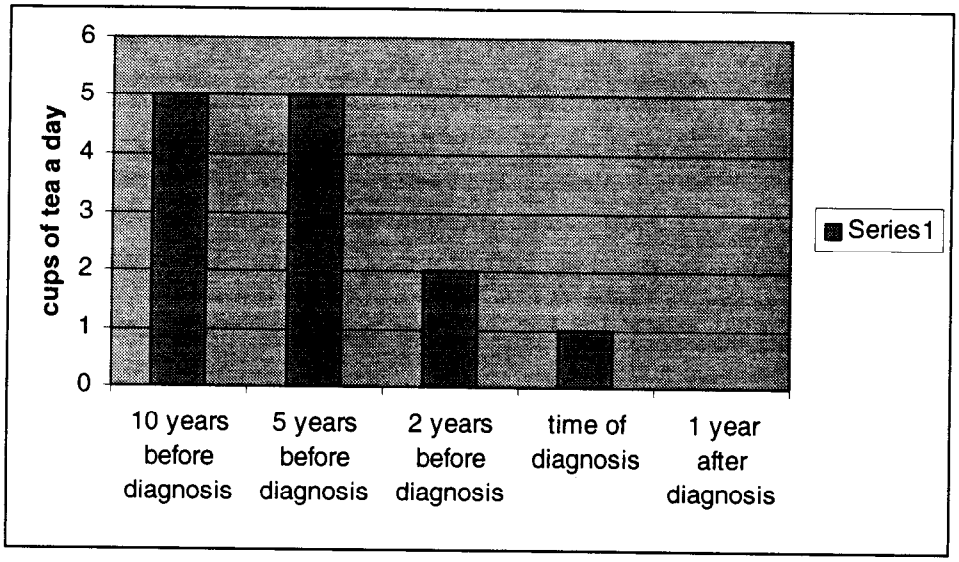
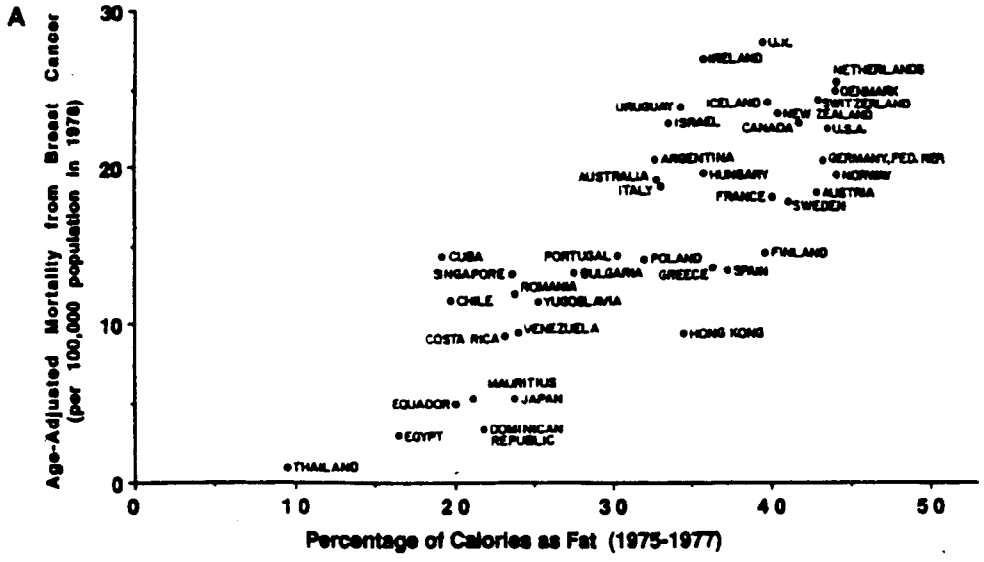
Cancer and diet: why can't they make up their minds? 2/11/03.

1. At least five studies over the years have shown that drinking green tea is associated with reduced risk of gastric cancer. That is, patients with gastric cancer report drinking less green tea, and patients without cancer report drinking more green tea. But a recent major study of Japanese tea drinkers found no significant benefit with respect to gastric cancer. To explain the disparity, this latest paper cited the following results:



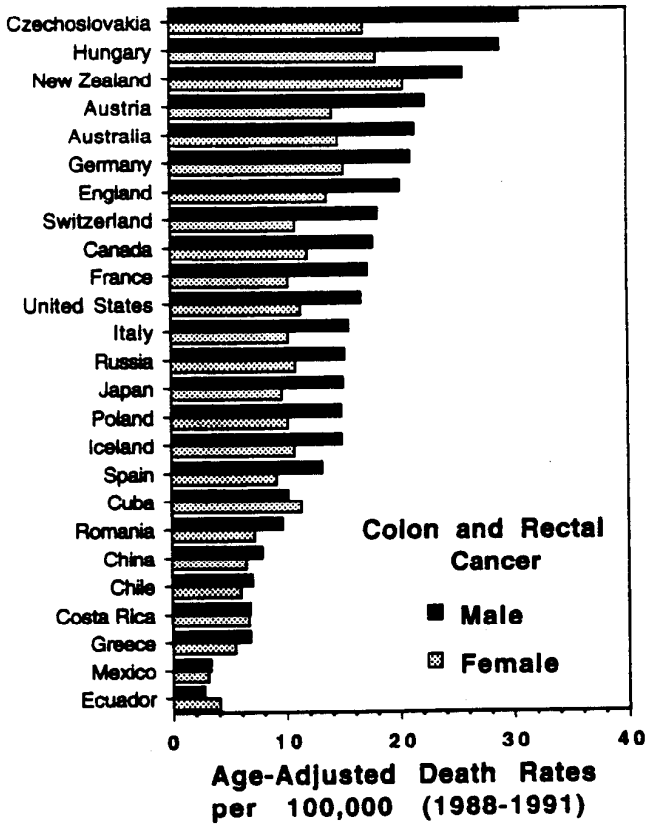
Why do the patients have this behavior, and how does it affect the interpretation of earlier green tea studies?

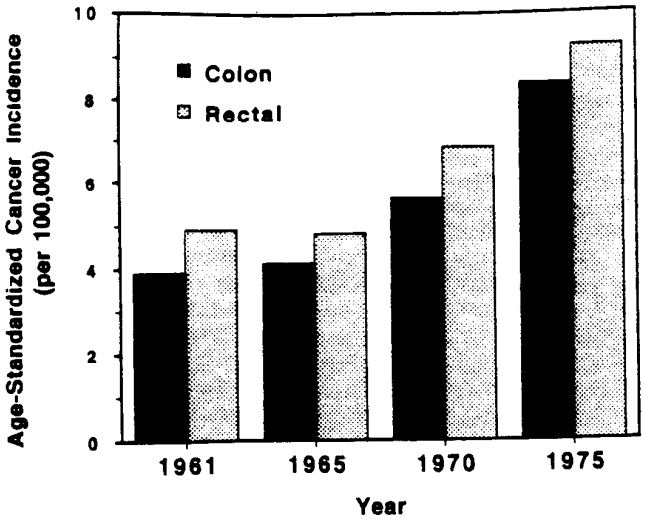
2. Consider the following graph.



a. What is the immediate conclusion a reader would draw?
b. Now think of as many alternative explanations of the data as you can. What confounding factors make it hard to interpret this graph? (Hint: one such factor may relate to health care differences.)

3. Consider the graph on the left first.





a. Give as many interpretations of this graph as you can. What racial, geographic, cultural, and socioeconomic causes of colon/rectal cancer might be operating, based on these data? Brainstorm with other groups to get as big a list of hypotheses as you can.
b. Now consider the graph on the right. Do the implications from this study shoot down or bolster any of your hypotheses from (a)? If so, which?

4. Read the article called, "Why drinking green tea could prevent cancer." Here's some background to help:
    - An active site of a protein is the part that performs the chemical reaction. In this case the authors also refer to the

active site as the catalytic triad. Fig. 1a is a picture of the protein interior.

- The "search for new uPA inhibitors" means the authors used computers to predict which molecules would bind to the uPA protein.
- Their Spectrozyme experiment is a way to see when uPA does its job. A more active protein produces more "cleavage of Spectrozyme."

a. What is uPA and what relation does it have to cancer?
b. Would a good drug increase or decrease the activity of uPA?
c. What are EGCG and amiloride?
d. Interpret Fig. 1b. What do the *x* and *y* axes mean? What do the authors conclude about EGCG's utility as a drug?
e. How do the authors use their results to explain green tea's possible anti-cancer effect?

5. Now read the article called, "Inhibition of carcinogenesis by tea." What are Yang's four objections to the Jankun article?

Why drinking green tea could prevent cancer

Epidemiological studies suggest that the consumption of green tea may help prevent cancers in humans; also, breast and prostate cancers in animal models are reduced by green, but not black, tea[1]. Here we offer a possible explanation. We have inferred (using molecular modelling) and subsequently demonstrated that one of the major ingredients of green tea inhibits urokinase, an enzyme crucial for cancer growth.

Tea is drunk in three forms: black (78%), green (20%) and oolung (2%). Green tea contains many polyphenols known as cathechins, including epigallocathechin-3 gallate (EGCG), epigallocathechin (EGC) and epicathechin-3 gallate (ECG). The brewing of black tea oxidizes the cathechins, destroying any beneficial effects[1]. Several mechanisms of anticancer activity of cathechins have been postulated, but none seems universal for all cancers[1-3].

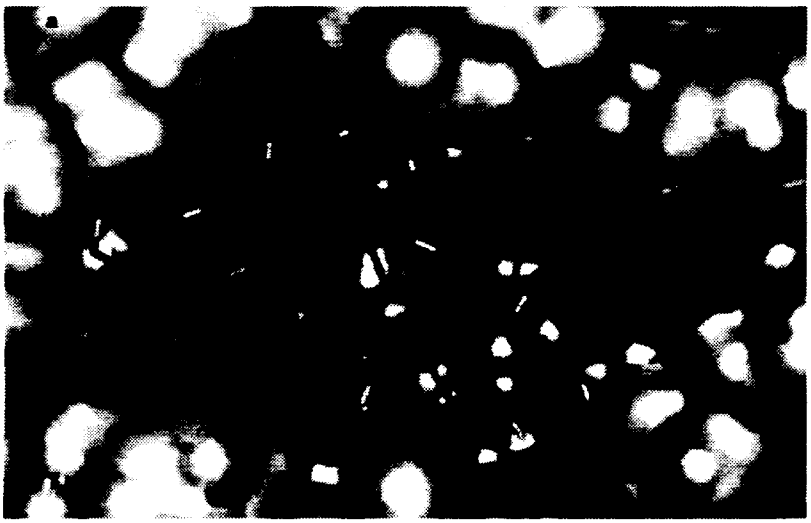
Human cancers need proteolytic enzymes to invade cells and form metastases. One of these enzymes is urokinase (uPA). Inhibition of uPA can decrease tumour size or even cause complete remission of cancers in mice[4,5]. The known uPA inhibitors are unlikely to be used in anticancer therapy because of their weak inhibitory activity or high toxicity.

We have searched for new uPA inhibitors by computer modelling using the active site of uPA as a template. Coordinates of human uPA were kindly provided by C. Phillips[6]; National Cancer Institute, MayBridge, and Merck 3D databases of 190,000 compounds were used to select inhibitors by Biosym LUDI and DOCKING programs. In these calculations, the relative position of inhibitor and receptor (uPA) with the minimum potential energy represents the most probable way of binding. Polyphenols, among other compounds, showed good inhibitory potential. One of them, EGCG (a component of green tea), binds to uPA, blocking His 57 and Ser 195 of the uPA catalytic triad and extending towards Arg 35 from a positively charged loop of uPA (Fig. 1a). Such localization of EGCG would interfere with the ability of uPA to recognize its substrates and inhibit enzyme activity[6].

We have verified our computer calculations using an amidolytic assay of uPA activity in the presence of different concentrations of EGCG. The uPA activity was quantified spectroscopically using Spectrozyme, which releases a chromogen on specific cleavage by uPA. EGCG from two different suppliers showed almost identical rates of uPA inhibition.

We have compared the ability of EGCG to inhibit uPA with that of a well-known inhibitor, amiloride, and a control sample where no inhibitors were used (Fig. 1b). EGCG is a weaker inhibitor than amiloride, but can be consumed in much higher doses without any toxicological effects. Amiloride is administered in a maximum dose of 20 mg per day, whereas a single cup of tea contains 150 mg EGCG, and some tea lovers consume up to 10 cups a day[1].

Such high levels of a uPA inhibitor are likely to have a physiological effect and could reduce incidence of cancer in humans or the size of cancers already formed. Theoretically, EGCG might inhibit cancer formation in many different ways; however, we postulate that the well-known anticancer activity of green tea is driven by inhibition of uPA, one of the most frequently overexpressed enzymes in human cancers.



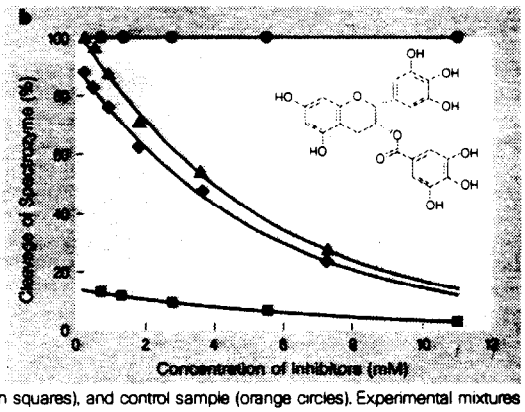


Figure 1a, Connolly surface of uPA showing the catalytic triad His 57, Asp 102 and Ser 195 (red) at the bottom, and Arg 35, Arg A37 (blue) at the brim, of the cavity. EGCG, well fitted into this cavity, is shown as a 'stick model' in green (C), red (O) and white (H). The calculated energy of intermolecular interaction between EGCG and uPA is −116.81 kcal mol^{-1}; LUDI score, 498; calculated K_i, 1.04×10^{-5} M. **b,** Cleavage of Spectrozyme by uPA in the presence of EGCG (inset) from Sigma (blue triangles), and from MayBridge, UK (purple diamonds); amilonde (green squares), and control sample (orange circles). Experimental mixtures (50 mM Tris with 0.01% Tween 80, 0.01% PEG 8000 buffer; pH 8.8) were incubated with 1 μg of uPA and decreasing amounts of inhibitor for 15 min. 100 μl of this mixture was incubated in a 96-well microplate with 50 μl (2.5 mM) Spectrozyme (carbobenzyl-L-(γ)-Glu(α-tBuO)-Gly-Arg-*p*-nitroanilide.$2C_2H_5OH$ from American Diagnostica Inc., Greenwich, Connecticut), for 10 min. Absorbance, which is inversely proportional to the uPA inhibitory activity[7], was measured at 405 nm on a microplate reader.

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Inhibition of carcinogenesis by tea

In the recent Scientific Correspondence[1] by J. Jankun *et al.*, entitled "Why drinking tea could prevent cancer", I was surprised to find that a review article by myself and Z.-Y. Wang about the effects of green and black tea on cancer[2] had been misquoted. In addition, I find the hypothesis in this report as to why drinking tea could prevent cancer misleading, and believe that the data were erroneously interpreted with regard to the mechanisms of cancer inhibition by tea. I would like to clarify four points.

First, Jankun *et al.* wrote that "breast and prostate cancers in animal models are reduced by green, but not black, tea"[1], citing my review[2]. But the review provides no evidence to support this statement. The review discussed the inhibitory activities of both green and black tea in animal models. In the lung tumorigenesis model with mice and the oesophageal tumorigenesis model with rats, green and black tea had similar inhibitory activities[3–5]. Black tea can inhibit the hyperproliferation of lung epithelial cells and the progression from adenoma to adenocarcinoma in the mouse lung[6]. There is no evidence from the review or elsewhere to support the statement that "the brewing of black tea oxidizes the cathechins, destroying any beneficial effects"[1]. Although the inhibitory activities of green and black tea have been demonstrated convincingly in animal studies, such activity has been shown in some, but not other, epidemiological studies[2,7]. The cancer-preventing effect of tea in humans requires further study.

Second, my laboratory has conducted several studies indicating that the blood level of epigallocatechin-3-gallate (EGCG) after consuming the equivalent of 2–3 cups of tea was 0.1–0.6 μM and for an equivalent of 7–9 cups was still lower than 1 μM[8–10]. In studies with mice and rats in which inhibition of skin, lung and oesophageal tumorigenesis was found, the blood EGCG level was 0.1–0.3 μM and the tissue (such as the lung) levels were very low — the highest tissue level, close to 1 μM, was observed in the oesophageal epithelia[9]. The effective concentration needed to inhibit urokinase (2–10 mM), as reported by Jankun *et al.*[1], was at least 3 or 4 orders of magnitude higher than the expected tissue levels. Given this information, how could inhibition of urokinase be related to cancer prevention in animal models and in humans?

Third, we and others have shown that micromolar concentrations of EGCG, other tea cathechins, and theaflavins (a characteristic group of compounds in black tea) inhibited the cell growth of many human cancer and other cell lines. Even in these cases, it cannot be concluded that this activity is related to the inhibition of tumorigenesis. EGCG binds strongly to many biological molecules and affects a variety of enzyme activities and signal transduction pathways at micromolar or even nanomolar concentrations[2,9]. Thus I believe that inhibition of urokinase at millimolar concentrations is unlikely to be a viable mechanism for the inhibition of tumorigenesis.

Fourth, as discussed in my review and in other publications[2,9], the inhibitory activity of tea against tumorigenesis is not due to a single compound, but rather the combined activities of several or many constituents of tea. The mechanisms of action of tea need to be further elucidated before sweeping conclusions can be drawn.

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