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# An Investigation of the Antioxidant Activity of Black Tea Using Enhanced Chemiluminescence

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Antioxidants are important species which possess the ability to protect the body from damage caused by free radical-induced oxidative stress. A variety of free radical-scavenging antioxidants exist within the body many of which are derived from dietary sources. There is currently much interest in the antioxidant role of flavonoids and other polyphenols found in tea, wine, fruit and vegetables. Enhanced chemiluminescence is a simple technique which can be used as a rapid and sensitive assay for measuring the antioxidant activity of beverages such as green and black tea. This article examines the impact of water temperature, stewing time, leaf concentration and the addition of milk upon the antioxidant activity of black tea solutions. The antioxidant activity of a range of commercially available black and green teas has also been measured.

*Keywords:* Antioxidant, catechin, flavonoid, free radical, oxidative damage, tea

*Abbreviations:* AOA, antioxidant activity; CV, coefficient of variation; ECL, enhanced chemiluminescence; HRP, horseradish peroxidase

## INTRODUCTION

The human body is constantly subjected to free radical oxidative stresses originating from both endogenous sources such as mitochondrial electron transport and exogenous sources such as environmental pollution including cigarette smoke and exhaust fumes or the products of ionising radiation.[1,2] The harmful effects of the superoxide radical are amplified in the presence of iron when it may undergo a Haber-Weiss or Fenton reaction leading to the formation of the highly reactive hydroxyl radical. Free radicals are capable of oxidizing lipids (lipid peroxidation) in membranes and lipoproteins, as well as proteins and DNA. The resulting damage leads to disruption and instability of biological systems followed eventually by death. Free radical-related oxidative damage has now been impli-

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cated in the pathogenesis of a variety of common diseases including atherosclerosis,[3] ischaemia-reperfusion injury,[4] inflammation,[5] and cancer.[6] Ischaemic heart disease resulting from atherosclerosis of the coronary vessels is a particularly common cause of death in the developed world. Although a range of risk factors for coronary atherosclerosis have been identified it is now known that oxidative modification of cholesterol-rich low-density lipoproteins is one of the final pathways leading to their deposition in the vessel wall.[3]

A wide variety of radical-scavenging antioxidants exist to protect against oxidation of important biological molecules. Most are available in the diet and include antioxidant vitamins such as ascorbate and α-tocopherol as well as a group of polyphenolic compounds known as flavonoids which are present in vegetables, fruit, tea and wine. Several studies suggest that a high consumption of flavonoids is associated with a reduction in mortality from ischaemic heart disease.[7–10] In Western diets tea consumption accounts for as much as 60% of the total flavonoid intake.[8] Therefore, it is not surprising that a number of epidemiological studies have also given support to the notion that tea consumption alone might also be associated with some protection from ischaemic heart disease.[11,12] Although many biological activities have been attributed to dietary flavonoids it is their potential to act as powerful antioxidants inhibiting oxidation of lipoproteins and other molecules that has attracted particular interest.[13–22]

Tea is grown in approximately thirty countries and is the most widely consumed beverage apart from water in the world. The most significant of all tea components are the polyphenols, in particular the catechins.[23] These are colourless, astringent, water-soluble compounds which are readily oxidized and are therefore powerful antioxidants. The structures of the six principal catechins found in significant quantities in leaf tea are shown in Figure 1. Green tea is consumed mainly in Japan and China and is manufactured from the fresh leaf during which the oxidation of the polyphenol content is minimal. Black tea is more widely produced and involves enzymatically catalysed aerobic oxidation of the leaf polyphenols followed by a series of chemical condensations. The oxidation process is initiated in the maceration step where there is intimate contact between polyphenol oxidase (present in the tea leaf), polyphenols and atmospheric oxygen. The catechin quinone products of oxidation undergo further condensation reactions to produce theaflavins, bisflavonols, theaflavic acids and theaflagallins which contribute to the colour and flavour of black tea and possess some antioxidant properties. Consequently, the catechin content of black tea is significantly decreased in comparison to that of green tea or fresh leaf.[23] The polyphenol content of tea, especially the catechins, has been shown to have powerful antioxidant properties in a variety of *in vitro* model systems.[13–16,18,21,22] Although catechins are hydrophilic they may be important inhibitors of lipid peroxidation by scavenging aqueous oxygen radicals thereby preventing their entry into the lipophilic environment and the subsequent oxidation of α-tocopherol.[14] It has also been suggested that catechins may regenerate active α-tocopherol by donating a hydrogen atom to the α-tocopherol radical formed during the termination of lipid peroxidation.[24]

We have previously demonstrated that enhanced chemiluminescence (ECL) forms a simple method of measuring free radical-scavenging antioxidant activity in biological fluids.[25,26] This paper examines the use of the ECL technique to describe the antioxidant properties of tea solutions.

## MATERIALS AND METHODS

### Enhanced Chemiluminescence Assay Theory

The ECL assay is based on the HRP-catalysed oxidation of the chemiluminescent substrate luminol by hydrogen peroxide. The addition of

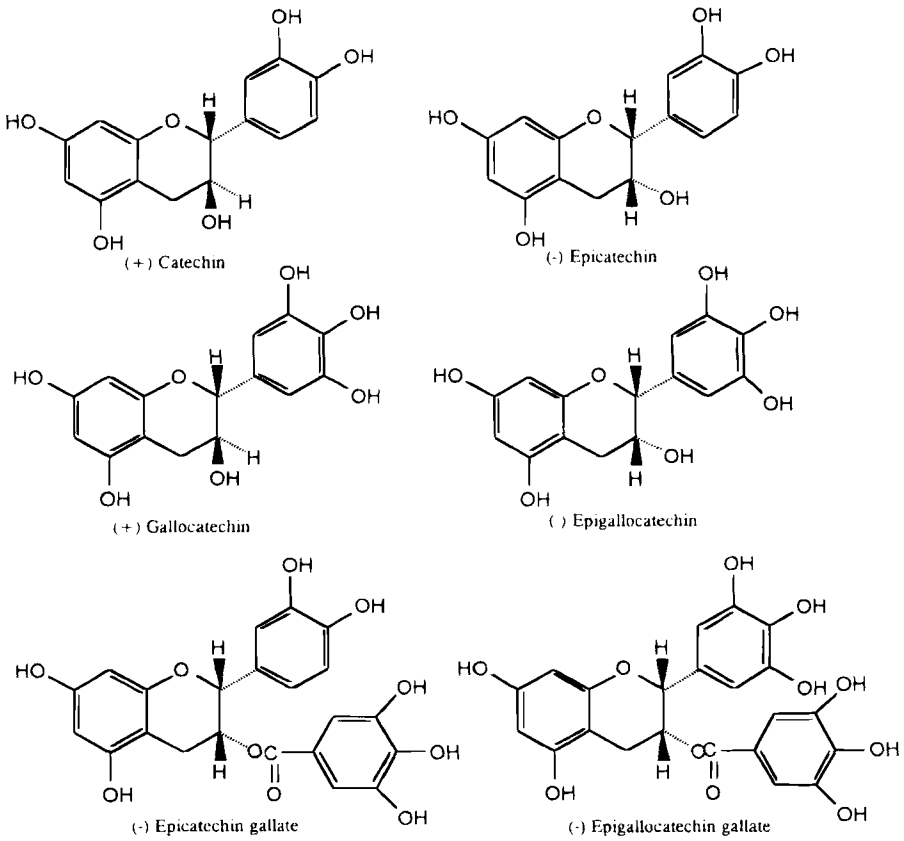


FIGURE 1 The six principal catechins found in leaf tea (23).



an enhancer phenol such as ρ-iodophenol produces a more intense, prolonged and stable light emission by overcoming the rate-limiting step in the reaction sequence. The continuous light emission from the reaction depends on the constant production of free radical intermediates derived from ρ-iodophenol, luminol and oxygen. The light emission is therefore sensitive to interference by radical-scavenging antioxidants such as those present in tea. The time period of light suppression produced by the addition of antioxidants is directly related to the concentration of antioxidant present in the reaction mixture.[25] When all of the added antioxidants have been consumed (oxidized) the light emission is restored.

## Materials

Enhanced chemiluminescent signal reagent and HRP conjugate (mouse IgG HRP linked whole antibody from sheep) were obtained from Amersham International (Amersham, Bucks, UK) as ECL Anti-oxidant Detection Pack NK8989. The signal reagent consisted of assay buffer, tablet A (luminol and enhancer phenol) and tablet B (oxidant, perborate). Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), a water-soluble tocopherol analogue, was obtained from Aldrich (Poole, UK). An aqueous 80 $\mu molL^{-1}$ solution was prepared from which aliquots were frozen at –70°C until use as the standard antioxidant solution. A luminometer (photocurrent measurement)

based on a side-window photomultiplier tube (EMI/ Type 9781A, 94 μA lumen$^{-1}$) was used to measure light emission. The kinetics of light emission were recorded using a chart recorder.

### Tea Samples

All tea samples used for antioxidant experiments were fresh. Unless otherwise stated standard tea solutions were prepared by the addition of 25 ml of de-ionised water to 0.5 g of tea leaves. All measurements in the ECL assay system were made using 1 in 500 dilutions of the tea solution unless otherwise stated.

### Antioxidant Assay Procedure

Working signal reagent was prepared by addition of tablets A and B to the buffer solution (without handling the tablets). The resulting solution was stable for two weeks at 4°C. The working HRP conjugate solution was prepared by adding 5 μl to 5 ml of deionised water. In order, 900 μl of deionised water, 100 μl of signal reagent and 20 μl of HRP conjugate were added to the luminometer cuvette (10 × 10 × 45 mm) and shaken well. The cuvette was placed in the luminometer and the light emission allowed to stabilise at a peak value (P). The cuvette was removed and 20 μl of trolox calibrant or test solution added. The cuvette was replaced in the luminometer at which point the light emission was almost completely suppressed if any radical-scavenging antioxidants were present. Measurement was continued until the suppression of the light emission returned to a second peak value (P′). The period of complete light suppression was represented by drawing a tangential line from the steepest point of the recovery curve to its intersection with the x-axis and measuring that time in seconds (t) (Fig. 2). The t-value for the sample can then be compared to the t-value for the trolox standard of known concentration and the total antioxidant activity (AOA) in μmol trolox equivalents per litre can be calculated using the following equation

$$\text{AOA} = \text{t-sample}/\text{t-trolox} \times 80\ \mu\text{molL}^{-1}$$

Figure 2 illustrates typical curves produced by trolox and tea antioxidants.

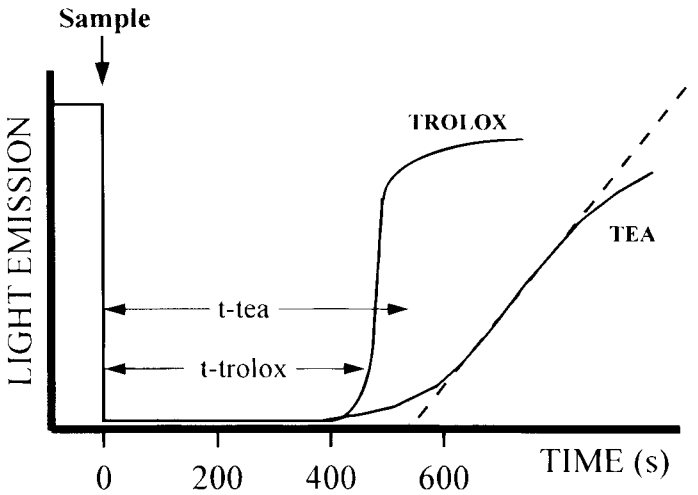


FIGURE 2 The kinetics of light emission from the enhanced chemiluminescent reaction following the addition of 20 μl aliquots of the trolox standard (80 μmolL$^{-1}$) and a 1 in 500 dilution of a tea solution.

## RESULTS

### Measurement of Antioxidant Activity of Tea Solutions

The antioxidant activity of tea solutions was many times higher than the trolox standard and for convenience of measurement tea samples were diluted 500 times in water before addition (20 µl) to the reaction cuvette. The antioxidant activity of most tea samples prepared in the standard way (0.5g tea leaves in 25 ml of water) fell in the range 5000–10000 $\mu molL^{-1}$. It should be noted that this is also many times greater than human serum (350–550 $\mu molL^{-1}$).[25] Figure 3 shows the linear relationship between the concentration of the trolox standard added to the reaction cuvette and the value of t-trolox. The figure also shows the the concentration-activity relationship for a single sample of Sainsbury Red Label tea prepared as above. This indicates that in this dilution range the relationship is essentially linear. The 'within-batch' coefficient of variation (CV) for repeated measurement of antioxidant activity in a single tea sample was 3.4%.

### Variation of Tea Antioxidant Activity with Water Temperature

Water (25 mls) was added to tea (0.5 g) and the the mixture was shaken every two minutes over a time period of thirty minutes. After the tea leaves had settled aliquots of the tea solution were removed at regular intervals for antioxidant analysis. All analyses were carried out at 20°C. Figure 4 illustrates the effect of three water temperatures on the subsequent antioxidant activity of four different teas. The antioxidant activity of tea solutions prepared with hot water (50°C) increased over time although the measurements sometimes fluctuated throughout the sampling period. When prepared with water at room temperature (20°C) a similar pattern was seen

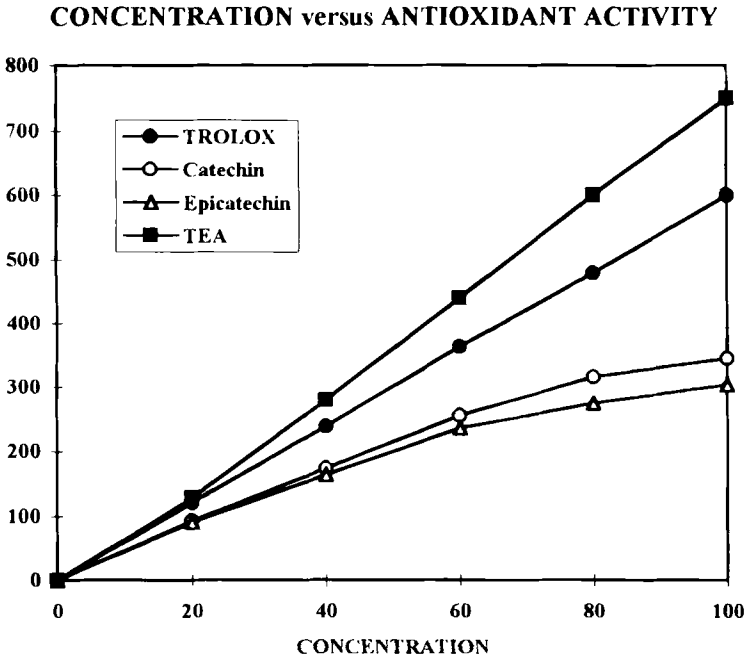


FIGURE 3 Concentration-antioxidant activity relationships for trolox, tea, catechin and epicatechin. The x-axis relates to the concentration of the antioxidant ($\mu molL^{-1}$) or in the case of tea the dilution (µl/5000 µl) of the 20 µl test sample added to the ECL reaction. The y-axis represents the t-value relating to the delay before resumption of light from the ECL reaction (seconds).

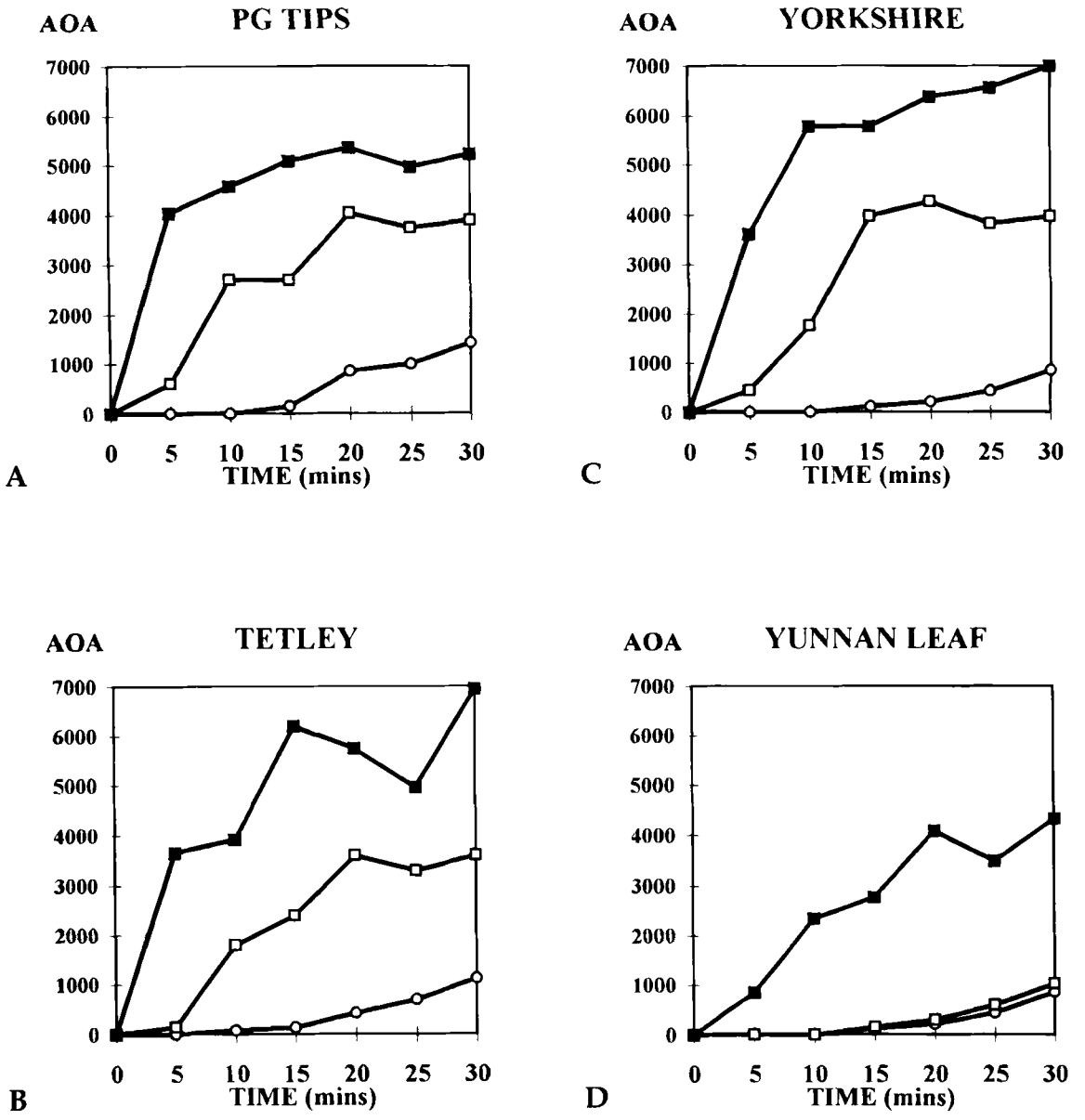


FIGURE 4 The effect of different water temperature on the antioxidant activity of solutions of four teas. Water (25 ml) was added to tea leaves (0.5 g) and the mixture shaken every two minutes over 30 minutes. The leaves were allowed to settle and aliquots removed a regular intervals for antioxidant measurements. (■) Hot water (50°C), (□) room temperature (20°C), (○) cooled water (10°C).

although the increase in antioxidant activity was smoother suggesting that the lability of tea antioxidants was decreased by lower water temperatures. This view was supported by the very smooth rise seen when cooled water (10°C) was used to prepare the tea solution. A further observation was that the rate of release of antioxidant activity into the tea solution was faster and the peak activity increased at higher water temperature. Even higher peak antioxidant activity values (43800 $\mu molL^{-1}$) were observed with water at 90°C. An intriguing finding was that after cool-

ing this solution on ice for twenty minutes the antioxidant activity decreased to 26000 $\mu molL^{-1}$ and on re-heating the levels rose again. This observation (as well as the lability of the measurements at higher temperatures) suggested that, in addition to the variable rates of release of polyphenols from the tea leaf into aqueous solution, the antioxidant activity may also depend on other temperature-dependent reactions occurring in solution.

A marked difference in peak antioxidant activity was seen when a solution of Sainsbury Red Label tea prepared with hot water (50°C) was not shaken during the sampling period, 3500 $\mu molL^{-1}$ seen when the solution was shaken. This suggested that agitation of the tea-water mixture as well as its temperature plays an important role in determining polyphenol release and the subsequent antioxidant activity.

### Relationship between Concentration of Tea Leaves and Antioxidant Activity

Figure 5 illustrates the changes in antioxidant activity over one hour after mixing 0.5 g, 1.0 g, 1.5 g and 2.0 g of Sainsburys Red Label tea with 25 mls water at room temperature (20°C) followed by regular shaking. The antioxidant activity increased rapidly at a rate and to an extent related to the concentration of tea leaves. There was little further increase in activity after 15 minutes. The study also showed that even up to a concentration of 2.0 g of tea leaves in 25 mls of water, a concentration considered undesirable for drinking, the solution was not saturated with antioxidants.

### Antioxidant Activity of Catechins in Pure Solution

Epicatechin and catechin are two of the more important antioxidants found in green and black tea (Fig. 1). Epicatechin was dissolved in deionised water and catechin in 50% dimethylsulphoxide and 50% deionised water because of its relative insolubility in aqueous solution. Figure 3 illustrates the relationship between antioxidant activity and concentration of these antioxidants in pure solution in comparison to the trolox standard. In contrast to tea and the

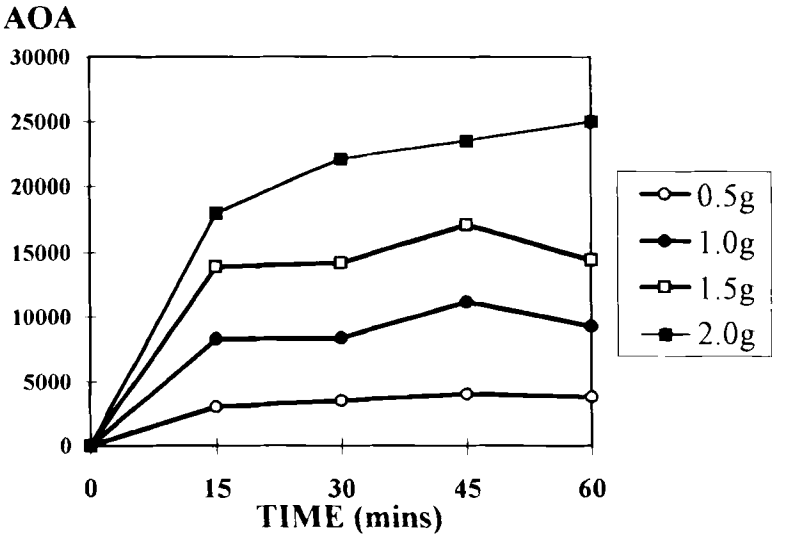


FIGURE 5 Variation in antioxidant activity of tea solutions with increasing concentration of tea leaves. Water (25 ml) at 20C was added to 0.5 g, 1.0 g, 1.5 g and 2.0 g of tea leaves. Solutions were shaken regularly and aliquots removed at fifteen minute intervals for antioxidant measurements. (○) 0.5g of tea, (●) 1.0g of tea, (□) 1.5g of tea, (■) 2.0g of tea.

trolox standard solution a non-co-operative relationship exists between concentration and antioxidant activity of both epicatechin and catechin. Linear regression analysis of the data obtained suggested that the stoichiometric equivalence for epicatechin is 0.63 and for catechin is 0.66 in relation to the trolox standard (1.0) in this concentration range.

### Antioxidant Activity of Commercially Available Black and Green Teas

Following earlier experiments that had shown an apparent temperature-dependent lability of the antioxidant activity of tea solutions the following comparisons were performed in hot and cooled tea solutions. Boiling water (25 mls) was added to the tea leaves (0.5 g) and the mixture was shaken every minute for thirty minutes whilst cooling at a room temperature of 20°C. After thirty minutes an aliquot was removed for immediate antioxidant measurement and a further one removed and cooled to 4°C over thirty minutes. Table I shows antioxidant activity measurements at high and low temperatures for a range of twenty commercially available teas. The results show firstly that there is a considerable variation in the antioxidant activity of the different teas. The two green teas included in the sample, Gunpowder green tea and Japanese green tea, were among the most potent antioxidants under these experimental conditions. The results suggested that there may be a relationship between strength of tea (based on taste) rated by the manufacturer and its antioxidant activity. Stronger teas such as Ceylon and Kenya tended to have higher antioxidant activities than weaker teas like Darjeeling. No relationship appeared to exist between the market price of a tea and the antioxidant activity it produced in solution. A

TABLE I Antioxidant activity in a range of commercially available teas. Boiling water (25 ml) has been added to 0.5 g of tea leaves and the mixture shaken every minute for thirty minutes whilst cooling at a room temperature of 20°C. An aliquot was removed for immediate antioxidant measurement and a further one removed and cooled to 4°C over thirty minutes before measurement. All results are expressed in $\mu molL^{-1}$ trolox equivalents as mean ± SD of three consecutive measurements of the same sample.

| TEA | WARM | COOLED |
|---|---|---|
| Gunpowder Green Tea | 12661 ±197 | 13774 ± 984 |
| Ceylon | 12429 ± 623 | 11786 ± 215 |
| Japanese Green Tea | 10482 ± 833 | 11061 ± 413 |
| Assam | 10370 ± 357 | 11414 ± 429 |
| Safeway | 10303 ± 455 | 9647 ± 712 |
| Jasmine | 10291 ± 521 | 11031 ± 197 |
| Kenya | 9799 ± 380 | 10647 ± 1007 |
| PG Tips | 8977 ± 0 | 10253 ± 332 |
| Sainsbury Red Label | 8856 ± 822 | 10381 ± 519 |
| Yorkshire | 8730 ± 159 | 8413 ± 692 |
| Asda | 8706 ± 603 | 8657 ± 150 |
| Tetley | 8443 ± 322 | 7413 ± 409 |
| Typhoo | 8052 ± 582 | 8975 ± 582 |
| Marks & Spencer 'Fine Flavours' | 7883 ± 146 | 9002 ± 223 |
| Nilgiri | 7744 ± 95 | 7777 ± 47 |
| Darjeeling | 7387 ± 483 | 7340 ± 659 |
| Yunnan | 7276 ± 669 | 7932 ± 1005 |
| Lapsang Souchong | 6756 ± 307 | 6909 ± 406 |
| Earl Grey | 6080 ± 480 | 6933 ± 333 |
| Gold Crown | 5539 ± 10 | 4911 ± 419 |

surprising finding was the close agreement of the results obtained following measurement of teas prepared at different temperatures in spite of the previous observations.

### Effect of Adding Milk to Hot Tea Solutions

Previous reports had suggested that milk proteins might complex with tea polyphenols and have the potential to reduce their antioxidant and other biological activities.[27] Antioxidant measurements were made for a variety of milks which were found to have minimal intrinsic activity in comparison to tea. For the following experiments full-fat milk, semi-skimmed and skimmed milk were used which had antioxidant activities of 142 ± 3.5 $\mu molL^{-1}$, 71 ± 4.2 $\mu molL^{-1}$ and 76 ± 3.5 $\mu molL^{-1}$ respectively. PG Tips tea was prepared by mixing 0.5 g tea leaf with 25 ml water (75°C). After 10 minutes the tea was removed from the tea leaves and mixed with either water or various dilutions of milk. These mixtures were cooled to 20°C and four aliquots were removed over a further 30 minutes for antioxidant measurement. The antioxidant activity of the tea solution was unaffected by mixture with skimmed milk at any dilution (Table II). However, the activity appeared to be decreased when either semi-skimmed or full fat milk had been mixed at a 1:1 ratio with the tea prior to measurement.

TABLE II The impact of milk upon antioxidant activity in tea solutions. Prior to the meaurement of antioxidant activity a tea solution (PG Tips, 0.5 g in 25 ml water at 75°C) was first diluted in an equal volume of (i) water, (ii) a 1:5 dilution of milk, (iii) a 1:2 dilution of milk and (iv) undiluted milk. Three kinds of milk of differing fat content were used. The results represent the mean of four measurements of antioxidant activity.

| | MILK-TEA RATIO | | | |
|---|---|---|---|---|
| MILK | 0 | 0.2 | 0.5 | 1.0 |
| Skimmed | 3830 | 3482 | 3410 | 4407 |
| Semi-Skimmed | 3895 | 3799 | 4117 | 3369 |
| Full Fat | 4175 | 4276 | 3707 | 2928 |

## DISCUSSION

Enhanced chemiluminescence has previously been shown to be a simple method for measuring radical-scavenging antioxidant activity in human serum in health[28] and disease.[29] These studies show its ease of application for studying the antioxidant status of aqueous beverages. The assay is based on the fact that the emission of light from the chemiluminescent reaction depends on the constant production of free radical intermediates (Fig. 6). These are generated by the catalytic cycle of the enzyme horseradish peroxidase driven by hydrogen peroxide. The completion of the cycle depends on the reduction of the enzyme intermediates which is linked to the oxidation of the enhancer phenol to phenoxy radicals. This is more favourable than the direct oxidation of luminol. The phenoxy radicals can themselves oxidize luminol to its radical form leading eventually to light emission. Chain-breaking antioxidants such as tea polyphenols are electron donors that can preferentially reduce the phenoxy radicals before their reaction with luminol. In this way the steps leading to light emission are prevented despite the continued generation of oxidizing equivalents by the catalytic cycle. It cannot be excluded that tea polyphenols react directly with the enzyme although the net effect is equivalent: oxidation of the chemiluminescent substrate is prevented.

The simple addition of water at different temperatures to a fixed amount of tea leaves produced predictable results. The rate and extent of the increase in antioxidant activity was closely related to the initial water temperature (Fig. 4). This can be simply attributed to a temperature-dependent release of polyphenolic compounds from the tea into solution. We also observed that the measurement of antioxidant activity tended to be rather erratic at higher temperatures during these experiments. This might be attributed to experimental error. However, we have previously found the of the ECL antioxidant assay to

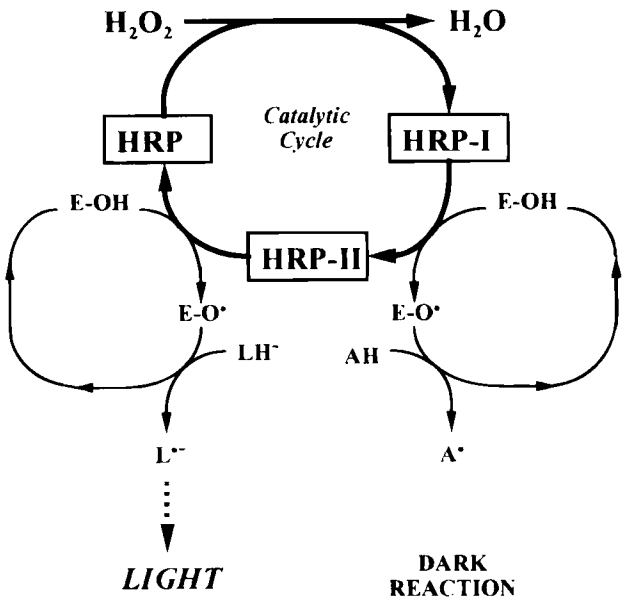


FIGURE 6 The putative reaction sequence leading to light emission in ECL reactions. Oxidizing equivalents are provided when horseradish peroxidase (HRP) is oxidized to Compound I (HRP-I) by hydrogen peroxide ($H_2O_2$). HRP-I is then reduced first to Compound II (HRP-II) and finally to HRP to complete the catalytic cycle of the enzyme. In the enhanced reaction these reductions are linked to the oxidation of the enhancer phenol (E-OH) to phenoxy radicals (E-O•) which is more favourable than the direct oxidation of luminol. The phenoxy radicals can themselves oxidize luminol to its radical form (L•-) leading eventually to light emission. In the process the enhancer is regenerated from its phenoxy radical form. Antioxidants (AH) are powerful reductants that can preferentially reduce the phenoxy radicals before their reaction with luminol. In this way the steps leading to light emission are prevented despite the continued generation of oxidizing equivalents by the catalytic cycle.

be very reliable in other situations with a high degree of precision and little variability.[25,26] A further intriguing finding was that even after removal of the tea leaves (preventing the entry of any more polyphenols into solution) the antioxidant activity could vary dramatically in response to changes in temperature. We hypothesize that the antioxidant activity of tea solutions may indeed be labile at higher temperatures. The chemistry of the polyphenolic compounds in tea is very complex but it is known that during tea manufacture many of them undergo condensation reactions to form more complex derivatives.[23] Many of these reactions are catalysed by polyphenol oxidase. Under the influence of higher temperatures some of the larger compounds may be broken down to smaller ones with the net effect being to release more phenolic groupings available for antioxidant activity. Such reactions may be reversible as the solution cools. We intend to try and resolve this question with further experiments under closer temperature control.

When larger amounts of tea were added to water the rate of increase and maximum antioxidant activity achievable increased (Fig. 5). This result was predictable since more tea leaves allow a greater surface area of contact with the aqueous solution and a greater total polyphenol content. This suggested that higher concentrations of polyphenols could be achieved if more than 2.0 g per 25 ml of water were added. However, few people would wish to drink tea at such high concentrations.

It has been suggested that some antioxidants have a co-operative interaction i.e. their activity on a molar basis is greater at higher concentrations. We found that the concentration-activity relationship of tea was essentially linear at dilutions where it could be compared with the trolox stan-

dard (Fig. 3). Since the activity of tea solutions is so high they were usually diluted 500 times to enable measurement in the ECL reaction system. Therefore, we cannot exclude co-operative interactions at the much higher concentrations found in drinking tea. In contrast to whole tea, the curves for epicatechin and catechin showed a non-co-operative pattern. This can be attributed to the likelihood of catechin radicals (formed during scavenging of radical intermediates in the ECL reaction) reacting with eachother. At low concentrations this is less likely allowing the catechin radical to react with (scavenge) a further reaction radical giving a stoichiometry of two radicals per catechin molecule. At higher concentrations an interaction between two catechin radicals is increased which will tend to allow only one radical scavenged per catechin molecule. It is not clear why a similar phenomenon was not observed with the related polyphenols found in tea.

Measurements of antioxidant activity in a range of commercially available teas under standard conditions showed considerable variability (Table I). The two green teas tested appeared to have the highest activity. This was anticipated since the manufacture of most black teas involves considerable oxidation of the native tea leaf polyphenols which are preserved in green tea manufacture.[23] The 'strength' of the tea rated by the manufacturer based on taste and flavour showed an association with its activity. This was expected since both parameters are influenced by the polyphenol content. Although these popular and commonly consumed beverages are clearly very powerful antioxidants in vitro (up to 30 times the antioxidant activity of human serum) their potential impact on the antioxidant status of human extracellular fluids is less clear. There remains considerable uncertainty about the fate of dietary polyphenols in the gastrointestinal tract and the extent to which they are absorbed and distributed to the tissues.[30] We have previously demonstrated that antioxidant activity in serum is significantly increased by red wine consumption,[31] another rich source of polyphenols. However, in spite of the foregoing observations, we were unable to demonstrate a similar effect in healthy volunteers following the consumption of 500ml of strong tea (without milk)![32] These results have recently been challenged by Serafini *et al.* who did find a significant increase in plasma antioxidant activity following ingestion of both green or black tea.[33]

Since black tea is commonly consumed with milk a further complication is the possible interaction of tea polyphenols with milk proteins and their subsequent inactivation as antioxidants.[27,34] Our results suggest that at higher ratios of milk to tea (1:1) there is a detectable decrease in the antioxidant activity of tea solutions (Table II). This was not found with skimmed milk suggesting that this effect may be related to the lipid fraction. Milk itself has relatively little intrinsic activity. Although such mixtures are not reflective of normal tea consumption this implies that milk could reduce some of the putative beneficial effects of a regular consumption of tea polyphenols. This belief has further support from Serafini and colleagues who suggested that the *in vivo* antioxidant effect of drinking tea was completely abolished when there was concomitant ingestion of milk.[33]

## CONCLUSION

Enhanced chemiluminescence is a simple method for the analysis of the antioxidant activity of solutions of black and green tea. Tea appears to be a rich source of antioxidant activity with comparable activity to red wine and much in excess of normal serum activity. The antioxidant status of tea solutions appears to be rather labile possibly as a result of the chemistry of tea polyphenols in solution. The addition of full-fat milk may have some impact on the antioxidant activity of tea. Further studies are required to investigate whether the antioxidants of tea have a significant impact on antioxidant status in vivo following ingestion.

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