



# Unilever Bestfoods

## *North America*

800 Sylvan Avenue  
Englewood Cliffs, NJ 07632  
25 September, 2001

Dr. Scott Masten  
Office of Chemical Nomination and Selection  
NIEHS/NTP  
P.O. Box 12233  
Research Triangle Park, NC 27709

*Rec'd 9/26/01*

Dear Dr. Masten,

**Re: Epigallocatechin-3-gallate**

The National Toxicology program (NTP) requested comments on the substances nominated to the NTP for toxicological studies and on the testing recommendations made by the NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC) in the *Federal Register* of July 25, 2001. According to the notice, the National Cancer Institute (NCI) nominated EGCG because of its use as a dietary supplement and its potential use as a chemopreventative agent. The ICCEC indicates that it recommends testing EGCG for genotoxicity and subchronic toxicity. It also states that it might consider testing green tea extract (GTE). Unilever Bestfoods would like to provide the following information and perspective on epigallocatechin-3-gallate (EGCG) and GTE.

As perspective, Unilever Bestfoods is the maker of Lipton teas, the largest tea brand in the United States. Unilever is the largest seller of tea in the world. We have extensive knowledge of the chemistry and health effects of tea and tea components. Based on this knowledge, we believe that none of the recommended testing is necessary. This letter outlines the basis for our conclusion.

Unlike what is stated in the "Summary of Data for Chemical Selection" prepared for NCI by Technical Resources International (obtained from NTP's web site), we believe there is a great deal of information in the published literature about both the safety and toxicology of EGCG and GTE. For example, a basic literature search of EGCG and cancer done on the Internet resulted in 442 citations (attached), many more references than are cited in "Summary of Data for Chemical Selection." We therefore believe the summary is incomplete. In the time available since the notice was published (and allowing for the events of the past two weeks), we have assembled a short evaluation of the data on EGCG and GTE with respect to the areas in question.

## Background on Tea

From its legendary discovery by the Chinese emperor Sheng Nung in 2737 BC, to the medicinal effects reported around 24 to 221 AD, to NCI's description of it as a cancer chemopreventative, tea has carved out a unique place in human culture (Balentine, 1997).

Tea is food, and has been consumed by humans – all ages, races, both sexes, throughout their lives – for millennia, thereby demonstrating its safety. In fact, tea is a healthy beverage without any indication of untoward effects unless extraordinarily high levels are drunk. Thus, EGCG, a major component of green tea, is also safe to humans at similar levels of intake.

Tea is made from the leaves of the tea plant, *Camellia sinensis*. It is currently consumed by two thirds of the world's population (Kuroda and Hara, 1999), and it is generally accepted that, apart from water, tea is the most popular beverage in the world. It has been estimated that the worldwide consumption per capita is 120 ml per day (Mukhtar and Ahmad, 1999). Of the total amount of tea produced and consumed, 78% is black, 20% green, and less than 2% is oolong (Kuroda and Hara, 1999). See (Balentine et al., 1997) for a discussion on how the various types of tea are made. Tea possesses antioxidant activity, which is derived from the presence of polyphenolic flavonoids (Wiseman et al., 1997). This antioxidant activity has been associated with tea's anticarcinogenic activity and other beneficial effects (Liao et al., 2001).

The most biologically important chemicals in tea are the polyphenols and caffeine. Polyphenols constitute 30-35% of the dry tea leaf. Catechins are the predominant form of these polyphenols, and the most abundant individual catechin in both black and green tea is EGCG. The amounts present in the beverage in green and black teas (from Kuroda and Hara, 1999) are:

<b>Catechin Class</b>	<b>Name</b>	<b>Conc. in Green Tea (µg/ml)</b>	<b>Conc. in Black Tea (µg/ml)</b>
Catechins	Catechin	21	20
Epicatechins	Epicatechin (EC)	98	37
	Epicatechin gallate (ECG)	90	73
	Epigallocatechin (EGC)	411	42
	EGCG	444	128
Theaflavins	Theaflavin	0	22
	Theaflavin gallate A	0	20
	Theaflavin gallate B	0	13
	Theaflavin digallate	0	9
Thearubigens	e.g., Procyanidine	3	≈700

## Exposure

Lifetime consumption of tea – sometimes at high levels – has been occurring for over 4000 years. No adverse effects are seen. EGCG has been a major part of this exposure, and, thus, it too is not toxic. The literature shows that only incredibly high amounts of tea (e.g., 14 L/d) show toxicity, which is primarily hypokalemia due to excessive diuresis (Trewby et al., 1998).

We do not have data to show whether the use of dietary supplements would replace the consumption of tea or would be in addition to it. Thus, two types of dietary supplements will be examined, then a dietary supplement plus tea beverage. (This requires some generalization because of the lack of standards for dietary supplements and the large number and forms of dietary supplements on the market.)

The amount of green tea in one dietary supplement in the form of a tea bag labeled has less tea than in Lipton green tea bags, so intake of EGCG and GTE will be less than consuming regular green tea. It seems unlikely that other supplements in this form would be significantly stronger than commercial tea bags.

To the best of our knowledge, only GTE (or green tea polyphenols) is available in capsules, not EGCG. This is likely due to the expense of producing “pure” EGCG, which is on the order of \$30,000/kg. Due to size limitations, the amount of material in capsules is generally no more than 500 mg. If a person took two pills of GTE three times a day, that would be equivalent to about five cups of green tea (assuming 700 mg solids/cup). If caffeine is present in the GTE, it also limits the amount of GTE that a person can take as a chemopreventative (see “Human safety data” below).

According to FDA, the 90<sup>th</sup> percentile (high, but not unreasonable) of tea consumption is 332 g/person/d, or about 1.5 servings (FDA, 1994). This would provide approximately 1050 mg of tea solids. If both tea beverage and a dietary supplement were consumed, the intake of tea solids would be approximately 4000 mg/d, or what is in six cups.

Based on internal Unilever information we believe the amount present in cosmetics does not contribute a meaningful amount to total body exposure.

Thus, GTE in dietary supplements may cause more people to ingest green tea material, but unlikely to be beyond what is consumed by devoted drinkers. When both tea and dietary supplements are taken together exposure is only slightly greater than with supplements alone. Even if one assumed that intake was doubled, as will be shown in the next section, this will not exceed a “safe” dose.

## Subchronic toxicity

A 13-week dietary study of EGCG in rats has been published in abstract form and a poster of the work was presented at the most recent Society of Toxicology meeting

(Johnson, et al., 2001; copy of abstract and poster attached). No adverse effects were seen. A no observed adverse effect level (NOAEL) of 500 mg/kg/d was established. A full report of the study is not available at this time.

NCI contracted subchronic studies with EGCG in dogs and rats, which were presented at a Society of Toxicology meeting (McCormick et al., 1999; copy of abstract attached). No effects were seen in dogs given up to 300 mg/kg/d by capsule. In the rat study, in which up to 500 mg EGCG/kg/d was administered by gavage, effects occurred in the intestinal system which cascaded to other organ systems. A likely explanation is that the bolus of EGCG upset the microflora in the rats' intestines, leading to changes in the architecture of the tissue, which had a secondary influence on other organs. The relevance to humans is questionable because of the differences in the gastrointestinal systems and the routes of exposure. A no effect level of 45 mg/kg/d was proposed.

Similar studies with green tea polyphenols (54% EGCG) also contracted by NCI were reported at the same meeting (Johnson et al., 1999). No effects were seen in dogs up to the highest dose of 600 mg/kg/d. Rats were administered up to 1000 mg/kg/d by gavage. The same effects were seen with EGCG, and a NOAEL of 90 mg/kg/d was proposed.

To the best of our knowledge, none of the studies has been published in full yet.

The dietary study reported by Johnson, et al. (2001) is the most appropriate of the rat studies for assessing safety in humans because the dietary route of administration is closer to how EGCG is consumed by people. Using 500 mg/kg/d as the NOAEL, and using a conservative safety factor of 10 (due to extensive human exposure), an acceptable daily intake of 50 mg/kg/d is established. That is 3500 mg EGCG/d for a 70 kg person, the amount in roughly 35 cups of green tea.

The dog study with a NOAEL of 300 mg/kg/d gives essentially the same acceptable daily intake because a smaller safety factor can be used with dogs.

It is important to recall that no adverse effects were seen in these studies, so the safe level for humans is likely even higher than what is calculated.

Taken as a whole, there are studies already completed that sufficiently address the subchronic toxicity of EGCG and additional studies would be redundant.

### Genotoxicity

The literature in this area is simply too vast to review in any meaningful way in this letter. Extensive reviews have been published (e.g., Kuroda and Hara, 1999) and they are commended to NTP.

That being said, some examples of *in vitro* and *in vivo* studies specifically investigating the mutagenic/antimutagenic effects of EGCG may be useful here to set the stage. EGCG has shown antimutagenic activities towards heterocyclic amines (Trp-P-2), benzo(a)pyrene diol epoxide, UVA and UVB, 4-nitroquinoline oxide, and ethyl methane sulphonate in microbial systems. In addition, EGCG has caused a decrease in gene mutations following exposure to 4-nitroquinoline oxide in Chinese hamster cell lines although it had no effects on ethyl methane sulphonate. The antimutagenic activities were dependent on the time of dosing with EGCG such that protective effects were only observed if administered post-treatment with 4-nitroquinoline oxide.

The effects of EGCG have also been investigated in a rat bone marrow micronucleus assay where its effects on aflatoxin B<sub>1</sub>-induced chromosome damage were investigated. EGCG reduced the incidence of micronuclei in the bone marrow following aflatoxin B<sub>1</sub> treatment but, as with mammalian cells *in vitro*, the time of dosing was critical. In this instance, protective effects were only manifested if the EGCG was administered 24 hours before aflatoxin B<sub>1</sub>, with no effect at 2 hours or 0 hours (Ito et al., 1989). Additional studies on GTE have demonstrated a reduction in DNA adducts following NNK, IQ, and aflatoxin B<sub>1</sub> treatment, and a decrease in chromosome damage following benzo(a)pyrene treatment (reviewed in Kuroda and Hara, 1999).

As noted in the "Summary of Data for Chemical Selection," a few studies show some evidence of genotoxicity. Considering all the studies that have been done and the high false positive rate in genotoxicity studies, this is to be expected. However, the preponderance of data indicate that black tea, green tea, and EGCG are antigenotoxic.

Based on all the studies that have been performed that show that tea and its components are antimutagenic, the weight of evidence indicates that there is no need for further testing in this area.

### Human safety data

Pisters et al. (2001) reported a phase I trial with oral GTE in adult patients with solid tumors. Dose levels of 0.5 to 5.05 g/m<sup>2</sup> every day and 1.0 to 2.2 g/m<sup>2</sup> three times a day were evaluated. The maximum tolerated dose was 4.2 g/m<sup>2</sup> once daily or 1.0 g/m<sup>2</sup> three times daily. The side effects were related to caffeine and reversed upon discontinuation. The authors recommended that 1.0 g/m<sup>2</sup> three times daily be used for future studies. According to them, this amount is equivalent to seven to eight small cups (120 mL) of green tea three times daily (21 to 24 cups/d). This is about 10 to 12 regular cups (240 mL) of tea a day, assuming the same strength of tea.

We are aware that other work has been done on patients with cancer. NTP should obtain any reports NCI might have before doing making any decisions about testing.

The "Summary of Data for Chemical Selection" refers to the potential for carcinogenicity of EGCG, even though the ICCEC recommendations do not. I would like to bring your attention to work by IARC (1991) reviewing the epidemiological data on green and black tea consumption, which concluded that there is *inadequate evidence* for the carcinogenicity in humans of tea drinking and there is *inadequate evidence* for the carcinogenicity in experimental animals of tea. The overall evaluation was that tea is *not classifiable as to its carcinogenicity to humans (Group 3)*. The analysis did uncover evidence suggesting that the *temperature* at which tea is drunk may be a more important determinant of risk than the chemical composition of the beverage. Thus, EGCG is unlikely to have any carcinogenic activity.

A more recent review of tea and the prevention of cancer in humans concludes that green tea decreases the risk of stomach cancer and does not increase cancer in any organ system (World Cancer Research Fund, 1997).

In conclusion, tea is a safe and healthy beverage consumed by people around the world for thousands of years. Increased intake of tea constituents is unlikely to occur through the use of dietary supplements. There is already a sufficient package of data on EGCG and GTE, including rats, dogs, and humans, that demonstrates the safety of EGCG. The data cover intake from both beverage use and dietary supplement use. Therefore, it would be an unnecessary use of animals as well as a misuse of taxpayers' money for NTP to do any more work on this compound.

I would be happy to provide more data if you believe it is necessary.

Sincerely,

A handwritten signature in black ink that reads "Richard W. Lane". The signature is written in a cursive style with a large, sweeping initial "R".

Richard W. Lane, Ph.D.  
Director of Scientific Affairs

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### SAFETY/RISK ASSESSMENT OF THE CONSUMPTION OF DOMESTIC AND IMPORTED PEAR BRANDIES CONTAINING METHANOL.

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Some pear brandies can contain methanol (MeOH), a compound associated with pronounced visual and central nervous system toxicity. This assessment addresses the potential health hazards due to MeOH exposure of acute and chronic consumption of domestic and imported pear brandy. Various pear brandy products were analyzed for MeOH content by gas chromatography with a flame ionization detector. The mean MeOH content of the domestic (n=51) and imported (n=83) products were 4827 and 2585 ppm, respectively. The estimate of exposure to MeOH from brandy consumption was based on brandy MeOH content and point estimates of brandy intake (USDA, CFSII, 1989-1992). Mean and 90-99th percentiles of the average MeOH exposures were estimated for one-day and chronic consumption of brandy. The tolerable daily intake (TDI, mg/kg/day) for MeOH for one-day exposure is 7.1 (human NOEL, FDA, 1984) and for chronic exposure is 0.5 (RfD, IRIS, EPA, 1993). The average one-day exposure to MeOH from ingestion of domestic pear brandy exceeded the TDI at the 90-99th percentile brandy intake levels; whereas, one-day exposure to methanol from ingestion of imported pear brandy only exceeded the TDI at the 99th percentile intake level. One-day MeOH exposure for the individual pear brandies exceeds the one-day TDI at the mean, 90th, 95th and 99th percentile intake levels 19.6, 88.2, 88.2, 98.0 % of the time for the domestic and 0, 21.7, 28.9, 78.3% of the time for the imported products, respectively. The average chronic exposure to MeOH associated with drinking domestic or imported pear brandies exceeds the chronic TDI at several intake percentiles (mean, 90-99th). Thus, based on the estimates derived in this assessment, exposure to MeOH associated with both acute and chronic consumption of pear brandy can exceed levels of toxicological concern. Also differences in the level of MeOH exposure, and thus, in the margin of safety, exist between drinking domestic and imported pear brandies.

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### 13-WEEK DIETARY TOXICITY STUDY OF EPIGALLOECATECHIN GALLATE (EGCG) IN RATS.

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Epigallocatechin gallate (EGCG), the most abundant polyphenol in green tea, demonstrates antioxidant, anti-inflammatory, chemopreventive, and other biological activities in several in vivo experimental model systems. A 13-week dietary toxicity study was conducted to evaluate the toxicity of EGCG, to establish a no observed effect level (NOEL), and to determine the reversibility of any observed toxic effects. Sprague-Dawley rats (10/sex/group) were fed EGCG at nominal dose levels of 0 (control; basal diet), 50, 150 and 500 mg/kg/day for 13 weeks. An additional 10 rats/sex in the high dose and control groups were held for a 4-week recovery period in which they were fed basal diet without supplemental EGCG. Blood levels of EGCG were determined after one day of feeding and again during week 13. Toxicologic endpoints included survival, body weight, food consumption, clinical signs, clinical pathology, ophthalmology, organ weights, gross pathology, and microscopic pathology. No treatment-related deaths occurred during the study, and no signs of systemic toxicity were seen in any animal during the 13-week feeding period. No treatment-related effects were seen on body weights or food consumption. Mean total body weight gain at the end of the 13-week feeding period was comparable across all groups for both sexes. Administration of EGCG did not induce ocular toxicity, and had no effect on either clinical pathology parameters or organ weights. No treatment-related histopathological lesions were seen in the high dose males or females sacrificed at the end of the 13-week treatment period. In conclusion, dietary administration of EGCG to Sprague-Dawley rats for 13 weeks did not induce any treatment-related toxicological or histopathological effects in either males or females at dose levels up to 500 mg/kg/day. Based on these results, the NOEL in this study was 500 mg/kg/day.

## 1539

### L-TARTARIC ACID-INDUCED NEPHROTOXICITY.

S. Ortega<sup>1</sup>, B. Mounho<sup>1</sup>, S. Kelley<sup>1</sup>, L. Harris<sup>1</sup>, Z. Shahrokh<sup>1</sup>, L. Khan<sup>1</sup>, E. Torres<sup>1</sup>, J. Nelson<sup>2</sup>, N. Gillett<sup>2</sup> and N. Dybdal<sup>1</sup>. <sup>1</sup>Genentech, Inc., South San Francisco, CA and <sup>2</sup>Sierra Biomedical, Sparks, NV.

We have evaluated the safety of a potential drug formulation containing L-tartaric acid. The dose regimen for the formulation was a 1-hour IV infusion protocol. The formulation was administered daily to cynomolgus monkeys (low dose = 1.8, mid-

dose = 18 or, high dose = 188 mg tartaric acid/kg/day) with the start of infusion staggered over 5 days. Clinical signs consistent with acute renal failure developed in several high dose monkeys starting on Day 2 following the start of dosing. As additional high dose monkeys developed renal failure, high dose infusions were stopped on Study Day 7 (Dosing Days 3-7), and the monkeys were euthanized. Acute renal failure characterized by clinical signs and by marked elevations in BUN and serum creatinine was confirmed histopathologically by the presence of acute tubular necrosis. Daily infusions in low- and mid-dose groups continued for 27 days as there were no clinical signs or clinical pathology abnormalities noted in these groups. At necropsy there was no gross or histopathologic evidence of acute renal failure in these groups. In summary, L-tartaric acid can be highly nephrotoxic to cynomolgus monkeys, and caused severe acute renal tubular necrosis when delivered IV over a 1-hour period at high concentration (188 mg/kg). L-tartaric acid, at the high dose, was deemed inappropriate for use in this drug formulation for this dosing regimen. Caution is warranted when new or rarely used excipients are being introduced into drug formulations, as excipients may be toxic under certain dosing regimens.

## 1540

### TOXIC RESPONSE FOLLOWING LATEX DRAIN IMPLANTATION.

E. T. Shriver, P. Nicolaysen, A. Hubbs, D. Weissman, P. Siegel and B. J. Meade. NIOSH, Morgantown, WV.

These studies were initiated to investigate the potential role of implanting latex surgical drains on the development of latex allergy. Protein and allergen levels of 5 different brands of Pentose drains were quantified by the Lowry method and an immunoassay. Protein values ranged from 44 to 2566 ng/mg drain material, and latex allergen ranged from 70 to 4528 pg/mg. Drain material (200 mg) was implanted subcutaneously in anesthetized female BALB/c or B6C3F1 mice 6-8 weeks old (N = 5/group). Serum was collected by tail-bleed prior to implantation and weekly, thereafter. Exposure to drain brand (A) induced significant elevation in total IgE (5748 ng/ml vs. 347 ng/ml for the sham control) by day 21. No elevation in IgE or systemic toxicity was observed with brand (B). IgE was not measured for mice implanted with brand (C) due to systemic toxicity within 14 hours. In a dose response study, implants of 10, 100 and 200 mg of brand (C) drain material produced dose-dependent clinical signs (severe vasodilatation, hypothermia, and ataxia) and elevations in serum alanine amino-transferase, blood urea nitrogen, and creatinine. Kidney and liver weights were decreased in the 200 mg group. The principal histopathologic alteration in the 200 mg group was acute, fibrinosuppurative dermatitis with suppurative vasculitis of dermal venules. No bacteria were seen in tissue sections. Hepatic changes were consistent with glycogen depletion. Endotoxin levels in the drain material were <1 EU/mg. Leaching drain material for 24 hours in methanol or acidic water reduced the toxic effects of brand (C), but leaching in sterile phosphate buffered saline, water and alkaline water had no effect on the toxicity of the material. In summary, responses other than latex sensitization that ranged from no effect to acute toxicity occurred after implantation of various Pentose drains. These studies were supported in part by NIEHS interagency agreement #Y1-ES-0049-03.

## 1541

### SAFETY OF A RIBOZYME TARGETING HER2 RECEPTOR mRNA (HERZYME™) IN CYNOMOLGUS MONKEYS FOLLOWING A SINGLE SUBCUTANEOUS OR AN INTRAVENOUS BOLUS INJECTION.

P. A. Lee<sup>1</sup>, E. A. Caputo<sup>2</sup>, T. E. Jackson<sup>1</sup> and J. A. Sandberg<sup>1</sup>. <sup>1</sup>Ribozyme Pharmaceuticals, Inc., Boulder, CO and <sup>2</sup>Sierra Biomedical, Inc., Sparks, NV.

A stabilized ribozyme (HERZYME™) targeting the mRNA of the human epidermal growth factor receptor-2 (HER2) has been developed. HER2 is a member of the epidermal growth factor receptor (EGFR) family. In normal adult tissues, HER2 expression is low. However, HER2 is overexpressed in at least 25-30% of breast and ovarian cancers. Overexpression of HER2 in malignant breast tumors has been correlated with increased metastasis, chemoresistance and poor survival rates. Thus, HERZYME™ is indicated for use in treating breast (and other cancers) in which HER2 overexpression may play a role in disease progression. In this study, cynomolgus monkeys received a single subcutaneous (SC), bolus injection of HERZYME™ at 100, 300 or 1200 mg/m<sup>2</sup>. One group of animals received HERZYME™ at 300 mg/m<sup>2</sup> as a single intravenous (IV) bolus injection. Control animals received an equivalent volume of saline as a SC and an IV bolus. Blood samples for toxicokinetic analyses were collected at various timepoints following administration of HERZYME™. Animals were followed for 14 days following HERZYME™ administration. There were no effects of HERZYME™ on body weight, food consumption, urinalysis, heart rate, blood pressure, clinical hemato-



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**267** ERYTHRITOL: AN INTERPRETIVE SUMMARY OF BIOCHEMICAL, METABOLIC, TOXICOLOGICAL AND CLINICAL DATA.

J C Munro<sup>1</sup>, W O Bernd<sup>2</sup>, J F Borzelleca<sup>3</sup>, G Flamm<sup>4</sup>, B S Lynch<sup>1</sup>, E Kennepohl<sup>1</sup>, A Bär<sup>5</sup>, and J Modderman<sup>6</sup>. <sup>1</sup>CanTox Inc., Consultants in Toxicology, Health and Environmental Sciences, Mississauga, ON, Canada; <sup>2</sup>University of Nebraska Medical Center, Nebraska's Health Science Center, Omaha, NE; <sup>3</sup>Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA; <sup>4</sup>Flamm Associates, Vero Beach, FL; <sup>5</sup>Bioresco AG, Binningen, Switzerland; and <sup>6</sup>Keller and Heckman, LLP, Washington, DC.

A critical and comprehensive review of the safety information on erythritol was undertaken. Numerous toxicity and metabolic studies have been conducted on erythritol in rats, mice, and dogs. The toxicity studies consist of long-term feeding studies conducted to determine carcinogenic potential, intravenous and oral teratogenicity studies to determine the potential for effects on the fetus, oral studies in which erythritol was administered over 1 or 2 generations to determine the potential for reproductive effects, and studies in bacterial and mammalian systems to determine mutagenic potential. The majority of the safety studies conducted were feeding studies in which erythritol was mixed into the diet at concentrations as high as 20%. The metabolic studies in animals have shown that erythritol is almost completely absorbed, not metabolized systemically and is excreted unchanged in the urine. The safety studies have demonstrated that erythritol is well-tolerated and elicits no toxicological effects. The clinical program for erythritol involved a series of single-dose and repeat-dose, short-duration studies which have been used to investigate the human correlates to the physiological responses seen in the pre-clinical studies. The clinical studies showed erythritol to be well-tolerated and not to cause any toxicologically relevant effects, even following high-dose exposure. Erythritol administered orally to humans was rapidly absorbed from the gastrointestinal tract and quantitatively excreted in the urine without undergoing metabolic change. At high oral doses, urinary excretion accounted for approximately 90% of the administered dose with minimal amounts appearing in the feces. A comparison of the human and animal data indicated a high degree of similarity in the metabolism of erythritol and this finding supports the use of the animal species used to evaluate the safety of erythritol for human consumption. It can be concluded, based on the available studies that erythritol did not produce evidence of toxicity.

**268** ORAL TWO-GENERATION REPRODUCTION STUDY IN RATS ON PHYTOSTEROL-ESTERS (PE) - A NOVEL FUNCTIONAL FOOD.

D H Waalkens-Berendsen<sup>1</sup>, A P M Wolterbeek<sup>1</sup>, M Richold<sup>2</sup>, P A Hepburn<sup>2</sup>. <sup>1</sup>TNO Nutrition and Food Research Institute, Toxicology Division, Zeist, The Netherlands and <sup>2</sup>Safety & Environmental Assurance Centre, Unilever Research, Colworth House, Sharnbrook, UK.

A new margarine has been developed by Unilever which contains the novel ingredient, phytosterol esters. PE are a natural component of vegetable oils which lowers blood cholesterol by blocking the absorption of cholesterol from the gut. As part of an extensive programme of safety evaluation studies we have conducted a two-generation reproduction study in rats, in which the possible effect of PE on male and female reproductive performance and on the growth and development of the offspring, was carried out. Wistar rats were fed diets containing various levels of PE (0, 1.6, 3.2 and 8.1% w/w) over two successive generations and a wide range of reproductive- and developmental parameters were determined. Histopathological examinations were performed on various organs of F1- and F2-weanlings and of F0- and F1-parental animals.

Daily clinical observations did not reveal any remarkable findings. In the highest-dose group, food intake and body weights were statistically significantly decreased. In both generations no effects of PE were observed on pup mortality calculated on litter basis, pre-coital time, mating index, male and female fertility, female fecundity, gestation index, duration of gestation and number of females with stillborn pups and post-implantation loss, pup development, sexual maturation and estrus cycle length. Apart from an increased relative liver weight of the F0-males of the 1.6 and 3.2% PE groups no differences were observed in organ weights of adrenals, brain, epididymides, pituitary, prostate, seminal vesicles, ovaries and uterus. Furthermore, no histopathological changes were observed in these organs.

In conclusion, dietary administration of up to 8.1% PE during two generations had no effects on reproduction of parental F0- and F1-generation Wistar rats and the development of the F1- and F2-pups.

**269** GENETIC TOXICITY *IN VITRO* OF BISPHENOL A-DIGLYCIDYL ETHER (BADGE) AND ITS HYDROLYSIS PRODUCT.

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BADGE is released from epoxy resins and organosoles, which are used for the interior lacquer-coating of food cans. Hydrolysis may occur at the epoxide rings leading to a diglycol, and, after metabolic hydroxylation, at the ether bonds leading to bisphenol A (BPA). The health implications of BADGE and its putative hydrolysis products are yet unclear. We have recently reported that BPA induces metaphase arrest and micronuclei with whole chromosomes in cultured Chinese hamster V79 cells, indicative of an aneuploidogenic potential (Mutat. Res. 390, 21, 1997). In the present study, the induction of micronuclei and gene mutations in V79 cells by BADGE and its epoxide hydrolysis product was investigated. BADGE was very stable in cell culture medium at pH 7.5 and 37°C: after 24 h, only unchanged BADGE was extracted and identified by HPLC. However, in the presence of rat liver microsomes or S-9 supernatant with or without NADPH-regenerating system at 37°C, BADGE disappeared completely within a few min, and the only product formed was the diglycol. At concentrations ranging from 10 - 50 µM, BADGE in the absence of S-9 mix clearly induced micronuclei with acentric chromosomal fragments and gene mutations at the HPRT locus. Higher concentrations were cytotoxic. In contrast, the diglycol of BADGE was devoid of clastogenicity and mutagenicity even at concentrations up to 200 µM. Contrary to BPA, neither BADGE nor its diglycol exhibited aneuploidogenic potential. The present study provides evidence that BADGE is a gene mutagen and very resistant against chemical hydrolysis, but is rapidly inactivated by enzymatic epoxide hydrolysis to a non-mutagenic metabolite. (Supported by Deutsche Forschungsgemeinschaft (Me 574/9-2).)

**270** SUBCHRONIC ORAL TOXICITY OF EPIGALLOCATECHIN GALLATE (EGCG) IN RATS AND DOGS.

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Human consumption of green tea is inversely associated with cancer risk in several organ sites. EGCG is the most abundant polyphenol in green tea, and has significant chemopreventive activity in animal carcinogenesis models. Ninety-day oral toxicity studies were conducted to characterize the toxicity of EGCG, and to establish its No Adverse Effect Level (NOEL). Sprague-Dawley rats (20/sex/group) received oral doses of 0, 45, 150, or 500 mg EGCG/kg/day via gavage; beagle dogs (4/sex/group) received oral doses of 0, 30, 100, or 300 mg EGCG/kg/day via capsule. Toxicologic endpoints included survival, body weight, food consumption, clinical signs, clinical pathology, ophthalmology, electrocardiography (dogs only), organ weights, gross pathology, and microscopic pathology. In dogs, EGCG induced no gross toxicity, body weight effects, alterations in functional parameters, or gross or microscopic pathology. By contrast, early deaths in the high dose group and a dose-related suppression of body weight gain were seen in both sexes of rats. Intestinal dilatation was a common finding both in rats dying or euthanized *in extremis* during the dosing period, and in rats surviving until the terminal necropsy; this change may be secondary to alterations in digestive processes and/or gut microflora. Microscopic findings suggesting possible alterations in gastrointestinal function included pancreatic necrosis and hepatic (peri-acinar) degeneration and necrosis; modest elevations in ALP, AST, and ALT were seen in female rats in the high dose group. High dose rats of both sexes also demonstrated necrosis/atrophy of the thymus. In dogs, the NOEL of EGCG is  $\geq 300$  mg/kg/day. Histopathologic data suggests that the NOEL of EGCG is 150 mg/kg/day in both male and female rats. However, body and organ weights appear to be more sensitive indicators of EGCG toxicity in male rats. On the basis of reduced body weight gain and decreased absolute and relative thymus weights, the NOEL of EGCG in male rats is 45 mg/kg/day. (Supported by NCI-N01-CN-55146.)

**271** SUBCHRONIC ORAL TOXICITY OF GREEN TEA POLYPHENOLS IN RATS AND DOGS.

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Consumption of green tea is inversely associated with human cancer risk in

several tissues; green tea contains a number of polyphenols whose biological activity is consistent with anticarcinogenic efficacy. These studies were designed to evaluate the subchronic oral toxicity of a green tea polyphenol (GTP) fraction [Polyphenon E; Mitsui Norin] whose major components are: epigallocatechin gallate (EGCG; 53.4%); epigallo-catechin (11.4%); epicatechin (9.1%); gallo-catechin gallate (5.1%); and epicatechin gallate (4.9%). Sprague-Dawley rats (20/sex/group) received daily oral doses of 0, 90, 300, or 1000 mg GTP/kg via gavage; beagle dogs (4/sex/group) received daily doses of 0, 60, 200, or 600 mg GTP/kg via capsule. Toxicologic endpoints included survival, body weight, food consumption, clinical signs, clinical pathology, ophthalmology, electro-cardiography (dogs only), organ weights, and gross and microscopic pathology. When administered to dogs, GTP induced no gross toxicity, suppression of body weight gain, alterations in functional parameters, or gross or microscopic pathology. By contrast, early deaths were seen in both male and female rats receiving the high dose of GTP. Dose-related suppression of body weight gain, food consumption, and relative and absolute spleen and thymus weights were also seen in both sexes. As seen with purified EGCG, intestinal dilatation was a common finding in early deaths, moribund kills, and in rats surviving until the terminal necropsy; gross intestinal pathology may be secondary to alterations in digestive processes and/or gut microflora. Microscopic findings included pancreatic necrosis, hepatic degeneration/necrosis, and thymic necrosis/atrophy. The NOEL of GTP in dogs (both sexes) is  $\geq 600$  mg/kg/day; the NOEL of GTP in rats (both sexes) is 90 mg/kg/day. Mortality patterns in rats indicate that GTP is more toxic than would be predicted based on its EGCG content alone, suggesting that polyphenols other than EGCG play a role in its toxicity. (Supported by NCI-N01-CN-55146.)

**272** ADRENERGIC AGONISTIC EFFECTS AND CYTO-TOXICITY OF CHINESE EPHEDRA (MA-HUANG) USED FOR WEIGHT REDUCTION.

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Ma-huang is a traditional Chinese medicinal herb derived from stems and branches of *Ephedra sinica* Stapf and other species of *Ephedra* plants. It contains 1 - 2% by weight of ephedrine alkaloids, of which ephedrine accounts for 40 - 90%. Through their  $\beta$ -adrenergic agonistic action, these alkaloids can induce thermogenesis and energy expenditure and hence sustain weight loss when ma-huang is taken on a long-term basis. However, these alkaloids produce myocardial stimulation through their  $\beta$  agonistic effect. They also constrict peripheral blood vessels via their  $\alpha$ -agonistic effect, causing an increase in blood pressure and heart rate. Recently, great volumes of ma-huang have been used as an active ingredient in some dietary supplements used for weight reduction, causing serious side effects and numerous cases of poisoning, some of which were fatal. In the present study, ma-huang extracts were prepared under various conditions for possible different potencies. For each extract, ephedrine content was analyzed by HPLC, biological activities were determined with mammalian cell lines stably expressing adrenergic receptors, and cytotoxicity to a hepatocarcinoma cell line (HepG2) was measured using MTT colorimetry. The results indicate that the potencies of adrenergic agonistic activity and cytotoxicity of ma-huang extracts are in correlation with their ephedrine contents. Grinding of herb was found to be a crucial condition for increased potency. (Supported by Hong Kong UGC PGS and Hong Kong ISF.)

**273** HEAT RESISTANCE CHARACTERISTICS OF *ESCHERICHIA COLI* O157:H7 ISOLATED IN JAPAN.

F U Kasuga, Y Itoh, and S Kumagai. *National Institute of Infectious Diseases, Tokyo, Japan.* Sponsor: W Norred.

Thermal resistance of enterohemorrhagic *E. coli* O157:H7 was compared between the strains isolated from large outbreaks occurred in Japan in 1996. Difference in the shape of heat resistance curves upon pH at the heating was also studied. Four strains isolated in outbreaks and one from meat specimen in Japan and one obtained from ATCC were cultured individually in Tryptic Soy Broth (TSB) for 16 hr at 37°C. The cultures were diluted in TSB (pH 7.3) to give  $1 \times 10^6$  cells per ml of cell suspension, and pH was adjusted to 4.0 or 5.0 with HCl when needed. Syringe vials (15 x 40 mm) were used instead of TDT ampules or plastic bags to heat the samples, in order to improve the handling efficiency and to enable to measure inner temperature during the heating process. Temperature of the contents was monitored using a type K thermocouple. Duplicate vials were removed at each prescribed

time interval, transferred to an ice bath, and the content of the vials was spiral plated on Tryptic Soy Agar. When the bacterial suspension was heated at 55°C in the medium with pH 7.3, D-value varied from 2.46 to 5.34 within the strains isolated in Japan. Heat resistance curve has been understood as logarithmically declining against heating time. However, the pattern of the curve was dramatically altered and accordingly different D-value was given when the samples were heated in the medium with different pH. Our observations suggest that there exist various types of *E. coli* O157:H7 strains from the viewpoint of thermal resistance, and arrest attention to the effect of pH on the thermal resistance of pathogenic bacteria when heating duration is estimated for food processing.

**274** STRATEGIES FOR ESTIMATING PROVISIONAL ACCEPTABLE RESIDUES (PAR) FOR EXTRALABEL DRUG USE IN FOOD ANIMALS.

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In 1996, the US Congress passed AMDUCA, which allows some veterinary drugs to be used off label. In order to implement this and protect the US consumer, tolerances, or safe concentrations are required before a withdrawal time can be adequately estimated for extralabel drug use. This paper presents several strategies that can be used to derive an equivalent safe concentration, referred to as a Provisional Acceptable Residue (PAR) for specific tissues, that may be used to estimate drug withdrawal times after extralabel use of several antibiotics and other drugs. Derivation of the proposed PAR ensures that daily intakes of drug residues in milk and/or edible tissues do not exceed Acceptable Daily Intakes (ADIs) specific for the US and also takes into account safety factors and food consumption values. Method A requires partitioning of 50% of the ADI to the target tissue and reserving the remainder for milk. Method B equally partitions the ADI into all edible tissues, including milk. Method C assigns 50% ADI to milk and equally partitions the remaining 50% ADI into edible tissues. These simulations showed that provided the safe concentration or its equivalent PAR is based on rigorous toxicology data (e.g., ADIs), the health of the most sensitive population may not be compromised. It is proposed that these PARs can be used for estimating withdrawal times after extralabel drug use and for purposes of trade harmonization.

**275** BELL-SHAPED RELATIONSHIP BETWEEN MOLECULAR WEIGHT AND DOSE-RESPONSE TO WHITE MINERAL OILS IN FISCHER 344 (F344) RATS.

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Dietary exposure to certain white mineral oils (WO) causes inflammatory effects in the liver and lymph nodes of F344 rats, but not in other strains or species. The average molecular weight (MW) of previously tested oils ranged from ~300-500. The inflammatory effects were inversely related to MW and were not seen with heavier paraffinic WO (MW 485). This study extends the relationship between MW and dose-response to lighter (MW<300) WO. Four groups of female F344 rats (n=15/group) were fed a diet with 0, 0.02, 0.2, or 2.0% of a light white mineral oil (MW=270) for 90 days. No effects were seen in the 0.02% group. Treatment related changes included increased relative liver and mesenteric lymph node (MLN) weights at 0.2%, MLN inflammation (macrophage accumulations) at 0.2%, and liver histopathologic changes (microgranuloma formation) at 2% dose level. These effects had a lower incidence and were less severe than those seen previously with heavier oils (MW=320-420), suggesting a bell-shaped structure activity relationship may exist for WO-induced inflammatory effects in F344 rats. Analysis of WO residues in livers from these study animals showed that lower hydrocarbons (C<sub>15</sub>-C<sub>21</sub>) were underrepresented compared to the original test material. The relative absence of lower MW hydrocarbons in liver tissue is most probably due to increased metabolism rather than decreased absorption of the WO.

# Phase I Trial of Oral Green Tea Extract in Adult Patients With Solid Tumors

By Katherine M.W. Pisters, Robert A. Newman, Brenda Coldman, Dong M. Shin, Fadlo R. Khuri, Waun Ki Hong, Bonnie S. Glisson, and Jin S. Lee

**Purpose:** This trial was designed to determine the maximum-tolerated dose, toxicity, and pharmacology of oral green tea extract (GTE) once daily or three times daily.

**Patients and Methods:** Cohorts of three or more adult cancer patients were administered oral GTE with water after meals one or three times daily for 4 weeks, to a maximum of 6 months, depending on disease response and patient tolerance. Pharmacokinetic analyses were encouraged but optional.

**Results:** Dose levels of 0.5 to 5.05 g/m<sup>2</sup> qd and 1.0 to 2.2 g/m<sup>2</sup> tid were explored. A total of 49 patients were studied. Patient characteristics: median age, 57 years (range, 27 to 77 years); 23 patients were women (47%); 98% had a Zubrod PS of 1%; 98% had PS of 1; and 21 had non-small-cell lung, 19 had head & neck cancer, three had mesothelioma, and six had other. Mild to moderate toxicities were seen at most dose

levels and promptly reversed on discontinuation of GTE. Dose-limiting toxicities were caffeine related and included neurologic and gastrointestinal effects. The maximum-tolerated dose was 4.2 g/m<sup>2</sup> once daily or 1.0 g/m<sup>2</sup> three times daily. No major responses occurred; 10 patients with stable disease completed 6 months of GTE. Pharmacokinetic analyses found accumulation of caffeine levels that were dose dependent, whereas epigallocatechin gallate levels did not accumulate nor appear dose related.

**Conclusion:** A dose of 1.0 g/m<sup>2</sup> tid (equivalent to 7 to 8 Japanese cups [120 mL] of green tea three times daily) is recommended for future studies. The side effects of this preparation of GTE were caffeine related. Oral GTE at the doses studied can be taken safely for at least 6 months.

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TEA IS A BEVERAGE made from the leaves of *Camellia sinensis* species of the theaceae family. This beverage is one of the most ancient and is, next to water, the most widely consumed liquid in the world. Tea leaves are primarily manufactured as green, black, or oolong, with black tea representing approximately 80% of tea products consumed. Green tea is the nonoxidized, nonfermented product and contains several polyphenolic components, such as epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate (EGCG).

The primary polyphenol in green tea extract (GTE) is EGCG. In 1987, Fujiki et al<sup>1</sup> first reported that EGCG significantly inhibited tumor promotion of teleocidin in a two-stage carcinogenesis experiment on mouse skin. Significant anticarcinogenic effects of EGCG and GTE on various organs, such as skin, stomach, duodenum, colon,

liver, pancreas, and lung in rodent models have been confirmed.<sup>2-12</sup> Possible preventive effects of tea consumption on cancer development in humans have been reported. Many studies have found no significant association,<sup>13-18</sup> whereas others have found an increase in cancer risk.<sup>19-21</sup> Other studies found a protective effect of tea consumption against cancer.<sup>22-27</sup> Many of the studies that found no effect or a negative effect of tea consumption were done in Western countries, while a cancer preventive effect was primarily seen in Asian countries, especially China and Japan, where inhabitants drink large amounts of green tea each day. The reason for these differing results may be due to variable consumption of tea, with much larger volumes typically being consumed in Asian countries.<sup>28</sup>

To determine the protective effects of green tea intake against cancer incidence, a prospective cohort study was undertaken to examine whether green tea prevented cancer development in a population that consumed large amounts of green tea.<sup>28</sup> A survey of 8,552 individuals over 40 years of age living in a town in Saitama prefecture in Japan was carried out. During the 9 years of follow-up (71,248.5 person-years), 384 cases of cancer were identified. A negative association between green tea consumption and cancer incidence, especially among females drinking more than 10 cups (cup = 120 mL) a day was found. A slowdown in increase of cancer incidence with age was observed among females who consumed more than 10 cups a day; consumption of green tea was associated with later onset of

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cancer. Age-standardized average annual incidence rate was significantly lower among females who consumed large amounts of green tea. Relative risk of cancer incidence was also lower among both females (RR = 0.57; 95% confidence interval [CI], 0.33 to 0.98) and males (RR = 0.68; 95% CI, 0.39 to 1.21) in groups with the highest consumption, although the preventive effects did not achieve statistical significance among males, even when stratified by smoking and adjusted for alcohol and dietary variables.<sup>28</sup>

These epidemiologic findings have spurred intense basic science research of green tea and its components. These studies have suggested several possible mechanisms of action of green tea. Antioxidant properties<sup>29</sup> or interactions with certain enzymes or proteins implicated in cancer biology such as urokinase,<sup>30</sup> ornithine decarboxylase,<sup>31</sup> NADPH-cytochrome P450 reductase,<sup>32</sup> protein kinase C,<sup>33</sup> steroid 5 alpha reductase,<sup>34</sup> TNF expression,<sup>35</sup> and nitric oxide synthase<sup>36</sup> have been proposed as possible mechanisms of action. More recently, an anticancer effect through antiangiogenesis activity of green tea and EGCG has been reported by Cao et al.<sup>37</sup> Naasani et al<sup>38</sup> have demonstrated that EGCG strongly and directly inhibits telomerase, an enzyme essential for unlocking the proliferative capacity of cancer cells by maintaining the tips of their chromosomes. Green tea may protect against cancer by causing cell cycle arrest and inducing apoptosis.<sup>39</sup> Investigators in Italy recently published evidence that green tea may exert its beneficial effect by impairing tumor invasion and nourishment through direct inhibition of two gelatinases (MMP-2 and MMP-9).<sup>40</sup> Despite these tantalizing reports, the exact mechanism underlying the anticancer effect of green tea remains elusive.

A study of oral GTE conducted in healthy Japanese volunteers found that the administration of 2.25 g of GTE given as three divided doses was safe. This was equivalent to approximately 10 cups (120 mL each) of green tea daily. We conducted this phase I trial, which is the first trial conducted in the world of oral GTE in cancer patients. The objectives of this trial were to determine the maximally tolerated dose (MTD) of oral GTE on a once daily and three times daily schedule in adult cancer patients. The safety and side effects of chronic daily GTE, clinical pharmacology of GTE, and antitumor activity of GTE were determined. Given the safe administration of 2.25 g of GTE (divided into three equal doses) proven in the volunteer study above, the first dose level of this study was 0.5 g/m<sup>2</sup>, once daily.

## PATIENTS AND METHODS

Forty-nine patients with histologic or cytologic proof of incurable malignancy who were either refractory to standard therapy or had a disease for which no standard therapy existed were entered between

Table 1. Composition of Green Tea Extract Capsules in This Study

Strength of capsule, mg	110
	200
	270
Composition of green tea extract, %	
Catechins, total	26.9
EGCg	13.2
EGC	8.3
ECg	3.3
EC	2.2
Caffeine	6.8
Protein	19.8
Lipid	0.1
Amino acids	4.5
Ash	10.8
Moisture	2.6
Other (carbohydrate, flavonoid, etc)	28.5

August 1997 and April 1999. Eligibility requirements included an estimated life expectancy of at least 16 weeks, a Zubrod performance status of zero or one, and age of 18 to 80. Patients could not have received chemotherapy or radiotherapy for 3 weeks before study entry (6 weeks for mitomycin or nitrosourea). Baseline laboratory parameters included a WBC count of more than 4,000/mL,<sup>3</sup> platelet count of more than 100,000/mL,<sup>3</sup> bilirubin of less than or equal to 1.5 mg/dL, ALT or AST of less than 1.25× normal, and creatinine levels of less than or equal to 1.5 mg/dL. Patients could not have a history of significant cardiac disease, metabolic disorder, infection, or brain metastases. Written informed consent was obtained from all patients. All patients were requested to participate in the pharmacokinetic studies, but participation in this was not mandatory. This study was reviewed and approved by the institutional review board of the University of Texas M. D. Anderson Cancer Center.

Before therapy, all patients had a complete history and physical examination. Pretreatment laboratory evaluation parameters included an electrocardiogram reading, complete blood count, platelet count, urinalysis, sodium, potassium chloride, carbon dioxide, blood urea nitrogen, creatinine, calcium protein, albumin, phosphorus, uric acid, alanine serum transferase, bilirubin, lactate dehydrogenase, alkaline phosphatase, cholesterol, triglyceride, prothrombin time, and partial thromboplastin time. During the first 4 weeks of the study, patients had a complete blood count, platelet count, sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, and creatinine assessment repeated weekly. Every 4 weeks, an interim history, physical, and pill count were performed, as well as the pretreatment laboratory examinations. Radiologic examinations to document tumor measurements and response were performed every 4 weeks. Standard criteria for response and the common toxicity criteria of the National Cancer Institute (NCI; version 2.0, NCI common toxicity criteria, CTCP) were used.

Green tea extract was supplied in capsule form by Ito En, Ltd. (Tokyo, Japan). The capsules came in three different strengths: 110, 200, and 270 mg. Composition of the capsules is listed in Table 1. Patients were instructed to take the GTE capsules as a once-daily dose (initial seven cohorts) or as a three-times-a-day dose (final three cohorts). Capsules were to be taken after meals with water. At least three patients were entered at each dose level; additional patients were added at levels at which toxicity was observed. Responding- or stable-disease patients were allowed to continue on study for a

maximum of 6 months. The initial dose level was 0.5 g/m<sup>2</sup>, and dose escalation proceeded at 100% until grade I toxicity was observed. Dose escalation then continued at 50% until grade II toxicity occurred and thereafter, at 25% increments until the MTD was defined. Dose escalation was not permitted in the same patient. Dose escalation proceeded from 1.0 g/m<sup>2</sup> to 5.05 g/m<sup>2</sup>. Once the MTD was determined for a once-daily schedule, the study was amended to explore a three-times-daily dosing schedule. The initial level evaluated was 1.7 g/m<sup>2</sup> tid. Dose escalation to 2.2 g/m<sup>2</sup> tid found that long-term use of the GTE was not possible secondary to the side effects observed. The dose was then reduced to 1.36 and 1.0 g/m<sup>2</sup> each given on a tid schedule, as toxicities were encountered. This dose de-escalation was performed to define the dose that was most likely to be tolerated on a long-term basis. Pharmacokinetic studies were done in as many patients as possible at each dose level. The MTD was defined as that dose that produced reversible toxicity ( $\geq 2+$  magnitude) in 70% of the patients or at least 3+ toxicity in 30% of patients.

### Pharmacokinetic Studies

Pharmacokinetic studies were performed on day 1 and again in the same manner at the end of weeks 4 and 8. Ten-milliliter blood samples were obtained before treatment and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, and 24 hours after drug ingestion for patients taking GTE on a once-daily basis. For patients entered on the three-times-daily schedule, a 10-mL blood sample was obtained before the each dose of drug and was also obtained 2 hours after the initial and second dose on day 1 and at the end of weeks 4 and 8. On the days of study, the GTE was taken at least 4 hours apart, beginning at 8 AM. Urine samples were collected only for patients on the once-daily schedule.

### Analytic Methodology

**Caffeine.** Caffeine was determined using a validated high-performance liquid chromatography (HPLC) assay. Plasma samples were extracted using Waters Oasis HLB cartridges (Waters Associates, Milford, MA). Aliquots (200  $\mu$ L) of sample plasma were loaded onto prepared cartridges that were then washed with water (1 mL). Caffeine was eluted using 1 mL MeOH that was then dried under nitrogen. Samples were reconstituted with MeOH (100  $\mu$ L), and an aliquot of this solution injected onto the HPLC. Analyses of caffeine was performed using a Spherisorb ODS analytic HPLC column (Phenomenex, Torrance, CA). The isocratic mobile phase consisted of 0.5% acetic acid and acetonitrile (80:20; v:v) and was run at a flow rate of 1 mL/min. Caffeine was detected at 276 nm. The HPLC instrument was a Waters Alliance model 2.690. Authentic caffeine was purchased from Sigma Chemical Co. (St. Louis, MO).

**Unconjugated (free) catechin assay.** Catechin standards (EGCg, ECg, EGC, and EC) were obtained from Ito En, Ltd. Spiked plasma samples and patient samples (500  $\mu$ L) were extracted using a modification of the method reported by Poon.<sup>41</sup> An aliquot of internal standard (ethyl gallate) was added to samples in a microcentrifuge tube. Proteins were precipitated by adding cold acetonitrile (500  $\mu$ L). Samples were then processed by centrifuge (23,000  $\times g$ ) for 5 minutes at 5 EC. The supernatant was transferred to a new tube to which was added 100  $\mu$ L 1M ammonium acetate and ethyl acetate (750  $\mu$ L). Samples were vortex mixed for 20 minutes and then processed by centrifuge (23,000  $\times g$ ) for 3 minutes. The upper organic layer of each sample was transferred to a clean glass tube. A second extraction of the sample using 750  $\mu$ L ethyl acetate was conducted and the extract supernatant solutions combined. These were then dried under nitrogen at 40 EC. The dried samples were reconstituted with 100  $\mu$ L 10:90

Table 2. Patient Characteristics (N = 49)

Sex	
Male	26
Female	23
Age, years	
Median	57
Range	27-77
Zubrod performance status	
1	48
2	1
Prior treatment	
Chemotherapy	39
Radiation	37
Surgery	38
None	3
Diagnosis	
Non-small-cell lung cancer, total	21
Head and neck cancer, total	19
Squamous	10
Adenoid cystic	5
Other salivary gland	3
Lymphoepithelioma	1
Mesothelioma	3
Thymoma	2
Other	4

acetonitrile:0.1% formic acid (pH 3.0) and mixed. The clear supernatant was then transferred to a sample vial for analysis by liquid chromatography (LC)/mass spectrometry (MS).

Prepared sample extracts were analyzed using a validated LC/MS method. Reconstituted samples (50  $\mu$ L) were injected into a Micro-Mass (Beverly, MA) VG platform mass spectrometer equipped with an electrospray inlet source using a Hewlett Packard 1,100 HPLC apparatus equipped with a photodiode array detector. Catechins were separated using an isocratic method with a total run time of 7 minutes. The mobile phase consisted of acetonitrile: 0.1% formic acid (pH 3.0; 20:80, v:v) and was run at 300  $\mu$ L/min. The column used was a YMC-basic S-5 column (2.0  $\times$  250 nm; YMC, Inc., Waters Corp., Milford, MA). Catechins were detected using selective ion monitoring (EGCg, *m/z* 457; EGC, *m/z* 441; ECg, *m/z* 305; EC, *m/z* 289; ethyl gallate, *m/z* 197) with the mass spectrometer operated in an electrospray-negative mode.

### RESULTS

Forty-nine adult cancer patients were entered onto this phase I trial between August 1997 and April 1999. Patient characteristics are listed in Table 2. Twenty-six (53%) were male, and the median age was 57 years. All patients had an excellent performance status. As is typical for a phase I population, the vast majority of patients had received prior anticancer therapy. Non-small-cell lung cancer accounted for the most common type of malignancy (21 patients, or 43%). Cancer of the head and neck comprised the second most common type of cancer diagnosis, and there were a

Table 3. Number of Green Tea Extract Capsules and Total Daily Dose

Dose (g/m <sup>2</sup> )	No. of Patients	No. of Capsules/ Dose	Total GTE Dose (gm)
Once daily			
0.5	3	4-10	0.8-1.1
1.0	3	6-11	1.5-2.2
1.5	3	10-20	2.3-2.6
2.25	3	18-19	3.2-4.9
3.37	6	21-32	5.5-6.4
4.2	6*	28-46	6.1-9.9
5.05	3*	31-39	8.2-10.1
Three times daily			
1.0	8†	7-10	4.8-6.7
1.36	7‡	9-20	6.6-8.9
1.7	4	12-15	8.9-11.4
2.2	3	14-18	9.9-13.6

\*One patient stopped after one dose.

†One patient refused therapy after registration.

‡One patient removed from study after 3 days for unrelated medical problem.

few patients with mesothelioma, thymoma, or other diagnosis.

The dose levels, number of patients entered at each level, number of capsules consumed per dose, and the total daily dose of GTE taken are listed in Table 3. Because of the strength of the capsules available for use in this study (110, 200, and 270 mg), many patients consumed a large quantity of capsules per dose. This is outlined in greater detail in Table 3; however, the range of capsules per dose was 4 (0.5 g/m<sup>2</sup> daily) to 46 (4.2 g/m<sup>2</sup> daily). Four patients were not evaluable for toxicity or response. Two of these patients refused further study medication after one dose (one each at 4.2 and 5.05 g/m<sup>2</sup> daily), one patient declined to participate in the study after registration, and one patient was removed from study after 3 days for an unrelated comorbid illness (perforated diverticulum).

Toxicity data is presented in Table 4. None of the patients developed hematologic toxicity as a consequence of GTE consumption. During the trial, two patients had a grade 1 increase in serum cholesterol, and four patients had a grade 1 increase in serum triglyceride. No trends were noted, and it was the opinion of the investigators that the minor differences in cholesterol and triglyceride values most likely reflected temporary changes in diet than any real effect of GTE. In addition, follow-up laboratory examinations found no significant changes in serum chemistries, coagulation, electrocardiographs, or urinalysis.

No toxicities were seen in the three patients treated at the initial dose level of 0.5 g/m<sup>2</sup> daily. Grade 1 toxicities were seen at dose levels 1.0, 1.5 and 2.25 g/m<sup>2</sup>. These toxicities were mild and included gastrointestinal (abdominal bloat-

ing, sore throat, and nausea), neurologic (insomnia, paresthesias, restlessness), and cardiovascular (palpitations) complaints. Two patients (one each at the 1.0 and 1.5 g/m<sup>2</sup> levels) complained of polydipsia and urinary frequency. The cohort treated at 3.37 g/m<sup>2</sup> was expanded to six patients after grade 2 toxicity was observed. At this dose level, similar toxicities to that seen in the previous three dose levels were observed, with gastrointestinal, neurologic, and cardiovascular toxicities observed. In addition, the constitutional complaints of diaphoresis and fatigue were seen. Because only one of six patients experienced grade 2 toxicity, dose escalation to 4.2 g/m<sup>2</sup> proceeded. There was no notable increase in toxicity seen at this level. One of six patients experienced grade 2 toxicity (fatigue and nausea) and declined further treatment after only one dose. At 5.05 g/m<sup>2</sup> daily, three patients were studied. The first patient had only grade 1 toxicity. The second patient initially took the prescribed GTE incorrectly on a divided-dose, three-times-daily schedule with no toxicity. When this error was corrected to a once-daily schedule, the same patient developed grade 1 diaphoresis, dyspepsia, and insomnia; grade 2 cough; constipation; headache and pain; and grade 3 tremors. The third patient entered at this level withdrew from the study after the initial dose, which had caused the patient to have grade 2 abdominal bloating and nausea.

On the basis of the toxicity seen at the 5.05 g/m<sup>2</sup> daily level, the protocol was amended to explore a tid schedule. The initial dose on the tid schedule was 1.7 g/m<sup>2</sup> three times daily (total daily dose of 5.1 g/m<sup>2</sup>). Four patients were entered at this level (one additional patient was entered at this level through a registration error). One patient experiencing no toxicity, two patients with grade 1 toxicity and one patient with grade 2 abdominal bloating, nausea, and emesis. Dose escalation to 2.2 g/m<sup>2</sup> tid (total daily dose of 6.6 g/m<sup>2</sup>) found similar toxicities to those reported previously with gastrointestinal complaints (abdominal bloating, flatulence, nausea and vomiting) being most common. Given the degree of symptoms that patients were experiencing, a dose de-escalation to further define a more tolerable dose for long-term use was undertaken. The third cohort of patients treated on the tid schedule received 1.36 g/m<sup>2</sup> (4.08 g/m<sup>2</sup> total daily dose) of GTE. The cohort was expanded to six patients after the third patient at this level experienced grade 2 fatigue and palpitations and grade 3 constipation. A additional 4 patients were registered to this level (one patient was not evaluable for toxicity after coming off study—ruptured diverticulum—after 3 days of GTE). The toxicities observed are noted in Table 4 and were similar to those previously seen. The final dose level was 1.0 g/m<sup>2</sup> tid (total daily dose of 3.0 gm/2). After the initial three patients entered had minor toxicities, the dose level was

Table 4. Toxicity of Oral Green Tea Extract

Side Effect	NCI Grade	Dose level, mg/m <sup>2</sup>										
		Once Daily							Three Times Daily			
		0.5 (n = 3)	1.0 (n = 3)	1.5 (n = 3)	2.25 (n = 3)	3.37 (n = 6)	4.2 (n = 6)	5.05 (n = 3)	1.0 (n = 8)	1.36 (n = 7)	1.7 (n = 4)	2.2 (n = 3)
<b>Cardiovascular</b>												
Hypertension	1	0	0	0	0	1	0	0	0	0	0	0
Palpitations	1	0	0	0	1	1	0	0	0	1	0	0
	2	0	0	0	0	0	0	0	0	1	0	0
<b>Constitutional</b>												
Diaphoresis	1	0	0	0	0	0	0	1	0	1	0	0
	2	0	0	0	0	1	0	0	0	0	0	0
Fatigue	1	0	0	0	0	2	0	0	0	1	0	0
	2	0	0	0	0	1	1	0	1	2	0	0
<b>Gastrointestinal</b>												
Abd bloating	1	0	0	1	1	1	0	1	0	1	2	2
	2	0	0	0	0	1	0	1	0	0	0	0
Anorexia	1	0	0	0	0	0	0	0	0	1	0	0
Constipation	1	0	0	0	0	0	0	0	0	2	0	0
	2	0	0	0	0	0	0	1	0	0	0	0
	3	0	0	0	0	0	0	0	0	1	0	0
Diarrhea	1	0	0	0	0	0	0	1	0	1	0	0
Dyspepsia	1	0	0	0	0	1	0	1	0	1	0	0
Dysphagia	1	0	0	0	0	0	0	0	0	0	0	1
Flatulence	1	0	0	0	0	2	0	0	1	2	0	2
	2	0	0	0	0	0	0	0	0	1	0	0
Nausea	1	0	1	3	1	2	3	1	1	3	2	0
	2	0	0	0	0	1	1	1	0	1	1	1
Odynophagia	1	0	0	1	1	0	0	0	0	0	0	0
Polyphagia	1	0	0	0	0	0	1	1	1	0	0	1
Vomiting	1	0	0	0	0	0	1	1	0	1	1	1
	2	0	0	0	0	1	0	0	0	1	1	1
<b>Neurologic</b>												
Agitation	1	0	0	0	0	0	0	0	2	1	0	0
Dizziness	1	0	0	0	0	0	1	0	0	1	0	0
Insomnia	1	0	0	1	0	2	0	0	3	2	0	0
Memory	1	0	0	0	0	0	0	2	0	1	0	0
Paresthesia	1	0	0	1	0	0	0	0	0	0	0	0
Restlessness	1	0	2	1	1	0	1	1	0	1	1	0
Tremor	1	0	0	0	0	1	0	3	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0
<b>Pain</b>												
Headache	1	0	0	0	0	0	1	0	1	1	0	0
	2	0	0	0	0	0	0	1	0	0	0	0
Pain	1	0	1	0	0	0	0	0	0	0	0	1
	2	0	0	0	0	0	0	1	0	0	0	0
<b>Renal</b>												
Dysuria	1	0	0	0	0	0	0	0	0	0	0	1
Polyuria	1	0	1	1	0	0	0	1	0	0	0	0
<b>Other</b>												
Cough	2	0	0	0	0	0	0	1	0	0	0	0
Myalgia	1	0	0	0	0	0	0	0	0	1	0	0
Polydipsia	1	0	1	1	0	0	0	0	0	0	0	1

expanded, enrolling a total of eight patients. One patient did not participate in the study after registration and did not take any GTE. Of the seven patients who did complete at least 1 month of GTE, three patients did not experience any toxicity.

The other four patients had grade 1 toxicity consisting of insomnia,<sup>3</sup> agitation,<sup>2</sup> fatigue,<sup>2</sup> or mild gastrointestinal complaints. On the basis of the tolerable nature of the side effects seen at the 1.0 g/m<sup>2</sup> level, the trial was concluded.

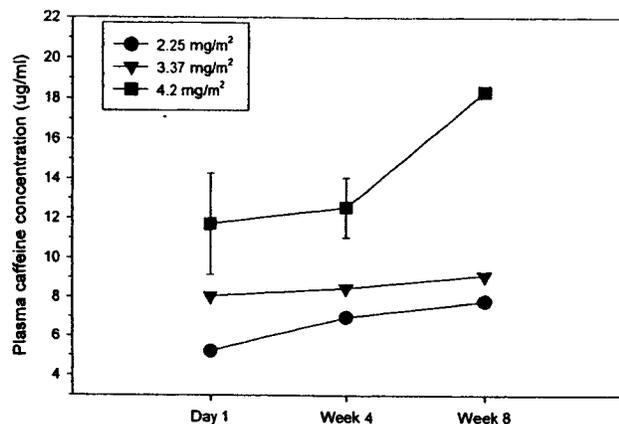


Fig 1. Dose-dependent increases in plasma caffeine concentrations as a function of duration of therapy with green tea extract (GTE). Data are presented as mean caffeine C<sub>max</sub> concentrations after the daily dose of GTE on day 1, as well as the initial day of therapy on weeks 4 and 8. In the highest dose group, data are presented as mean  $\pm$  SD.

No major or minor antitumor responses were seen during the trial. The median duration of therapy was 2 months. Two patients refused further participation in the study after one dose. One patient came off study after 3 days when he developed a perforated diverticulum that was judged unrelated to therapy. Fifteen patients completed 1 month, 16 patients completed 2 months, 3 completed 3 months, one took 4 months of treatment, and 10 patients with stable disease completed the full possible 6 months of oral GTE.

#### Pharmacokinetic Results

**Caffeine.** As shown in Fig 1, plasma caffeine C<sub>max</sub> concentrations were dose-dependent on the once-daily schedule, and average concentrations ranged from 5  $\mu$ g/mL at 2.25 g/m<sup>2</sup> to 11.4  $\mu$ g/mL at 4.2 g/m<sup>2</sup>. Within dose levels, plasma caffeine concentrations were observed to increase over the 8-week investigation period. As seen in Fig 2, however, there was considerable variation between patients with respect to relative caffeine pharmacology. For example, there was no detectable increase in caffeine C<sub>max</sub> or clearance throughout the duration of the study for the patient in Fig 2A. In contrast, the patient data in Fig 2B show a clear time-dependent accumulation of caffeine with C<sub>max</sub> levels reaching 23  $\mu$ g/mL by week 8. On the tid schedule at 1.0 g/m<sup>2</sup>, the dose recommended for phase II trials, plasma caffeine levels also exhibited considerable interpatient variability and ranged from 1.5 to 5  $\mu$ g/mL throughout the daily doses.

**Catechins.** Catechins exist in plasma as either free compounds or in several conjugated forms.<sup>42</sup> Because only free catechins are believed to possess an antioxidant activ-

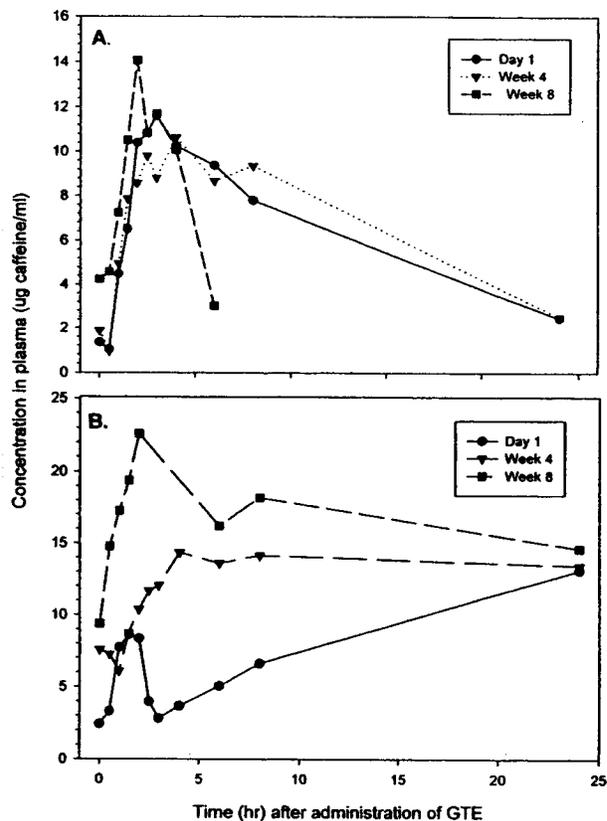


Fig 2. Individual variation in caffeine pharmacokinetics. Data show plasma caffeine concentrations versus time plots after administration of green tea extract (GTE) at 4.2 mg/m<sup>2</sup> to two separate patients. (A) This patient showed reproducible caffeine pharmacokinetics throughout the 8-week trial of GTE. (B) This patient showed clear evidence of time-dependent accumulation of plasma caffeine concentrations; these data were indicative of approximately 33% of all patients entered at this dose level.

ity,<sup>43</sup> no attempt was made to hydrolyze conjugated catechins to provide a measure of total catechin content. The method employed in the present study routinely permitted quantitation of both EGCG and ECG in patient plasma samples. Plasma levels of ECG and EC were low and variable. The dose-response relationship of GTE to plasma EGCG concentration is shown in Fig 3. In contrast to caffeine, there was no indication of any time-dependent accumulation of catechin over the 8-week period of study. Although numbers of data sets per dose level were small, determination of EGCG in plasma revealed a T<sub>max</sub> of 1 to 3 hours and C<sub>max</sub> levels of 100 to 225 ng/mL. No EGCG was detected in the urine. Analyses of plasma from patients on the tid schedule revealed EGCG concentrations of 35 to 55 ng/mL with no accumulation of catechin over the 8-week period of the study.

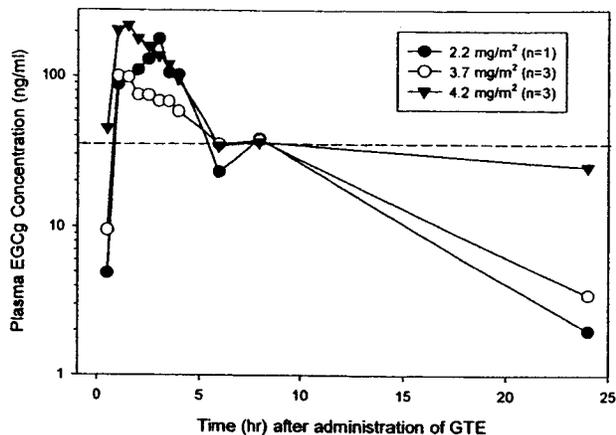


Fig 3. Dose-related plasma epigallocatechin gallate (EGCG) concentrations as a function of time after administration of green tea extract (GTE). EGCG pharmacokinetics were similar at all dose levels. Numbers in parentheses indicate the number of patient data sets at each dose level. EGCG plasma concentration-time data in patients administered GTE at 1 mg/m<sup>2</sup> on a tid schedule were greatly reduced from levels reached in the daily single-dose regimen (data not shown).

#### DISCUSSION

This phase I study of oral GTE was performed after epidemiologic studies suggested a protective effect of tea consumption and preclinical laboratory studies of green tea constituents demonstrated anticancer activity.

This phase I study of oral GTE in adult cancer patients found dose-limiting side effects of gastrointestinal complaints (abdominal bloating, dyspepsia, flatulence, nausea, and vomiting) and CNS stimulation (agitation, dizziness, insomnia, tremors, and restlessness). These side effects were likely related to the 7% caffeine content of the GTE employed in this study. Patients treated at the 1.0 g/m<sup>2</sup> three-times-daily level had tolerable side effects. This dose could likely be administered on a long-term basis, as would be utilized in a chemopreventive setting. This dose of GTE is roughly equivalent to drinking seven to eight Japanese-style cups of green tea three times daily. A decaffeinated product might be better tolerated. This study was done with a caffeinated product because the epidemiologic data in support of this trial<sup>28</sup> was based on consumption of caffeinated green tea. Because drinking 20 to 25 cups of green tea on a daily basis is impractical and, some would say, unpleasant,<sup>44</sup> the development of a capsular alternative of green tea may prove beneficial. The MTD of oral GTE once

daily was 4.2 g/m<sup>2</sup>. This dose is not recommended for further study because it was not as well tolerated as the divided schedule.

The toxicities observed in this trial appear to be related to relative plasma caffeine levels. Hence, one might consider the possibility of using a decaffeinated GTE product. However, caffeine has been implicated as an important component of the chemoprevention activity of tea. For example, Chung et al<sup>45</sup> have shown that in addition to the polyphenolic compounds in tea, caffeine seems to contribute significantly to its inhibitory activity against lung carcinogenesis in rats. A more recent study<sup>46</sup> has directly compared oral administration of tea, decaffeinated tea, and caffeine itself on the formation and growth of tumors in mice previously treated with ultraviolet B light. The decaffeinated teas were inactive or less-effective inhibitors of tumor formation than the regular teas; adding caffeine back to the decaffeinated teas restored biologic activity. Interestingly, as in the prior study, caffeine alone in the drinking water inhibited the formation of nonmalignant and malignant tumors.

Plasma concentrations of free (unconjugated) catechins were determined in this study. On the single daily-dose schedule, levels of EGCG reached 225 ng/mL after administration of 4.2 g/m<sup>2</sup>. This can be compared with total (free plus conjugated) EGCG plasma levels in human volunteers of 326 ng/mL after administration of decaffeinated GTE 4.5 g/m<sup>2</sup> orally.<sup>47</sup> On the 1.0 g/m<sup>2</sup> tid schedule, plasma EGCG levels were, as expected, approximately one third of those observed in patients on the single daily-dose regimen.

The recommended dose for future trials of GTE is 1.0 g/m<sup>2</sup> three times daily. A three-times-daily dosing schedule is recommended over a daily dose because this was better tolerated and allowed administration of more GTE. The side effects of this preparation of GTE were related to caffeine. Oral GTE can be safely taken for at least 6 months. Future trials exploring the use of GTE in patients with oral leukoplakia are planned. These studies will incorporate translational research with biomarker studies. At this point, it would seem that GTE may have more potential as a chemopreventive agent rather than a cytotoxic one.

#### ACKNOWLEDGMENT

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## Case report

## Teapot myositis

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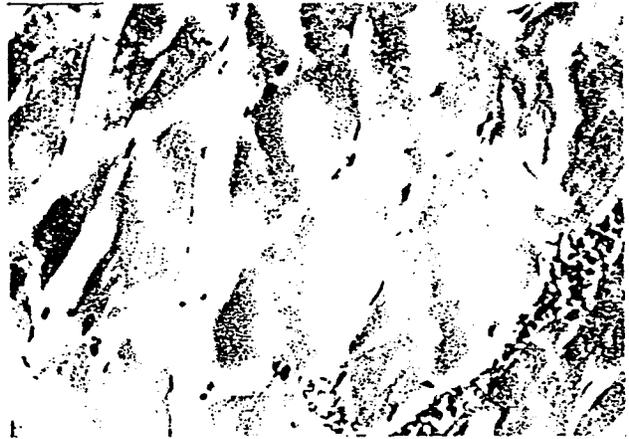
A 70-year-old man was admitted to hospital in October, 1996, with a 1-week history of increasing muscle pain and weakness, first noticed when pushing his wife's wheelchair uphill. By the time he was admitted to hospital he was unable to walk, lift his arms, or raise his head from the pillow. 20 years previously a laparotomy for small-bowel obstruction showed Crohn's disease of the small intestine. He had a small-intestine resection leaving an estimated 3 m of jejunum-ileum. He had been free from bowel symptoms since, and had no other relevant past history. On admission to hospital he was receiving hydroxocobalamin injections monthly and taking ferrous sulphate 200 mg per day. He smoked 15 cigarettes a day and drank less than 10 units of alcohol a week. Examination showed striking, mainly proximal, muscle weakness in both upper and lower limbs (grade 1/5). There was no muscle tenderness, fasciculation, or wasting. Investigations showed: sodium 144 mmol/L, potassium 1.7 mmol/L (in two samples), urea 5.7 mmol/L, creatinine 122 µmol/L, chloride 100 mmol/L, bicarbonate 24 mmol/L, creatine phosphokinase 5690 IU/L, aspartate aminotransferase 152 IU/L, lactic dehydrogenase 714 IU/L, bilirubin 7 µmol/L, albumin 34 g/L, alkaline phosphatase 146 IU/L, alanine aminotransferase 145 IU/L (NR 0-40), calcium 2.18 mmol/L, phosphate 0.82 mmol/L, haemoglobin 13.3 g/dL, white-cell count  $11.6 \times 10^9/L$ , platelets  $233 \times 10^9/L$ , erythrocyte sedimentation rate 12 mm/h, C-reactive protein less than 10 mg/L, and a negative test for autoantibodies. Coagulation studies, serum hydroxocobalamin, folate, immunoglobulins, thyroid function, prostatic specific antigen, and ferritin were normal. Chest radiograph and abdominal ultrasound were normal. There were flat T waves and prominent U waves on the electrocardiogram.

Histology of a biopsy specimen from the deltoid muscle showed variable degenerative changes with fibre splitting, internalisation of nuclei, and vacuolation, which are consistent with a metabolic myopathy (figure). Although the patient initially received intravenous steroids on a presumptive diagnosis of polymyositis, the laboratory results pointed to myositis due to hypokalaemia. With intravenous potassium he improved within 72 h. Steroids were stopped and 1 week later his strength had returned to normal and serum potassium and creatine phosphokinase were normal and have remained normal

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Haematoxylin and eosin-stained section of deltoid muscle

since. The cause of his hypokalaemia was unclear on presentation. On further questioning however, it became apparent that he drank around 14 L of tea a day, and had done for many years—a pot of tea was always “on the go”. He was able to reduce his intake without difficulty and remains well 1 year later.

My hero

Hypokalaemia increases the intracellular/extracellular potassium ratio, resulting in hyperpolarisation across excitable membranes. Many well-recognised complications may arise from this, including paralysis and rhabdomyolysis. Depending on seasonal variations, tea (*Camellia sinensis*) contains 2.5 to 5.5% caffeine, and trace amounts of theobromine and theophylline, and infused tea up to 60 mg caffeine per 100 mL.<sup>1</sup> Our patient would have been taking 6 g or more of caffeine per day. Previous reports of caffeine poisoning have shown hypokalaemia.<sup>2</sup> Theophylline, a closely related xanthine and metabolite of caffeine,<sup>3</sup> is a recognised cause of hypokalaemia and may cause rhabdomyolysis in overdose.<sup>3</sup> The probable mechanism is redistribution of potassium into cells mediated by increased concentration of intracellular 3,5 cAMP, at the expense of the extracellular pool.<sup>4</sup> Our patient's small-intestine resection might have been a further factor in his loss of control of extracellular potassium by reducing the surface area available for compensatory passive potassium absorption.<sup>5</sup> However his serum potassium remains normal 1 year later with no other intervention other than a reduction in tea intake and we therefore believe that xanthines derived from drinking tea were the cause of his hypokalaemia and profound muscle weakness.

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