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TEA AND HEALTH W W D Modder A M T Amarakoon

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The Tea Research Institute of Sri Lanka

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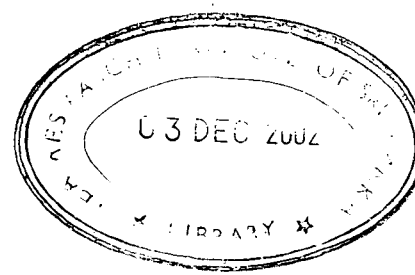
2002



The Tea Research Institute of Sri Lanka

ISBN 955-9023-03-9

"Current Research Shows Tea Contains Specific Antioxidants & Health Promoting Ingredients, Lowering the Risk of Heart Disease, Stroke & Certain Types of Cancer ..."

Dr. John Weisburger,
Director Emeritus
American Health Foundation

From an advertisement issued by the Tea Board,
Government of India (*The Hindu*, 25 September 2001)

DEDICATED

to

those millions

who had not known that tea

might have given them happier and

more fulfilling years

About this Monograph

When we commenced writing this monograph, we were told, with some condescension, that claims for the beneficial effects of tea on those who drank it were not new, and that these claims went back over several centuries. Indeed this is true; but not centuries, rather millenia.

Modern scientific research is now discovering, to a remarkable extent, that those ancient claims regarding tea's health-giving attributes are not merely myths and old wives' tales, but rather that tea could indeed have singular properties that prevent illness and prolong life.

We are glad to have the opportunity to write this monograph on Tea and Health, which has become a subject of pivotal importance, not only to the global tea trade and tea-producing countries including our own, but also to continuing and better health for us all. We have put down, in the pages that follow, scientific information and commentaries on Tea and Health drawn from a variety of sources (papers published and unpublished, presentations and discussions at conferences, and informal discussions) and from the experience of primary research carried out by one of us (A.M.T.A.) in this specific field.

We conceived this book as aimed at the Intelligent Layman who is wondering what all the fuss is about, in this matter of tea and health. However, we should be delighted if professional scientists and medical practitioners also find it of some use, at least to point them at sources and informed opinions.

In case certain of the Chapters prove hard-going to the lay or casual reader, we hope they will skip to Chapter 17 which is

rather more assimilable. It is an overview of the subject, and if they read at least that (of course together with the introductory portions at the beginning), they can still have the essence of the whole.

The monograph was written during January – April, 2002 as part of our Institute's contribution to the Sri Lankan Government's Hundred Days Programme from the Ministry of Plantation Industries.

It had its genesis in a suggestion in 1999 to one of the authors (W.W.D.M.) from Dr R.O.B. Wijesekera, Chairman, National Science and Technology Commission (NASTEC). We are gratified that it has been included, concurrently, as one of NASTEC's contributions to the Hundred Days Programme.

Dr Wijesekera had requested the late Dr R. L. Wickremasinghe, formerly Director, The Tea Research Institute of Sri Lanka (1978-79), to write an introduction to tea for the monograph. This Dr Wickremasinghe very fortunately did, a few weeks before his death in October 1999. The authors consider it a special privilege to include Dr Wickremasinghe's introduction as the Foreword of their monograph.

W. W. D. Modder
A. M. T. Amarakoon

The Tea Research Institute of Sri Lanka
April, 2002

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Of course any errors and omissions are entirely due to the authors themselves.

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Foreword

TEA - A BRIEF INTRODUCTION

Dr R L Wickremesinghe
Formerly Director, The Tea Research Institute of Sri Lanka,
and later Consultant to the Tea Industry

1. THE ORIGINS OF TEA

The word comes from the Chinese Amoy dialect word "t'e" pronounced "tay", which in the Cantonese dialect was rendered "cha". Tea is very much taken for granted by the millions of people in the world who sip their "cuppa" with no thought given to its fascinating history dating back to several centuries, or to its unique chemical composition. The discovery and origin of tea is generally attributed to the Chinese Emperor Shen Nong who, in 2737 BC, is said to have observed that a brew of fresh tea leaves had a wonderful stimulant effect. He studied its properties further and in "The Herbal Canon of Sheng Nong," claimed that tea was able to detoxify 72 different kinds of poison. It was considered to be a valuable commodity and became so much in demand that in 1066 BC, at which time tea was apparently used half for medicinal purposes, people living in South West China were required to include tea as an item of tribute payable to the Emperor. The ecology of Tea trees was described in detail in the Erh Ya published in 130 BC, at which time tea was used both for medicinal purposes as well as a social drink. Recently excavated tombs in the Yunnan province of China also revealed that tea was one of the items included in the list of burial objects.

Records indicate that tea was initially restricted to the Yunnan province of South West China until about the 5th Century BC. In this connection the discovery was reported in 1961 of a 1700-year-old tea tree, growing wild in the Menghai country of that province. This tree which were 21.1 meters in height and 1.03 meters in diameter still produced leaves, which could be processed to quality tea. From Yunnan tea was disseminated to other provinces of China, and the use of tea was encouraged by the spread of Buddhism in China, and the edict of the Imperial Court that tea should replace the use of wine. It was during this time (780 AD) that Lu Yu published his famous classic "Cha Ching", wherein he extolled the virtues of tea. By the end of the 13th century major tea plantations were widely dispersed in South East China too and tea was now a sought after commodity. It was reported in 1398 AD that the barter price of 250,000 kg of tea was 13,584 fine horses, which were scarce in regions of inland China. By this time, tea had become a necessity of life in China, and people would rather go without rice for three days rather than forego tea for a single day.

2. THE SPREAD OF TEA

The spread of tea from China to other parts of the world is said to have begun as early as 221 BC, with the migration of minority nationalities to Vietnam, Burma, Laos and Thailand. These migrations were the result of wars which were common at the time. Although these migrations occurred so many centuries ago, the methods of processing have not undergone any significant changes from those used in ancient China.

During the 5th Century AD the custom of tea drinking became widespread as a result of the establishment of trade ties between

China, Istanbul, Rome, Arabia, Iran, India, Afghanistan, Pakistan, Korea and Japan. It was at this time that trade on the famed Silk Road reached zenith. The opening of sea-lanes led to further expansion of trade with China, and during the 7th century AD tea ceremonies were held first in Korea and later in Japan. Shortly after, tea seeds and the techniques of tea cultivation were transferred to Japan and Korea.

3. THE TEA HABIT

Earliest records of tea in the West are the diary (850 AD) of an Arab traveller, Soliman, and two books (559 A.D.) by the Venetian writer, Giambatista Ramusio. However, the first export of tea to then coffee drinking Europe occurred only in 1610, and it was not until 1657 that the first tea packet from China was sold in Galway's Coffee House in London - where the beneficial effects of drinking tea were eulogized. Subsequently in 1684, J.I.L.L. Jacoboson, a Dutch tea taster was able to penetrate the well guarded and forbidden tea gardens of China and, at great personal risk, succeeded in transferring tea seeds to Indonesia - and so was broken the monopoly of China in tea production.

Soon after tea drinking became a very popular, but expensive habit. The first authenticated attempt to commercially cultivate tea in India followed the discovery by Major Robert Bruce, in 1823, of tea growing wild in the Sibsagar district of Assam, whence it found its way in 1839, to the Botanical Gardens in Peradeniya. Thereafter the story is well known how James Taylor demonstrated at Loolecondera Estate, Hewaheta, Kandy, that tea cultivation was the answer to the devastation of the island's coffee plantations by the coffee rust. The Assam variety of tea discovered by Robert Bruce, known botanically as *Camellia sinensis* var.

assamica soon replaced the Chinese variety designated as *Camellia sinensis* var. *sinensis*. It was also soon found that the Assam variety, was suitable for processing to fermented black tea, whereas the *sinensis* variety was more suitable for processing to unfermented green tea. The fundamental difference between black tea and green tea processing is that the former gives full rein to the activity of the oxidizing enzyme present in the tea leaf, whilst the first step in green tea production is the inactivation of this enzyme by heat, which may be dry heat (parching) as in China or moist heat (steaming) as in Japan. Furthermore the chemical compositions of the Assam and *sinensis* varieties are distinct and it would be inadvisable to process the former to green or the latter to black tea. Apart from fully fermented black tea, and unfermented green tea, there is also a third group which merits special mention viz. Semi fermented teas, of which oolong tea is the most popular, especially in Taiwan. It is unfortunate that the advantages of oolong tea are not exploited in Sri Lanka. These advantages include solubility in ice cold water, an acceptable flavour, and the health benefits of both black and green tea — it is tailor made for the production of canned tea. A further group of tea is reprocessed or scented tea where the natural flavour of tea is enhanced by the incorporation of flowers (e.g. jasmine) or other extraneous additives (e.g. lemon, oil of bergamot, cinnamon oil etc.). The tea produced throughout the world even under similar processing conditions differ from each other, depending on the clone of tea, climatic and soil conditions, method of cultivation, fertilizer applied, nature of shade, type of processing machinery used etc. In fact the types of green tea and black tea are so numerous that they bring to mind the remark made by Lu Yu in 780 AD in his aforementioned classic that "there are a thousand and ten thousand teas". Of those black tea is the

CHAPTER 1

A History of Tea as Medicine and Beverage

1.1 Tea: Origins and Present Demand

The name, tea, derives from the Chinese ideograph *ch'a*, which came into use about 725 A.D. The plant belongs to the genus *Camellia* (family Theaceae), and in addition to the "true" tea species, *Camellia sinensis* (L.) O. Kuntze, the genus also contains nearly 90 wild and ornamental non-tea forms. The genus appears to be native to the south-east Asian land mass, and the "true" tea species to south-western China from where it spread to central China and southern Japan 1,000–2,000 years ago.

The earliest record of tea cultivation and consumption is found in ancient Chinese literature dating back to 1100 B.C. As it is still drunk today, tea was taken as an infusion after the leaves had been brewed in hot water. Tea consumption evolved into a part of Chinese religious symbolism and culture (indeed the first monograph on tea, about 780 A.D., was called *Ch'a Ching*, 'Tea Scripture'), and continues to this day in the elegant and ornate

tea ceremonies of far-eastern countries. Made green tea was brought to Japan in the thirteenth century by Zen Buddhist monks returning from China, and *Chado*, the 'Way of Tea' (the discipline of tea preparation and consumption), arose in the fifteenth century. These rituals, still extant, have distinct reverential and aesthetic connotations.

Today many so-called herbal 'teas' are retailed around the world, but only the terminal shoots of *C. sinensis* are used for manufacturing the ubiquitous and singular product, tea *sensu strictu*, the 'true' tea that feeds a huge global demand. World tea production was 2,903 million kilogrammes in 2000, and it is estimated that 3.5 billion cups, glasses or bowls of tea are consumed every day worldwide at the present time.

It is the 'true' tea that has been found, by independent scientific studies in tea-producing and non tea-producing countries alike, to have a remarkably beneficial effect on human health.

1.2 The Earliest Use of Tea in China and Japan

According to Chinese legend, this drink, the cheapest after water and consumed now in such prodigious quantities, was discovered when leaves from a tea tree fell by accident into drinking water intended for the emperor, Shen Nung, in 2737 B.C. The emperor, finding the resulting brew more pleasant to drink than hot water, came in time to believe that it had beneficial effects on health, and recommended it as a remedy for diverse ailments, "for kidney trouble, fever, chest infection and tumours that come about the head". In the light of recent research, this is amazingly perspicacious.

Tea is said to have been used in prehistoric times, in north-western China, as medicine and food (Sheng, 2001). Reference

most widely consumed (about 3 million tones), followed by green tea (about 500,000 tones). In addition similar quantities of instant tea (about 700,000 kg.) are produced in few countries, including Sri Lanka.

4. VALUABLE CONSTITUENTS OF TEA

Mention has already been made of the oxidizing enzyme present in the tea leaf and its pivotal value in differentiating black tea from green tea. In addition to the enzyme, tea leaves also contain an interesting compound, known as theanine, which is not found in any other plant in the world. Its unique occurrence in tea leaves plays an important role in tea chemistry and serves to distinguish tea from all other herbal beverages. Tea also contains other compounds of interest - a sterol known as spinasterol, the carotenoid rhodaxanthin and the depside theogallin - which though not unique to tea, are an indication of its early evolution in the vegetable kingdom. Tea is certainly a plant which originated several millennia ago in biological terms. However its commercial use from several centuries ago and the fact of its continuing popularity as a beverage is due not only to its pleasant taste and stimulant properties, but also to its many beneficial effects. To mention a few, tea has been found to be anti-carcinogenic and to possess anti bacterial, cholesterol lowering, rejuvenating and anti-diabetic effects. Tea also alleviates the problems of hypertension and plays an important role in the prevention of tooth decay. In addition tea root contain a high levels of saponins in combination with a high level of the amino acid, theanine, suggesting that tea root could yield a herbal hair shampoo with unique cleansing deodorizing and freshening properties. Recently Indian workers have reported that tea root extract possesses anti- carcinogenic activity. Immature tea seeds

have been found to contain useful amounts of the valuable pipeolic acid. Besides providing a refreshing beverage, tea leaves are also a source of useful food dyes, and spent tea leaves contain protein at levels which make it feasible for incorporation into animal feed. Tea has also been used as an ingredient of cooked foods, cosmetics and even for stuffing pillows. It has been processed to various canned teas, tea candy, tea wines, tea jelly and a high fibre tea drink. It is evident that the narrow image of tea as an invigorating and healthful beverage needs to be revised.

These and other aspects will be dealt with in greater detail in other chapters of this presentation.

Note by Dr R.O.B. Wijesekera:

Dr R.L. (Robbie) Wickremesinghe, unfortunately, died a few weeks after he had presented this overview to NASTEC. His dedication to his duty and the subject which was his lifelong interest was exemplified in this final act, where he characteristically fulfilled his commitment. He was to depart for a holiday with his wife to be with his daughter and son-in-law in Dhaka, but fate deprived him of this. This simple dedicated scientist made a great contribution to the world's knowledge of Tea and to his country's tea industry, for which he received the nation's highest recognition for science - the Presidential Award.

to its use as an antidote in the New Stone Age in China is found in literature on the legendary Shennong: "Shennong tastes hundreds of herbs, meets 72 toxins everyday, counteracts them by tea" (Dexian and Yongming, 2001).

Tea is on record as having being used in China in 500 A.D. primarily as a medicine. The date of its first use as a beverage in Chinese upper-class society is given as 589 A.D., at the beginning of the Sui Dynasty. More than three centuries later, during the After-Leang Dynasty (907–923 A.D.), the practice spread to the lower classes.

In Japan, in 951 A.D., the beverage was used against the plague. Here again, although the ornate, quasi-religious Tea Ceremony was initiated in Japan towards the end of the Heinan period, about 1159 A.D., the Ceremony was brought within the reach of the Japanese middle classes over four centuries later, about 1582 A.D., by the high priest Sen-no Rikyu.

Thus tea was, in east Asia (or the "Far East" in Euro-centric nomenclature), in the first instance in therapeutic use, and then for many centuries, only a drink for emperors, kings, nobles and rich men. Only much later did it become a part of the diet of ordinary folk.

1.3 The Use of Tea in Western Europe

Tea was discovered by Europeans travelling to the Far East during the 1500s and early 1600s, and later became restricted to the nobility and the rich in western Europe owing to its great cost. In the 1600s and 1700s, tea was assiduously imported from the Far East and highly prized. As in the Far East, in Europe too it was considered to have medicinal properties. In the European Age of Enlightenment, tea like tobacco and chocolate, all brought

in from the new colonies in the wondrous places of the world, became one of the fads and status symbols of the ruling classes, the *literati*, and the *nouveau riche* merchant class.

However, it was not without its detractors. In 1635, a German physician called Simon Paulli wrote against the immoderate use of tea and tobacco which, with our advantage of hindsight, seems sound advice in respect of tobacco but not so in respect of tea. In 1648, a learned doctor in Paris, Guy Patin, wrote that tea was "the newest impertinence of the century", and reported that a favourable account of tea by a young doctor called Morriset had been violently condemned by the medical profession.

Tea-drinking became a fashion in London soon after 1662, when the new Queen of Charles II, the Portuguese Catherine, insisted on tea rather than beer on first arriving in the capital of England (Young, 2001). The great maritime nation of the time, Portugal, who dominated the global trade routes, appeared to have been more advanced than Great Britain, in having knowledge of tea and preferring it to beer. Tea replaced beer and ale as the national drink of England during the 1700s, although at the present time this status has been doubted (albeit in a tête-à-tête with one of us) by no less than the President of the U.K. Tea Council.

The British East India Company, which monopolised the country's trade with the East, began to import tea commercially and on a large scale, beginning in 1678 (Young, 2001). A pound of tea (about 0.45 kg) then cost more than a skilled craftsman might earn in a week. Both green and black teas were drunk, the black being much cheaper.

From a medicinal concoction, tea became a symbol of wealth and lavish hospitality. The British developed formalised social

and domestic tea rituals of their own, both in the mother country and in the far-flung outposts of Empire, in the late 1600s and early 1700s. These were comparable to the much earlier east Asian tea ceremonies. British tea-drinking rituals have survived to this day, as in Morning and Afternoon Tea, and as the 'Tea Break' in the stately flow of the British game of cricket, now continued with even more distinction in the ex-colonies.

In 1657, tea was first sold to the public in Thomas Garraway's Coffee House in London as a medicinal drink.

According to Garraway:

> "..... It maketh the body active and lusty
>
> "It helpeth the Headache, giddiness and heavyness thereof
>
> "It removeth the obstructions of the Spleen
>
> "It is very good against the Stone and Gravel, cleaning the kidneys and Uriters, being drank with Virgins Honey instead of sugar
>
> "It taketh away the difficulty of breathing, opening obstructions
>
> "It vanquisheth heavy dreams, easeth the brain and strengtheth the Memory....."

In the same year, 1657, a Frenchman Dr Jonquet described it as "the divine herb" and compared it to nectar and ambrosia. This indicates that there was now rather more acceptance of it in medical circles in France. A similar eulogy had been made earlier, in 1641, by a Dutch doctor of medicine, Nicolas Dirx yclept "Nicolas Tulp", in his treatise *Observationes Medicae*. In 1679, another Dutch physician Cornelis Bontekoe published a commendatory tract on tea.

The shifting views of the medical profession aside, there were lay writers who praised it. Thomas Garraway's broadsheet, a famous London "newspaper" of the time, published the "Vertues" of tea in 1660, the year when Samuel Pepys recorded in his celebrated Diary the drinking of his first cup of tea. In Genoa, Simon de Mollnaris published *Asian Ambrosia, or the Virtues and Use of the Herb Tea* in 1672. In similar vein, in London, John Ovington, an English churchman, published in 1699 his *Essay upon the Nature and Qualities of Tea.*

However, the prominence of tea in the society of the time attracted the attention of some who presumably regarded it as witches' brew. John Wesley, the English religious reformer and preacher, who founded the Wesleyan or Methodist Christian denomination, exhorted his flock, in 1748, to stop drinking tea, rather as he would have exhorted them against the demon alcohol. Jonas Hanway, described as an English merchant and philanthropist, wrote a bitter denunciation of tea in 1756, to which the celebrated Dr Samuel Johnson, in 1757, responded in like manner, on behalf of tea, "with a broadside of ridicule".

To Dr Johnson, tea was not for the 'lower classes'. Its "proper use" was "to amuse the idle, and relax the studious, and dilute the full meals of those who cannot use exercise, and will not use abstinence" (Young, 2001). In 1743, again apropos of the 'lower classes', another patrician complained that tea had become "so common, that the meanest families, even of labouring people ..... make their morning's meal of it, and thereby wholly disuse the ale, which heretofore was their accustomed drink."

All this notwithstanding, far-eastern and British rituals have survived and transmuted into the widespread, modern habit of drinking tea, either hot or cold, with or without milk and sugar, as a pleasant, relaxing and beneficial beverage.

CHAPTER 2

Awareness of the Link Between Tea and Health

2.1 Confirming the Ancient Wisdom

The possibility that tea consumption is beneficial to human health has become a subject of serious scientific and medical enquiry only comparatively recently, over the last two decades. Arising from the accumulation of data in independent laboratories around the world, it would seem that inveterate tea drinkers have a lower risk of contracting a remarkably wide array of chronic diseases that includes cancers, coronary heart disease, stroke, diabetes, osteoporosis, liver ailments, bacterial and viral infectivity. Most of the conditions that tea is effective against are the so-called "modern" diseases of a degenerative, progressive nature, which arise from changing life-styles, including chronic subjection to stress patterns, and an increasingly polluted environment.

Contemporary research is confounding the sceptics (who now

seem to be diminishing with every new finding), and confirming what appears to have been the virtually instinctive knowledge of the ancient users of the tea plant: that the components of tea brew is not only beneficial in a general way, but could more specifically stave off diseases and even cure them. We are literally watching myth and mysticism being transmuted into modern scientific know-how.

Primarily because the initial research studies originated, about 1970, in the Far East where green tea is consumed as a matter of course, it was suggested that this type of tea, and the compounds called catechins they contain in large amounts, were alone beneficial to health. With the extension of research, from about 1985 and in other parts of the world, to black tea which comprises about 80 per cent of global tea consumption, it has now become clear that black tea and green tea are not at all different in their health-promoting attributes. Thus, they both decrease the risk of heart disease and many kinds of cancer (Weisburger, 1999 a).

2.2 Flavonoids: Tea's Magic Bullet

A class of polyphenols, phytochemicals or chemicals found naturally in plants, and known as flavonoids, are found in high concentrations in fruit and vegetables, but particularly in black and green tea and red wine.

Modern research shows that flavonoids are protectants against several chronic diseases and conditions because of their antioxidant and anti-inflammatory properties. Exhaustive studies carried out in independent research centres in different parts of the world, over the past decade, have shown that flavonoids are the most effective antioxidants found in nature.

Epigallocatechin gallate (EGCG), a flavonoid present in the tender shoots of the tea plant from which tea is made, is said to be one of the most powerful antioxidants known.

2.3 Conferences on Tea and Health

The results of the new research on tea and health, that had been carried out from about 1985, was presented in 1991 at the First International Symposium on Tea held in New York in the U.S. (Weisburger, 1999 a). These results came from three types of study: epidemiology; laboratory studies in biochemistry, physiology and pharmacology with animal models; and *in vitro* studies on the chemical compounds present in tea.

Other conferences followed, mainly in the U.S. and Japan, either wholly or in part on tea. In India, international tea conferences during this period invariably incorporated papers on the health aspects of tea (thus, in Calcutta in 1993 and in Delhi in 1996). In our own country, in August 1997, a Tea and Health Seminar was organised by the Sri Lankan Tea Board in Colombo where there was eminent foreign participation.

The Second International Scientific Symposium on Tea and Human Health was held in Washington, D.C. in 1999. It is described by Ringer (1999) of the American Cancer Society as a "balanced presentation of science", identifying new cell functions affected by tea components, and giving new information on the ability of antioxidants in tea to reduce oxidative stress (for definition, see Section 8.3.2).

As summarised by Weisburger (1999 a), of the American Health Foundation and a prominent, independent researcher on tea and its effects, this Second Symposium documented clearly that the earlier assumptions regarding the health benefits of tea were correct.

Epidemiological studies showed that tea drinkers had a lower risk of heart disease. Data was presented to demonstrate that the powerful antioxidants in tea may decrease the oxidative processes associated with the formation of arteriosclerotic plaques in the vascular system and heart.

As regards research on tea and cancers, exacting and reliable animal models to mimic the human system are available, and the use of these, together with epidemiological work, confirmed a lower risk of cancer of the stomach and oesophagus in China in regular consumers of green tea. Most of the presentations at the Second Symposium were on the mechanisms of action of tea antioxidants in several reliable animal models. Tea was shown to have a powerful preventive action on cancers of the oral cavity, oesophagus, stomach, colon, lungs and skin in mice and rats. With tea, there is a lower rate of cell proliferation decreasing the development and growth of cancer cells, but more importantly decreasing the growth of early cancer types such as those on the skin and the mammary glands. Weisburger believes that eventually these remarkable findings may mean a lowered risk of cancer in humans, and prove useful not only in primary cancer prevention, but in adjuvant cancer therapy.

Before 1999, however, the epidemiological studies had not yet revealed that regular use of black tea, as compared with green tea, reduced the risk of cancer common in western countries. Epidemiological work evaluated after 1999 indicates that black tea is effective as well.

At the First Symposium, the suggestion was strong that the chemical structure of polyphenols, the complex ring systems, would result in tea polyphenols passing unabsorbed through the intestinal tract. The Second Symposium laid these fears to rest:

new work and reviews of older work showed clearly that tea polyphenols are absorbed.

Weisburger (1999 a) believes that there is an adequate body of knowledge on the beneficial action of tea components, as demonstrated at the Second Symposium and in scientific reviews world-wide, and that this knowledge could be used in public health promotion.

The knowledge presented at the Second Symposium has been encapsulated in a special issue of the Official Journal of The Society for Experimental Biology and Medicine (Volume 220, Number 4, April 1999). This issue forms a valuable resource base and starting point for future researches on the associations between tea and health.

Similarly, the Proceedings of the 2001 International Conference on O-Cha (Tea) Culture and Science held in Shizuoka, Japan in October are a comprehensive and up-to-date source of valuable and interesting information.

This present review makes use of these and other sources but since our review is primarily intended for the intelligent layman, we recommend that professional scientists carrying out, or interested in commencing, research on tea and health consult these sources directly and in more detail than we are able to do here.

2.4 The FAO Project on Tea and Health

Since the first research began in the 1970s on the association between tea consumption and health, the evidence for such a link escalated remarkably, until in the mid-1990s the need was felt for serious coordinated research on the subject, by independent, disinterested scientists in state-of-the-art laboratories across the world.

An international project for such research, coupled to the generic promotion of tea, was organised under the aegis of the FAO with a fund of US $ 4.61 million contributed by the Common Fund for Commodities, the tea trades of the U.K., the U.S.A. and Canada, and four major producing countries, namely India, Indonesia, Kenya and Sri Lanka. The first phase of the project was independent scientific research, in demarcated subject areas, to stringently examine and confirm the health benefits of tea, and the second was to mount a promotional campaign as more and more positive evidence emerged from the research. Whether its positive health attributes could be used to promote tea was to be tested in four, widely differing markets: in Europe (Catalonia in Spain and the Czech Republic), in Africa (Zimbabwe) and in Asia (East Java in Indonesia).

The successful outcome of the project was reported to the FAO's Intergovernmental Group on Tea in September, 1999 in Ottawa, Canada. A logo showing a tea cup with the strap line "Tea – Discover the Goodness" was devised and registered under the name of the FAO.

2.5 Public Awareness of Tea

As a result of information in television and radio broadcasts, and in articles in popular papers and magazines, people in general are becoming increasingly aware of the dangerous entities (the 'bad guys') in their bodies called free radicals (FRs), and their destruction by antioxidants (the 'good guys'). They are mostly aware now that both black and green tea, indeed tea of all types, are rich in polyphenols, which are highly effective antioxidants, and that tea's health-giving and beneficial effects have been scientifically and medically upheld by the remarkable convergence of independent research in different countries.

CHAPTER 3

The Chemicals in Tea Shoots and their Health Effects

3.1 General Composition

The tender shoots of tea, comprising two or three of the topmost, immature leaves and the bud, referred to as the 'flush', are harvested or plucked for processing into 'made tea'. The water-soluble chemical constituents of the tender shoots, and their water-soluble derivatives obtained during the processes of manufacture, are responsible for the taste and quality of brewed tea. The soluble and insoluble chemical constituents in tender shoots of tea are given in Table 1.

TABLE 1

The Molecules in Tender Tea Shoots

Cold water-soluble	g/100 g dry weight
Flavanols: Epigallocatechin gallate (EGCG)	9 – 13
Epigallocatechin (EGC)	3 – 6
Epicatechin gallate (ECG)	3 – 6
Epicatechin (EC)	1 – 3
Gallocatechin (GC)	1 – 2
Catechin (C)	1 – 2
Flavonols and their glycosides	3 – 4
Leucoanthocyanins	2 – 4
Phenolic acids	4
Total polyphenols	**27 – 40**
Caffeine	3 – 4
Amino acids: Theanine	2
Others	2
Carbohydrates	4
Organic acids	0.5
Volatile compounds	0.01
Partially hot water - soluble	
Polysaccharides: Starch	2 – 5
Other	12
Protein	15
Ash (inorganic material)	5
Water-insoluble	
Cellulose	7
Lignin	6
Lipids	3

(after Hilton, 1973)

3.1.1 Does Tea cause Kidney, Bladder and Gall Stones?

The organic acids in tea include oxalic acid, and tea brew therefore contains relatively high amounts of oxalate. Kidney and bladder stones are formed mainly of calcium oxalate, but there is no danger of tea-drinking resulting in the formation of these stones because the absorption of tea oxalates by the digestive tract, or their bioavailablity, is known to be low. Thus, the bioavailability of oxalates from tea brew, and from tea with added milk, were found to be 0.08 and 0.03 per cent, respectively (Brinkley *et al.*, 1990).

Caffeine in tea contributes to increased calcium excretion (Massey and Whiting, 1993), but this has not been linked with stone formation in the kidneys or bladder. Caffeine is a mild diuretic, and the increase in urinary volume may contribute to a flushing effect in the kidneys, and so diminish the risk of the formation of kidney and bladder stones.

Studies have not found any association between stone formation and tea consumption. Rather, an increased fluid intake in the form of tea would be beneficial in reducing the risk of stone formation (Borghi *et al.*, 1999; Curhan *et al.*, 1998).

Since caffeine increases calcium excretion, studies have also been carried out to determine if there is a link between caffeine consumption and the onset of osteoporosis, which is a condition resulting from the withdrawal of calcium from the bones. No association has been found between moderate caffeine intake and osteoporosis (Hegarty *et al.*, 2000).

On the other hand, those who have already suffered from kidney, bladder or gall stones are advised not to drink more than three cups of tea per day, as there could be a risk of recurrence.

3.2 Polyphenols

The most abundant class of chemical compounds in fresh tea flush is the polyphenols (made up of many phenol molecules). The phenol molecule has a single hydroxyl (-OH) group attached to an aromatic ring; the polyphenols have a number of these –OH groups.

Of the polyphenolic categories, the flavonoids are the most abundant in tea flush. Flavonoids are 2-phenyl benzopyran-based compounds, classified into six groups: flavones, flavanones, isoflavones, flavanols (including catechins or flavan-3-ols), flavonols and anthocyanidins.

Flavanols and flavonols are the main components in fresh leaf. Catechins (flavan-3-ols) are the major flavanols in tea (Table 1: EGCG, EGC, ECG, EC, GC and C), and they undergo oxidative dimerisation or polymerisation during black tea manufacture.

Flavonols (such as quercetin, kaempferol, myricetin and their glycosides) and anthocyanidins are also found in the flush in appreciable amounts, but they do not undergo much change during black tea manufacture.

The tea leaves contain two enzymes, polyphenol oxidase and peroxidase, which are involved in the oxidation of polyphenols.

3.3 Vitamins and Minerals

In addition to the components shown in Table 1, tea leaf contains vitamins and several minerals. Vitamin C is lost during the processing of the fresh tea leaf, but carotenoids and vitamin K are present in made tea in amounts that would be significantly useful in the human body.

The mineral components include potassium, aluminium, manganese and fluoride.

3.3.1 Is too much Aluminium taken up from Tea?

The tea plant, especially its leaves, accumulate aluminium. As a result, there was a school of thought that believed tea consumption would result in an excessive intake of aluminium. However, it has been found that the accumulation of aluminium is several times more in the mature tea leaves, than in the tender parts of the shoot, which are used for manufacturing tea.

Anandacoomaraswamy and Sivapalan (1990) reported that tea brews prepared from various Sri Lankan, Indian and Chinese teas (2.5 g in 125 ml) had an aluminium content ranging from 1.6 to 7.1 mg/l (average value: 4.2 mg/l). The comparable values for 13 commercial brands available in the U.K. were 2.2-4.5 mg/ml (Baxter *et al.*, 1990), and for Japanese tea infusions 1.49-5.58 mg/l (Matsushima *et al.*, 1993).

A study with 12 healthy volunteers showed that the consumption of aluminium in tea, up to a level of 1.6 mg, does not influence aluminium levels in the serum, when compared with the consumption of 0.001 mg of aluminium in water (Drewitt *et al.*, 1993). Powell *et al.* (1993) have shown that only 5 per cent of the aluminium in tea is available for absorption from the small intestine.

These studies suggest that tea drinking does not contribute significantly to an increase of aluminium in the body.

CHAPTER 4

The Process and Chemistry of Tea Manufacture

Although a large number of types of tea, many showing only subtle differences, are manufactured from the tender terminal shoots of *Camellia sinensis* in the various tea-producing regions of the world, teas can be grouped into three main types: black, green and Oolong teas. In each case, the manufacturing methods employed are different.

At the start of the manufacturing process, shoots are either withered (for making black tea), or steamed or subjected to dry heat (for green tea).

4.1 Black or Fermented Tea

4.1.1 Withering

Batches of shoots are spread out on tats or netting, or in troughs on mesh, for withering, in layers less than 30 cm thick. In trough withering, fans are used to force air through the shoots

in order to prevent a temperature rise caused by internal respiration of the tissues, and to reduce the moisture content of the leaf to 50-55 per cent, if necessary by pre-heating the air stream depending on the prevailing relative humidity.

Withering is generally restricted to 6-20 hours, but most of the chemical changes occurring then are completed in 6-10 hours. These changes include modification of the catechins, and the release of chemicals called 'flavour compounds', amino acids and caffeine. The permeability of cell membranes increases during withering, allowing smaller molecules and ions to pass through them.

During withering, the activity of the enzyme, polyphenol oxidase (PPO), increases two- to three-fold, mainly owing to its renewed synthesis. (PPO catalyses oxidative changes to the polyphenols, or catechins, in the following stage of manufacture, rolling.)

An increase of free amino acids occurs owing to the enzymic breakdown of proteins by proteases. These amino acids are precursors of 'aroma compounds' that develop in subsequent stages of processing.

Caffeine is also synthesised during withering, the last step in the synthesis being the enzymic methylation of theobromine to caffeine. Withering therefore influences the amount of caffeine in black tea.

Simple carbohydrates or sugars increase in quantity owing to the breakdown of more complex carbohydrates. Increases in organic acids also occur.

4.1.2 Rolling

Moisture reduction during withering prepares the leaf for the next stage of manufacture called rolling. With a reduced water content, the leaf juices tend not to run out when the leaf is macerated or cut during the rolling process, and the essential components of the juice are retained in and on the surface of the leaf particles.

The withered leaf is either broken up or macerated in machines called rollers (in the 'orthodox' manufacturing process), or cut into minute particles of less than one mm, in a CTC (or Cut, Tear and Curl) machine (in the 'CTC' process).

These macerating and cutting processes allow the mixing and reaction of the substrates, the polyphenols, present in the leaves' palisade cells, and the PPO enzyme system, present in their epidermal cells, which initiate the production of molecules characteristic of black tea.

In severe maceration methods which give smaller particles, the oxidative reactions take place at a faster rate owing to a more thorough mixing of the reactants, and quicker and easier access of atmospheric oxygen to reaction sites within the particles.

4.1.3 'Fermentation'

In this stage, the macerated leaves (called 'dhool') are laid on 'fermenting' tables for 1-3 hours, to allow the reactions initiated during rolling to progress to the extent desired. The colour of the dhool gradually changes from green to black as the reactions progress. Its aroma also changes owing to the formation of so-called 'aroma compounds' from precursor molecules.

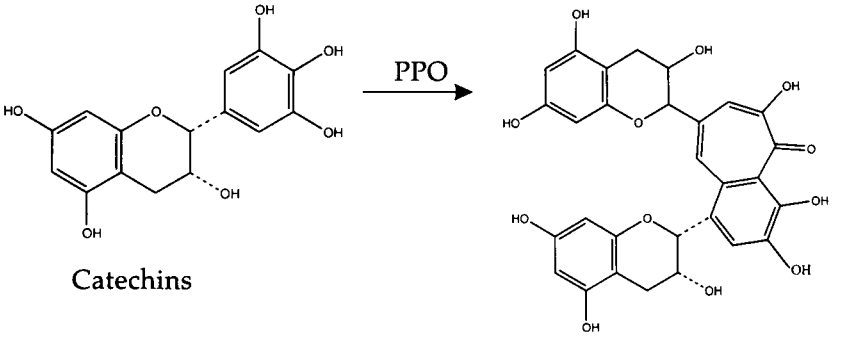
The polyphenols in the dhool (specifically the flavanoids or catechins) are oxidised by atmospheric oxygen, with the PPO acting as catalyst, and more polyphenol units (or monomers) are added on to the oxidised polyphenols to form dimers (two monomers) or polymers (more than two monomers).

Athough the chemical process involved is polymerisation by oxidation, it is incorrectly referred to in hoary tea parlance as 'fermentation'. The use of this term sometimes lead to a confusion with alcoholic fermentation, 'fermented' or black tea being considered erroneously by some as containing alcohol, and therefore perhaps taboo. 'Unfermented' or green tea may, on the other hand, be considered acceptable to non-alcohol consumers because it is regarded as non-alcoholic. In point of fact, neither black or green teas as usually manufactured are alcoholic.

Although originally thought to be the case, micro-organisms are not involved in the 'fermentation' process.

Di- and polymerisation of polyphenols, or more correctly of the catechins, result in simple dimeric, orange-red theaflavins (TFs) and more complex, predominantly polymeric, dark-brown thearubigins (TRs) (Fig. 1).

FIGURE 1
Conversion of Catechins to Theaflavins and Thearubigins



Catechins

Theaflavins
+
Thearubigins

This is the major biochemical change taking place during black tea manufacture. Over 80 per cent of the catechins in black tea is oxidised or 'fermented', and the resulting mixture of dimeric and polymeric pigments, TFs and TRs, may be referred to as oligomeric polyphenols.

No individual structures have been elucidated for TRs as yet, although the involvement of hydrolysable strictinin in the TR structures has been suggested (U.H. Engelhardt, 2001, unpublished).

TFs and TRs contribute mostly to the taste and character of black tea, and also to a large extent to its health-giving attributes.

4.1.3.1 The Efficacy of TFs and TRs as Antioxidants

It used to be thought, until relatively recent research showed otherwise, that the antioxidant activity, which makes tea beneficial to health, was due primarily to catechins in green teas. However,

it is now known that the TFs, found exclusively in black teas, are equally effective antioxidants, and even more beneficial as a counter to the onset of certain health conditions.

The antioxidant and anti-mutagenic activities of TRs have also been demonstrated by Yoshino *et al.* (1994) and Catterall *et al.* (1998), respectively. TRs reduce the damage to DNA by chemical carcinogens (Lodovici *et al.*, 2000; Gupta *et al.*, 2001).

Leung *et al.* (2001) showed that TFs possess the same antioxidant potency as catechins present in green tea, and that the conversion of catechins to TFs during black tea manufacture does not significantly alter FR-scavenging activity. They did this by comparing the antioxidant activity of the green tea catechins, EC, ECG, EGC and EGCG, and the black tea flavonoids, theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin-3,3'-digallate, using human LDL oxidation as a model.

4.1.4 Drying or 'Firing'

In this stage, the moisture level is brought down to only 3-4 per cent, using hot air in a machine called a drier. Reduction of moisture at this stage is essential for the keeping qualities of made tea.

At the beginning of drying, the reactions taking place in the dhool are accelerated owing to the gradual increase in temperature. However, the heat-sensitive enzymes are soon deactivated by the heat, and the reactions initiated in the fermentation stage are stopped. In the drying process, most of the undesirable low-boiling volatile compounds are removed, yielding a tea with better aroma.

Seasonal stress conditions in the field (bright days, cold nights and dry desiccating winds) increase the production of chemicals

in the growing tea plant that give a desirable flavour and aroma (called 'seasonal quality') to manufactured tea.

Carotenes and amino acids in the leaf are thought to produce so-called 'flavour' or 'aroma' compounds during manufacture, by reacting with the oxidised polyphenols, early in fermentation. However, this may not be the only mode of production of these compounds. Carotenes can be thermally degraded to one type, ß-ionones, during the drying or firing stage of manufacture. Others such as geraniol, linalool and its oxides, and methyl salicylate, present in the leaf as glycosides (that is, coupled with one sugar molecule) or as primeverosides (coupled with two sugar molecules), undergo changes throughout processing (the withering to the drying stages). The final 'aroma complex' comprises about 600 compounds.

4.1.5 Grading and Storage

The grading stage is solely a mechanical sorting of manufactured (or made) teas, according to particle size by sieving, into different 'grades'.

Made tea, which is hygroscopic, is best stored in sealed containers to prevent moisture absorption. Moisture increase, resulting from unsuitable packaging and storage, leads to the growth of micro-organisms, certain of which would be pathogenic, producing dangerous toxins such as aflatoxins. Micro-organisms multiply to undesirable levels in made tea at moisture levels of six per cent and over.

Moisture and high temperatures during storage could also break down some of the lipids in made tea. Both micro-organisms and lipid break-down result in an inferior, or 'off ', taste.

Proper packaging and storage, even under household conditions, are important to derive the best benefit from tea consumption.

4.2 Green or Unfermented Tea

In the manufacture of green tea, initial steaming of the leaves, or dropping them into a heated pan for a short time (pan firing, by dry heat), denatures and makes the polyphenol oxidase inactive at the very outset, and therefore no fermentation occurs. Green tea is thus rich in monomeric polyphenols or catechins.

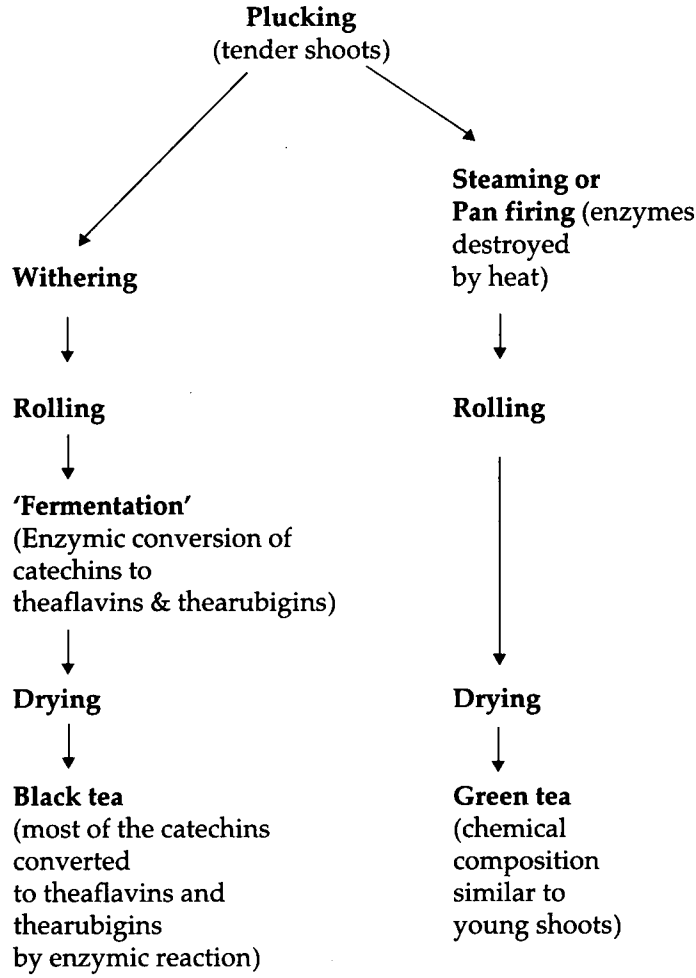
The chemical composition of the made green tea is similar to that of the fresh green leaf. The predominant chemical component in green tea are the catechins, which contribute largely to the taste and character of green tea.

4.3 Oolong or Semi-Fermented Tea

Oolong tea is mainly produced in mainland China and Taiwan. Although during its manufacture the enzymes in the leaf are not destroyed by heating as in green tea manufacture, insufficient time is given for the enzymic reactions to go to completion. The chemical composition of Oolong tea may therefore be regarded as intermediate between that of green tea and black tea, the chemical composition, as well as the taste, depending on the time allowed for fermentation. The characteristics of Oolong tea would be more akin to that of green tea if the fermentation time is short, or to black tea if the fermentation time is longer.

Oolong tea contains dimeric polyphenols and catechins. The percentage of catechins against total polyphenols in Oolong teas is 50-60, as against a value of less than 20 in black teas and over 65 in green teas.

FIGURE 2
Steps in the Manufacture of Black and Green Tea



CHAPTER 5

THE DIETARY VALUE OF TEA

Tea is drunk in a host of different styles: tea unadorned ('plain' tea), with milk, with sugar, with both milk and sugar, sometimes with spices, salt, butter, fruit juices or flavours; as a hot or as a cold, ready-to-drink (RTD) beverage.

The tea brew *per se* contains the hot-water soluble components of made tea which are extracted into the boiling water used for infusion.

5.1 Water

Importantly, tea is a pleasant source of water, a minimum volume of which is needed for health and well-being. The average water requirement for a person living in a temperate climate is about 1.7 litres, and in a tropical climate about 2 litres, per day. Approximately one litre of water is obtained as moisture in solid food and as water produced within the body by metabolic reactions, such as the breakdown of carbohydrates and fat. It is essential, therefore, to consume at least one litre (or six to eight glasses) of fluid or water, per day.

The tea-drinking habit provides an easy and pleasurable way of meeting the minimum water requirement. No calories need be consumed, if tea is taken without sugar and milk or other additives. The calorific value of the tea brew itself is almost zero. Other drinks and beverages usually have components which may be rich in calories, and for this and other reasons, may therefore be undesirable.

5.2 Nutrients

The nutrients, or the major components of food for energy-production, and the building and replacement of body tissues, are carbohydrates, fat and proteins. The amounts of these in tea are not large compared to that in other foods (Table 1).

TABLE 2

The Composition of Black and Green Tea Brews

(for flavonoids, see Table 4)

	% solids extracted into the brew
Theanine	3
Amino acids	3
Peptides/Proteins	6
Organic acids	2
Sugars	7
Other carbohydrates	4
Lipids	3
Caffeine	3
Other methylxanthines	<1
Potassium	5
Other minerals/ash	5
Volatiles	Trace

(after Harbowy and Balentine, 1997)

5.3 Non-Nutrients

On the other hand, tea brew contains significant amounts of non-nutrient components: compounds which do not contribute primarily to energy production and body growth, but which have other important biological functions. The major non-nutrient compounds in tea are polyphenols and a methylxanthine, caffeine. Polyphenols and caffeine have beneficial pharmacological effects and give tea its unique dietary significance. Although in excess caffeine can be harmful, together with polyphenols, as in tea, it is not.

5.3.1 Caffeine

Caffeine is a well-known stimulant acting on the central nervous system. It increases alertness and reduces feelings of drowsiness and fatigue. One cup of tea (170 ml) contains 20-70 mg of caffeine (Balentine, 1997) and 10-12 cups a day are within the limits for most people.

5.3.2 Polyphenols

The polyphenols, particularly those called flavonoids, are strong antioxidants (see Chapter 8). Flavonoids include catechins, theaflavins and thearubigins, and are mainly responsible for the health-giving, beneficial effects of tea.

A large number of *in vitro* studies have shown that flavonoids can inhibit, and sometimes induce, a large variety of mammalian enzyme systems (Middleton *et al.*, 1994), which catalyse metabolic pathways that regulate cell division and proliferation, the aggregation of platelets (the cell fragments in the blood that bring about clotting), detoxification, and inflammatory and immune responses. As a result, flavonoids inhibit various steps in cancer formation or carcinogenesis (Wattenberg *et al.*, 1992), and

influence the immune system and homeostatic balance in physiological systems (Middleton *et al.*, 1994).

Flavonoids also have potent anti-bacterial, anti-fungal and anti-viral activity.

Polyphenols are found in most plants, and hence in food derived from plant sources. However, tea is unique in having huge amounts of polyphenols (up to 40 per cent of the solids extracted into the tea brew).

Analyses have shown that, where tea is drunk, it is the main source of dietary polyphenols. Tea is the major source of flavonoids for the populations of Western countries (Geleijnse *et al.*, 2002).

On the basis of the United States Department of Agriculture 'Serving Sizes' (for tea: 240 ml), Balentine (2001) showed that tea is one of the richest sources of flavonoids in food (Table 3). Black tea and green tea from the major brands available in the United States market are comparable in milligrams of flavonoids per serving, and are even somewhat better than red wine in this respect.

TABLE 3
Dietary Sources of Flavonoids

	(mg/serving[1])
Black Tea	120 – 300
Green Tea	100 – 200
Red Wine	40 – 140
Apples	6 – 15
Soy Beans (Dry)	76 – 207
Tofu	35 – 63
Onions	28
Blueberries	2 – 36
Tart Cherries	26 – 33
Kale	22
Leaf Lettuce	17

[1] *USDA Serving Sizes (Handbook 8)*

(Balentine, 2001)

A comparison of the content of the different flavonoids in black and green tea brews is given in Table 4.

TABLE 4
Flavonoids in Black and Green Tea Brews

	% solids extracted into the brew	
	Black tea	Green tea
Catechins	8	70
Theaflavins	12	0
Thearubigins (TRs)	71	0
Flavonols	10	10
Polymeric flavonoids	0	20 (excluding TRs)

(adapted from Balentine, 2001)

The other chemical components of tea brew are the same, whether it be of black or green tea, and are given in Table 2.

5.4 Vitamins and Minerals

5.4.1 Vitamins

Tea brew contains thiamin, riboflavin, niacin, folic acid pantothenic acid, biotin and inositol (Stagg and Millin, 1975). Vitamin E is also present (Tirimanna and Wickramasinghe, 1966), its content being recently reported as 183.3 mg/kg of made black tea (Ching and Mohamed, 2001).

Tea could therefore make a contribution to the daily intake of vitamins in heavy tea drinkers. However, many other common foods and beverages either contain vitamins or are supplemented with vitamins, and the contribution from tea would usually be negligible.

5.4.2 Minerals

Tea brew is a rich source of dietary minerals (with potassium predominating, but inclusive of magnesium, calcium, manganese, zinc and iron). It can contribute substantially to daily requirements, depending on the levels and bioavailability of the minerals for absorption in the gastro-intestinal tract. Owing to its high concentration in tea brew compared to that in other foods, manganese in tea makes a significant contribution to the average daily intake (Table 5).

TABLE 5
The Contribution of Minerals in Tea to the Daily Dietary Intake
(based on bioavailability)

Element	Average daily dietary intake (% in one litre of tea)
Al	2.8
Ca	0.2
Cu	0.9
Fe	< 0.002
Mg	2.2
Mn	45.8
K	2.3
Na	< 0.05
Zn	0.44

(after Powell *et al.*, 1998)

A characteristic feature of tea is its low sodium content. This makes it ideal for people with hypertension for whom a low sodium intake is recommended.

Tea is a rich dietary source of fluoride (2-3 mg/kg of made tea). Fluoride helps in the prevention of tooth decay (see Chapter 14).

5.5 Tea taken with Milk

Tea is often consumed with milk and sugar, particularly in Britain and its ex-colonies. Therefore, tea in this form is reasonably rich in nutrients. It is reported that in Britain, half of the tea consumed is taken with reduced-fat milk, while two thirds of tea drinkers do not add sugar.

The nutrition provided by a cup of tea (170 ml), with semi-skimmed milk, as estimated by Walker (1996), is given in Table 6.

TABLE 6
The Contribution of Tea with Added Semi-Skimmed Milk to the Recommended Nutrient Intake

	Contents in one cup, 170 ml	% recommended nutrient intake
Energy	52 kJ (12 kcal)	0.7
Protein	0.89 g	2.0
Carbohydrate	1.25 g	0.5
Fat	0.36 g	0.5
Minerals:		
Calcium	29 mg	4
Manganese	1.8 mg	11
Potassium	78 mg	2
Zinc	0.18 mg	3
Vitamins:		
Thiamin (B1)	18 µg	2
Riboflavin (B2)	71 µg	7
Pyridoxine (B6)	18 µg	2
Folate	5 µg	3

(Walker, 1996)

5.6 Derivatives of Tea

5.6.1 Alcoholic Tea Drinks

Tea wines and sherries are available in world markets. A tea-based alcoholic beverage with an alcohol content of 10 per cent has been developed by the Tea Research Institute of Sri Lanka.

5.6.2 Non-Conventional Tea Products

In Japan, a wide range of health products, based on green tea and its catechin components (such as catechin pills for a variety of ailments), are available. Freshly-picked leaves are used as a vegetable. Made green tea in powder form is incorporated into common items of food such as noodles, rice and bread, and into toothpaste. Tea extracts and catechins extracted from tea are used to enrich drinks and bagged tea, and in chewing gum, laxatives, mouth-washes, deodorants, soaps, cosmetics and sun-blocks.

Tea is re-processed and used for making sweets, jams and jellies.

Tea is used for stuffing pillows, and in bathing sponges, massage oils, air-purifiers and molluscicidal sprays.

CHAPTER 6

Research Methodologies

The association between tea consumption and its health benefits has been made in three types of studies: firstly biochemical, *in vitro* laboratory tests of tea extracts and tea components; secondly physiological and pharmacological, *in vivo* experimental treatment and observation of animal models; and thirdly epidemiological, either interventionist or purely observational assessments of human groups and populations.

While each of these methodologies has its own drawbacks, if there is a certain positive congruence between results from different approaches, the assumption can probably be made in most cases that valid correlations exist.

6.1 *In Vitro* Studies

In vitro findings in the laboratory could appear to be far-removed from the real world of human physiology and metabolism, particularly since not every set of molecules entering the lumen of the gut, and which when tested give good

correlations in the test tube, would necessarily appear at the site in the body where it would be effective. This is the vexed question of bioavailability of ingested molecules.

6.2 Animal Models

With regard to animal studies, even if they are carefully chosen to mimic conditions in the human, direct extrapolation of results from non-human species to humans is often open to criticism.

Although it has been shown, in many of these controlled laboratory and animal-model experiments, that tea brew and tea components should have beneficial effects on human health, there could still be doubts as to whether they would actually do so in real life. The health of an individual is affected by a complex of factors, such as heredity, dietary habits, life-style, social status, societal dynamics and environmental influences. For this reason, epidemiological studies are designed and carried out to find the statistical effect of a certain factor or factors on human groups, in existentialist conditions.

6.3 Epidemiological Studies

Classical epidemiology was the study of epidemics. Today, it is defined as "the study of the distribution and determinants of health-related states and events in populations". A full discourse on epidemiology is beyond the scope of this book, but a brief description is given here to assist an understanding of the experimental data given in later chapters.

There are two main types of epidemiological research, experimental and observational. In experimental epidemiology, the researcher intervenes or can intervene. In observational epidemiology, he does not, or cannot for ethical or other reasons.

For example, in studying the effects of a new drug on a carcinogen like cigarette smoke, he can only conduct an interventionist experiment with the drug if there are a test group of people who are willing to, or normally do, smoke, in order to compare them with a control group of non-smokers. In an observational study, the researcher does not intervene, but merely compares two or more groups of people with pre-existing and contrasting habits or life-styles.

In epidemiology, data collection can either be prospective or retrospective. In a prospective study, data are gathered primarily during the study itself. In a retrospective study, data already collected, perhaps for some other purpose, are used.

The most commonly used strategic designs in epidemiology are referred to as cross-sectional surveys, ecological, or cohort, or case-control studies, and randomized controlled trials and cross-over studies.

In cross-sectional surveys, a cross-section of people are interviewed, or have questionnaires administered to them, in trying to find the association between a cause and an effect. This is an inexpensive and simple method to find an association, but it does not establish causation.

In ecological studies, people in the same geographical area are studied. Thus, in cancer research, a particular type of cancer may be studied in one geographic area which may be a country, a province or a state, or a smaller, circumscribed area. Ecological studies reveal useful associations, for example between dietary habits and cancer.

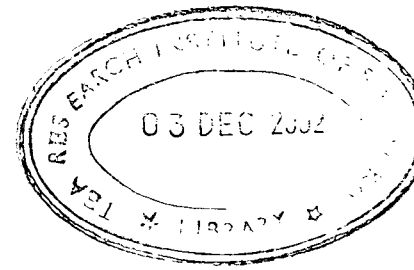
A cohort in epidemiology means a broad group of people who share some attributes or statistical characteristics. Cohorts could be, say, a group of people doing the same job, or in the same age

range or gender, or a group of university students or prisoners. A study of such cohorts could be prospective or retrospective.

In case-control studies, the selection of groups for study are made on the basis of an outcome or condition. Thus, the habits and circumstances of mothers of children born with, and without, birth defects can be studied in order to find the cause of the defects.

In randomized controlled trials, groups are selected and studied at random as, say, in a trial with a new drug where a group receiving the new drug is compared with a control group that does not receive it.

Cross-over studies are randomized controlled trials, in which the treatment is received by one group and not by another group for a certain period of time. This is followed by another period of time during which the treatment is switched to the group which did not receive it the first time, and not given to the group which did receive it.



CHAPTER 7

BIOAVAILABILITY AND METABOLISM OF TEA POLYPHENOLS

7.1 Variations in Bioavailability

It is important to keep in mind that although tea polyphenols and caffeine, according to many reports, clearly have potential health effects, their fate after absorption into the body, that is to say their bioavailability and pharmacokinetics in different tissues of the body, are largely unknown. However, it is known that polyphenols undergo degradation by colonic bacteria and enter the enterohepatic circulation. It is likely that, during their transport and distribution in the body, polyphenols are bound to proteins.

Differences in polyphenol bioavailability at different sites, under varying conditions, may be one reason for the variable results obtained in studies investigating the protection that tea gives against cancer and cardiovascular diseases. The different types of tea polyphenols show different degrees of bioavailability (Das and Griffiths, 1969).

7.2 Techniques

Recent advances in biochemical techniques have given some information on how the body handles tea components following their ingestion. In some studies, the chemical constituents of tea were measured in tissues directly. In other studies of an indirect nature, an increase in a single parameter, such as the antioxidant activity in tissues, have been made, both in animal models and in humans.

7.3 Absorption and Sequestration of Polyphenols

Consumption of three cups of strong tea per day (2 g of tea per cup), for two weeks, increases total polyphenol levels in human blood by 25 per cent (He and Kies, 1994). Although it has been suggested that milk added to tea binds polyphenols and reduces their bioavailability, Hollman *et al.* (2001) have shown that the addition of milk does not alter the bioavailability of tea flavonols.

This section reviews findings which demonstrate that polyphenols from tea, taken orally, are absorbed from the digestive tract and reach, or are sequestered by, body tissues.

7.3.1 Absorption from the Gut

In a study by Nakagawa and Miyazawa (1997) with rats, 60 minutes after a single oral administration of the catechin, epigallocatechin-3-gallate (EGCG), at 500 mg/kg body weight, the levels of EGCG were: plasma 12.3 nmol/ml, liver 48.4 nmol/g, brain 0.5 nmol/g, small intestinal mucosa 565 nmol/g and colonic mucosa 68.6 nmol/g. These values indicate that EGCG is absorbed from the digestive tract, particularly through the intestinal mucosa.

7.3.2 Blood, Urine and Faeces

He and Kies (1994) investigated the impact of green and black tea consumption on polyphenol concentration in blood, urine and faeces. Ten healthy adults received a laboratory-controlled and -measured, constant diet comprising normal food.

A 56-day study period was divided randomly into four 14-day periods in which no tea, green tea, black tea or decaffeinated black tea were given three times a day. Green tea consumption resulted in the highest urinary and faecal elimination, and the highest retention of polyphenols in the blood, followed by black tea and decaffeinated black tea.

Warden *et al.* (2001) assessed the bioavailability of prominent black tea catechins in humans drinking tea throughout the day.

After 5 days of consuming a low-flavonoid diet, subjects drank a black tea preparation containing 15.48, 36.54, 16.74, and 31.14 mg of epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), respectively, at four time points (0, 2, 4 and 6 h). Blood, urine and faecal specimens were collected over a 24- to 72-h period and catechins were quantified by HPLC.

Plasma concentrations of EGC, EC and EGCG increased significantly relative to the baseline concentrations. Plasma EGC, EC and EGCG peaked at 5 h, and ECG peaked at 24 h. Urinary excretion of EGC and EC, which peaked at 5 h, was raised relative to baseline amounts, and faecal elimination of all four catechins was increased relative to baseline. Approximately 1.68 per cent of ingested catechins were present in the plasma, urine and faeces, and the bioavailability of the gallated catechins was lower than that of the non-gallated forms.

A study was conducted by Shahrzad (2001) to find the bioavilability of gallic acid (GA) in humans. Black-tea brew was found to contain 93 per cent of its GA in free form. After the administration of a single oral dose of acidum gallicum tablets or tea (each containing 0.3 mmol GA) to 10 volunteers, plasma and urine samples were collected over various time intervals.

Concentrations of GA and its metabolite, 4-O-methylgallic acid (4-O-MGA), were determined. GA from both tablets and tea was rapidly absorbed and eliminated with mean half-lives of 1.19 ± 0.07 and 1.06 ± 0.06 h, and mean maximum concentrations in the plasma of 1.83 ± 0.16 and 2.09 ± 0.22 μmol/l, respectively. After oral administration of tablets and black tea, 36.4 ± 4.5 and 39.6 ± 5.1 per cent of the GA dose were extracted in urine as GA and 4-O-MGA, respectively.

The relative bioavailability of GA from tea, compared with that from the tablets, was 1.06 ± 0.26, showing that GA is as available from drinking tea as it is from taking GA tablets.

Plasma and urinary concentration of the flavonols, quercetin and kaempferol, were measured after intake from onions and black tea (De Vries *et al.*, 1998).

Fifteen human subjects were given black tea, 1600 ml/day, or fried onions, 129 g/day. Tea provided 49 mg of quercetin and 27 mg of kaempferol/day, while onion provided quercetin only, at 13 mg/day. The concentration of quercetin after tea increased four-fold from baseline levels, and three-fold after onion. The concentration of kaempferol after tea increased about six-fold.

7.3.3 Body Tissues

Kohri *et al.* (2001) studied the absorption of radioactive EGCG in rats.

After oral administration of [4-(3)H] EGCG, the radioactivity in blood, the major tissues, urine, and faeces was measured at periods of time.

The radioactivity in blood and most tissues was low at 4 h, began to increase after 8 h, peaked at 24 h, and then decreased. Major urinary elimination of radioactivity occurred in the 8-24 h period, and the cumulative radioactivity excreted by 72 h was 32.1% of the dose.

Suganuma *et al.* (1998) directly administered radioactive [^{3}H] EGCG solution into the stomachs of male and female mice, and the radioactivity in the digestive tract, various organs, blood, urine and faeces was measured after 1, 6 and 24 h. Radioactivity was detected after one hour in all organs.

Radioactivity was detected in the digestive tract, liver, lung, pancreas, mammary glands, skin, brain, uterus, ovary and testes. Within 24 hours, 6.4 per cent (males) and 6.6 per cent (females) of the total administered radioactivity were excreted in the urine, and 33.1 per cent (males) and 37.7 per cent (females) were eliminated in the faeces. A second administration after 6 h increased the radioactivity in blood, brain, liver, pancreas, bladder and bone by four to six times.

All of these results suggest that frequent tea consumption would maintain a high concentration of polyphenols in the body.

7.3.4 Blood Plasma

Following the oral administration of EC, EGC, ECG and EGCG to rats, the presence of all these compounds was detected in the blood of the portal vein and measured using HPLC and MS (Okushio *et al.*, 1996).

Similarly, the absorption of EGCG into the circulatory system of rats was studied after oral administration of 50-mg doses (Unno and Takeo, 1995). The concentrations in the plasma peaked about half an hour after administration and then decreased quickly.

Lee *et al.* (1995) studied the human plasma levels of EGCG, EGC, ECG and EC after consumption of 1.2 g of green tea in warm water. One hour later, the levels were EGCG 46-268 ng/ml, EGC 82-206 ng/ml, and EC 48-80 ng/ml. ECG was not detected.

In a study by Nakagawa *et al.* (1997), healthy human subjects were given 3, 5, or 7 capsules of green-tea extract orally (corresponding to 225, 375, and 525 mg of EGCG and 7.5, 12.5, and 17.5 mg of EGC, respectively). The plasma EGCG and EGC concentrations before administration were both below the detection limit (< 2 pmol/ml), but 90 min after administration the concentrations increased to 657, 4300, and 4410 pmol of EGCG/ml, and 35, 144, and 255 pmol of EGC/ml, in the subjects who received 3, 5, and 7 capsules, respectively. These concentrations of EGCG and EGC in the plasma were statistically different, and dependent on the dose of green-tea extract given.

Nakagawa *et al.* (1999) investigated the effect of green-tea catechin supplementation on the antioxidant capacity of human plasma. Eighteen healthy male volunteers, who orally ingested green-tea extract (254 mg of total catechins/subject), had 267 pmol of EGCG/ml in the plasma 60 min after administration. The levels of a plasma oxidant, phosphatidylcholine hydroperoxide (PCOOH), attenuated from 73.7 pmol/ml in the control, to 44.6 pmol/ml in the catechin-treated subjects, being correlated inversely with the increase in plasma EGCG level.

These results show that green tea consumption contributes to increased plasma antioxidant capacity in humans.

7.4 Polyphenol Metabolism

Following their absorption, approximately 20 per cent of the polyphenols are distributed in human body tissues.

Polyphenols, unlike many other compounds, have the ability to penetrate to the tissues of the brain, overcoming the so-called blood-brain barrier. This, together with their antioxidant and iron-chelating capacity, may lead to their becoming useful in the treatment of neurodegenerative diseases resulting from oxidative stress (Levites *et al.*, 2001).

7.4.1 Catechins

Of the total catechins consumed by humans, a large percentage passes out unchanged in the faeces (Hara, 1997) and, of that absorbed into the body, approximately 60 per cent are excreted in the urine, and the rest in the bile (Brown and Griffiths, 1981). Catechins are absorbed into the blood in the portal vein of humans (Hara, 1997) and rats (Okushio *et al.*, 1996), and are thereby conveyed from the gastrointestinal tract to the liver.

Catechins undergo extensive metabolism, and within eight hours of ingestion over 90 per cent of the catechins are excreted. After 24 hours, they are not detectable in the blood.

Although bactericidal, catechins do not affect the useful lactic acid bacteria in the human digestive tract (Hara, 1997). Tea catechins in the diet for several weeks decrease the products of putrefaction of the food residues, and increase organic acids in the gut by lowering pH. These changes were observed in tube-fed patients when 100 mg of tea catechins (equivalent to that in a cup of green tea) were administered three times daily with meals for three weeks. When catechin administration was discontinued, the effects reversed after one week.

The propensity of tea to remove 'malodours' (in this case that given by the compound, methylmercaptan) was determined *in vitro* by Wu and Hwang (2001). There was good correlation between total phenolic compounds or catechins and malodour removal. Green tea and semi-fermented teas were better than black tea in this respect.

Infusions of catechins and theaflavins were compared with whole tea infusions by Wu and Hwang. Malodour removal was related to dosages of catechins and theaflavins in green and semi-fermented teas, but not in black tea. It is assumed that, in addition to catechins and theaflavins, there are other deodorants such as gallic acid, thearubigins and other unidentified compunds in black tea.

CHAPTER 8

ANTIOXIDANT ACTIVITY

8.1 Human Metabolism and Oxygen

The human and animal body makes use of free oxygen (O_2) from the air for converting nutrients in ingested food into cell components and energy. This is done by means of a multiplicity of chemical reactions that interlock into each other to form a network of pathways and cycles. The whole complex network of chemical reactions is called metabolism.

The oxygen molecule consists of a pair of oxygen or O atoms, as its formula, O_2, indicates. Atoms in general consist of a nucleus around which electrons orbit along fixed circuits, or 'orbitals'. Each orbital can accommodate only a single pair of electrons. If pairs of electrons occupy the orbitals, as in the oxygen molecule, the molecule is stable and unreactive, and the firm bond between the constituent atoms of the molecule, resulting from paired electrons, is called a covalent bond. The energy in molecules is released when covalent bonds are broken, that is when electrons are torn away from their orbitals and electrons become unpaired.

During metabolism, the energy released from covalent bonds is not dissipated uncontrollably but is stored in ATP (adenosine triphosphate) molecules. ATP is therefore referred to as the currency of energy in biological systems. Energy from ATP is used for building new material in cells and for driving biological processes.

8.2 Free Radicals and their Damaging Effects

Nearly all the molecules in the body have paired electrons and are therefore stable and unreactive, except when they enter into a metabolic process.

When oxygen molecules split into oxygen atoms in the ATP-producing process, not all the oxygen atoms are utilised in energy production. Those left over become 'free oxidant radicals' (or simply 'free radicals', FRs). Apart from these left over oxygen atoms (O or singlet oxygen FRs), there are other FRs produced in diverse ways.

Within mitochondria (the powerhouses of the cell where energy is generated), there are chains of molecules called electron carriers along which electrons pass in the energy-producing process of cell respiration. When some of these electrons accidentally leak off the chain, they reduce O_2 converting it into a FR, the reactive superoxide anion, O_2^-. Hydrogen cations, H^+, react with superoxide anions producing other FRs, the even more reactive hydroperoxides such as hydrogen peroxide, H_2O_2. Hydroperoxides, in turn, generate more FRs, the hydroxyl, OH^-, and the peroxyl radicals. These chain reactions, in this case the proliferation of superoxide and hydrogen peroxide to give other, more reactive FRs, are a feature of FR production in general.

FRs are generated when the hormone modulators,

prostaglandins, a class of fatty acids, are synthesised. Xenobiotic molecules, entering the body, are metabolised by the cytochrome P-450 enzyme system in the liver, and here again FRs are produced.

Apart from their fortuitous formation, FRs are produced because they act as regulatory molecules in some metabolic processes. White blood cells called lymphocytes, and cells called fibroblasts which form connective tissue, constantly produce superoxide radicals in small amounts as growth regulators.

In addition to these so-called reactive oxygen species (ROSs), it was discovered in the 1990s that some FRs are reactive nitrogen species: nitric oxide, NO, and peroxynitrite. NO is produced by the cell-lining of blood vessels, the vascular endothelium, to function as a regulator in smooth muscle relaxation, and also in phagocytes (cells which engulf foreign matter) in the brain. Although physiologically useful, NO can become toxic in excess.

Since they have unpaired electrons, FRs are highly reactive, and react, virtually indiscriminately, with other molecules (proteins, carbohydrates, polyunsaturated fatty acids, and nucleic acids such as DNA) and with membrane, the primary structural and functional component of cells.

FRs play a useful rôle in the immune system. When phagocytic cells engulf potentially harmful invaders, such as bacteria and toxins, they release FRs that chemically destroy the engulfed cells or particles. These FRs, escaping into the extracellular medium, add to the pool of destructive FRs bathing the body cells.

The chronic exposure of body cells to FRs brings about damage to the nucleus, cell membrane, the cellular proteins and the genetic

material, DNA. Much of the damage to DNA is caused by ROSs. This damage of cells and tissues by FRs lead to the production of more FRs in chain reactions, and as a result a worsening of the initial lesions.

In addition to their production during normal metabolism (either for a regulatory purpose or fortuitously), FRs are also produced within the body by extraneous agents. Extraneously produced FRs then activate the immune system to produce even more FRs. Agents and factors effective in FR production are industrial chemical fumes and other environmental pollutants, cigarette smoke, many agro-chemicals (pesticides and herbicides, most of which are metabolised by the cytochrome P-450 system), as well as exertion, stress, anxiety and depression. The energy in electromagnetic radiation (ultraviolet radiation and sunlight) produce FRs in the skin.

In the modern world, chemical pollutants are unfortunately all around us in the environment and in much of the food we consume. However, it is possible to reduce FR production and to help in their elimination in our bodies, by choosing the correct foods, by not smoking, and by consciously adopting life styles that are stress-free and developing mind-sets that lead to contentment.

Damage by FRs is now believed to be responsible for the onset of ageing processes, and for the development of more than 50 degenerative and chronic diseases, such as atherosclerosis and cardiovascular disease, chronic inflammatory diseases, neurodegenerative and auto-immune diseases, cancers, cataract, dementia and radiation injury.

8.3 Antioxidants

Antioxidants are defined as molecules that can delay or prevent the oxidation of a substrate, when present in small amounts relative to the substrate.

8.3.1 Antioxidant Defence Systems

There is an interrelated array of extra- and intra-cellular antioxidant systems which together defend body cells by 'scavenging' or neutralising FRs. They may be natural antioxidants, as well as non-nutrient, dietary antioxidants that enhance the defences of the body against FRs.

8.3.1.1 The First Group of Defences

The first group of antioxidant defences are enzymes which control FRs that are formed when O_2 accepts electrons, such as superoxide and hydrogen peroxide. Containment of ROSs formed in this way is the first level of defence. The enzymes involved include superoxide dismutase, catalase and the glutathione enzyme system (glutathione peroxidase and glutathione transferase) (Diplock, 1994).

Superoxide Dismutase

Interestingly, it was the identification of superoxide dismutase (SOD) as an ubiquitous enzyme in the tissues of animals, with apparently no function other than to reduce superoxide radicals, that first drew the attention of scientists to the importance of free radicals and the antioxidant defences against them.

The liver, the kidney and the adrenal gland contain relatively large amounts of SOD. It catalyses the conversion of superoxide to hydrogen peroxide and molecular oxygen (Harris, 1994).

$$2\,O^{\bullet}_2 + 2\,H^{+} \longrightarrow H_2O_2 + O_2$$

Both catalase and the glutathione peroxidase system integrate with SOD by removing hydrogen peroxide. This is important as hydrogen peroxide could generate even more reactive hydroxyl radicals and ions through the so-called Fenton reaction in the presence of free metal ions.

e.g. $H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$

$H_2O_2 + Cu^{2+} \longrightarrow Cu^{3+} + OH^{\bullet} + OH^{-}$

Catalase

Catalase is a haemoprotein present in most aerobic cells within organelles called peroxisomes. In the human body, relatively high concentrations are found in the liver and in red blood cells or erythrocytes. It eliminates the hydrogen peroxide formed from SOD activity.

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2$$

The Glutathione Enzyme System

Glutathione plays an important role in the defence against xenobiotic compounds, ROSs and other FRs (Meister and Anderson, 1983). The reduced form of glutathione (GSH) reacts with them to give the oxidised form of glutathione (GSSG).

$$GSH + R^{\bullet} \longrightarrow GS^{\bullet} + RH$$

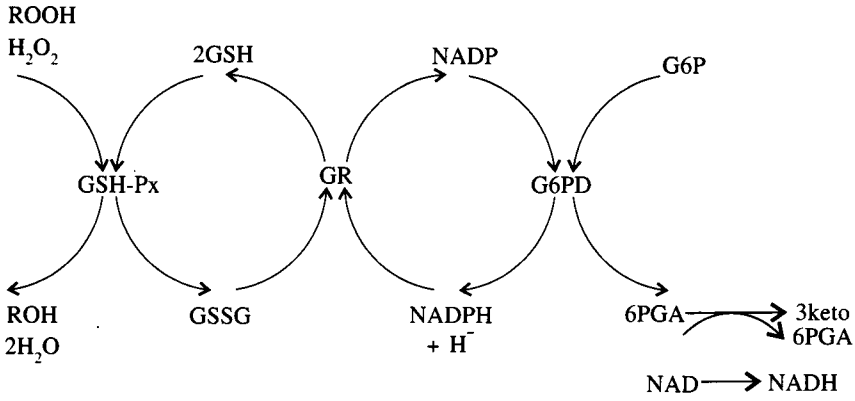
$$GS^{\bullet} + O_2 \longrightarrow GSOO^{\bullet}$$

$$GSOO^{\bullet} + GSOO^{\bullet} \longrightarrow GSSG + 2\,O_2$$

Glutathione can function as an antioxidant on its own (as shown in the equations above), or as a part of a larger antioxidant enzyme system (Fig. 3).

FIGURE 3

The Glutathione Redox Cycle



GR - Glutathione reductase, GSH-Px - Glutathione peroxidase, G6P(D) - Glucose 6 Phosphate (Dehydrogenase), PGA- Phosphoglucuronic acid

In the Glutathione Redox Cycle, the enzyme, glutathione peroxidase (GSH-Px), catalyses the reduction of organic peroxides (ROOH) and hydrogen peroxide to organic hydroxides (ROH) and water. Oxidised glutathione (GSSG) is regenerated by the action of glutathione reductase (GR) with the involvement of NADPH.

8.3.1.2 The Second Group of Defences

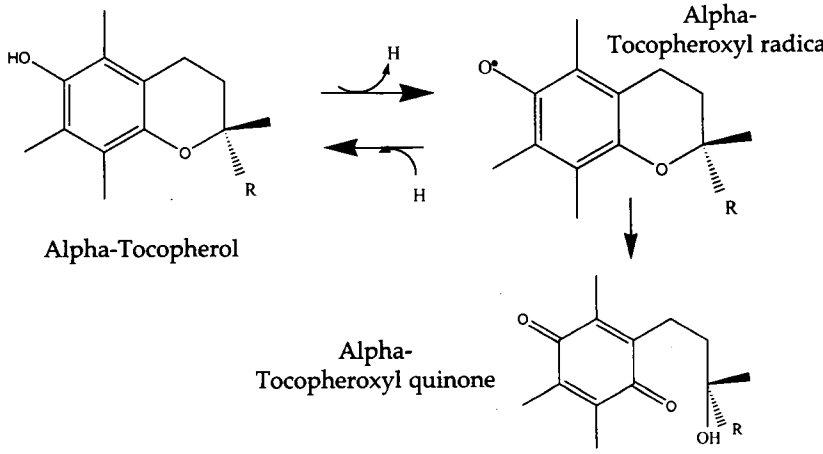
The second group of antioxidants, vitamins E and C, and carotenoids, are molecules that act against secondary FRs, formed when ROSs attack other molecules (Palozza *et al.*, 1992). These secondary FRs can cause damage by initiating proliferating chain reactions such as lipid peroxidation: the formation of peroxyl radicals from polyunsaturated fatty acids in membranes and low density lipoproteins (LDL).

Vitamin E

Vitamin E consists of eight chemically similar tocopherols, which may exist in a number of different steric forms. The tocopherol with the highest antioxidant activity is alpha-tocopherol (αTOC). Tocopherols are lipid-soluble and therefore widely distributed in membranes and lipoproteins. They play an important rôle in preventing damage to these structures by peroxidation of the unsaturated fatty acids within them (Diplock, 1985). Other than peroxyl radicals, tocopherol also can react with hydroxyl and superoxide radicals.

The α-tocopheroxyl radical (αTOC•) is fairly stable owing to the delocalisation of its unpaired electron. However, it could undergo further oxidation but only small amounts of these further oxidised products are detected in cells. It is now believed that αTOC is regenerated by vitamin C and glutathione (Englard and Seifler, 1986).

FIGURE 4
Radical Trapping Action by Vitamin E



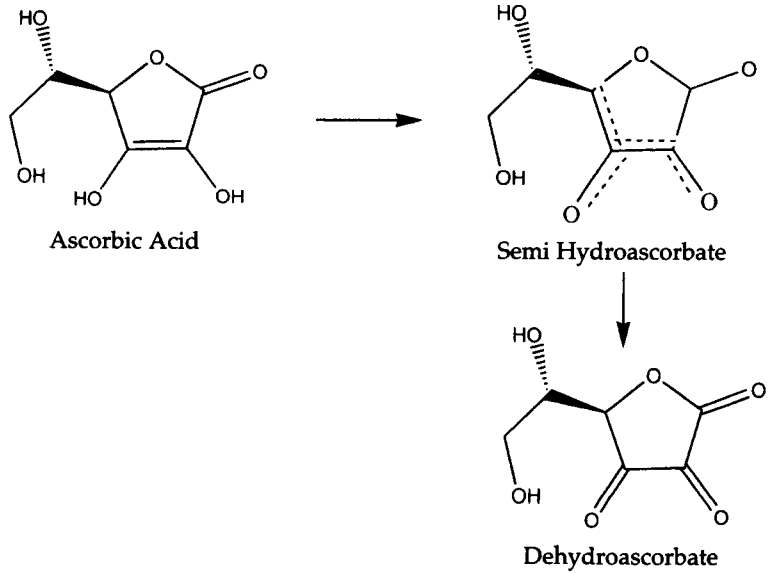
Vitamin C

Vitamin C (ascorbic acid) is water soluble. Its antioxidant action arises from its donation of one electron to FRs, resulting in the production of the semihydroascorbate radical with electrons de-localised between three oxygen atoms. Further oxidation gives dehydroxyascorbate.

Dehydroxyascorbate could break down further. However, there is an efficient mechanism by which it is reconverted to ascorbic acid and in which glutathione is involved.

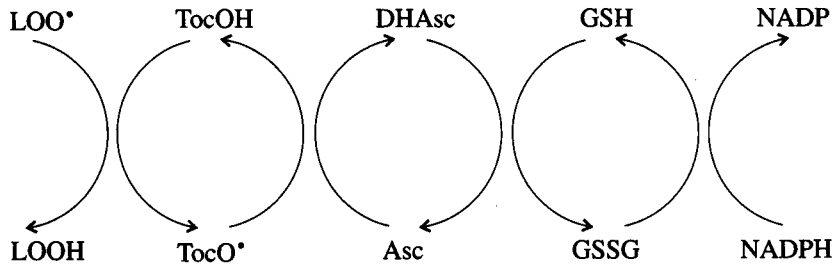
Dehydroxyascorbate + 2 GSH ⟶ Ascorbate + GSSG

FIGURE 5
Radical Trapping Action by Vitamin C



In addition to this function, it is believed that vitamin C may be involved in regenerating vitamin E (Packer *et al.*, 1979).

FIGURE 6
Regeneration of Vitamin E



(LOO• - Lipid peroxyl radical, LOOH - Lipid hydroperoxide, TocOH - Tocopherol, TocO• - Tocopheroxyl radical, DHAsc - Dehydroxyascorbate, Asc - Ascorbate, GSH - Reduced glutathione, GSSG - Oxidised glutathione)

Carotenoids

In animal tissues, β-carotene scavenges FRs including singlet oxygen at low partial pressures of oxygen. Palozza *et al.* (1992) found that a combination of β-carotene and αTOC was much more effective than the sum of the two compounds, acting separately, in preventing lipid peroxidation in membrane lipids. β-Carotene may be acting synergistically with αTOC, since TOC is effective at higher oxygen concentrations.

8.3.1.3 The Third Group of Defences

A third group, molecules such as caeruloplasmin and transferrin, are antioxidants because they bind to ("chelate with") metal ions which act as catalysts in FR production (Plonka and Metodiewa, 1980). Several other molecules, such as uric acid and albumin, are also involved in the control of the oxidation process, and help the function of this group of antioxidants (Halliwell, 1988).

Caeruloplasmin

Caeruloplasmin, the major copper transport protein, is an alpha 2 globulin protein containing 6-8 copper atoms. It is a positive 'acute phase' protein, that is one that makes a rapid response to injury or inflammation. Caeruloplasmin concentrations in plasma increases during acute phase responses (Fleck, 1989). Caeruloplasmin catalyses the conversion of ferrous (Fe^{2+}) to ferric (Fe^{3+}) ions, thus reducing the ferrous ions available for reactions, the Fenton reactions, in which harmful hydroxyl radicals are produced from hydrogen peroxide. It also binds free copper ions, which are involved in Fenton reactions.

It is reported, further, that caeruloplasmin is able to dismutate superoxide radicals (Plonka and Metodiewa, 1980).

Transferrin

Transferrin is a β-globulin protein containing two iron atoms. It is the major iron transport protein in blood plasma. The antioxidant function of transferrin is through the binding of free iron in an acute phase response. Transferrin decreases and the iron-storage protein, ferritin, increases during inflammation. These actions reduce the availability of ferric ions for Fenton reactions (Bullen and Griffith, 1987).

Albumin

Albumin is a large protein molecule, accounting for 50 per cent of the total protein in serum. It has been recognised as an antioxidant. The concentration of albumin decreases following injury or infection, in an acute phase response. Halliwell (1988) has suggested that this decrease occurs through the role that albumin plays as a 'sacrificial antioxidant'; that is where albumin

binds copper and ferrous ions and, as a consequence, FRs such as hydroxyl radicals react with the albumin molecule leading to its catabolism, or destruction. This 'sacrifice' on the part of the albumin molecule prevents FRs attacking more important molecules such as DNA.

Uric acid

Uric acid is found in high concentrations in plasma (Ames *et al.*, 1981). Its double-ring structure with a hydroxyl group makes it capable of scavenging FRs. It is reported to be a chain-breaking antioxidant.

8.3.1.4 The Fourth Group of Defences: Dietary Flavonoids

A fourth group of molecules, the flavonoids in the diet, are antioxidants because they accept or donate hydrogen by going into equilibrium with their quinone form, act in metal-ion chelation, and bind with activated molecules (some of which are carcinogens) which would otherwise initiate chain reactions (Rice-Evans, 1995).

Flavonoids are the major component of tender tea shoots (Table 1), used for manufacturing tea. Flavonoids are extracted into the solution when tea is brewed and they are the major component in the brew as well. Tea is therefore an important dietary source of flavonoids (Hertog *et al.*, 1993 b; Ho, 1992). Following their antioxidant activity, altered flavonoid molecules, which are fairly stable, are eliminated from the body.

8.3.2 Oxidative Stress

The antioxidant defence systems diminish oxidative damage by maintaining a balance between free oxidant radicals and

antioxidants. When there is a disturbance in the oxidant/ antioxidant balance in favour of the oxidant, the body is subjected to 'oxidative stress' which, if prolonged, leads to pathophysiological conditions and degenerative diseases.

8.3.3 Dietary Antioxidants

Some of the antioxidants are obtained directly from the diet, particularly from vegetables and fruit. The diet therefore plays an important role in overcoming oxidative stress. Prominent dietary antioxidants are β-carotene, tocopherols (vitamin E), ascorbic acid (vitamin C) and selenium. Selenium does not itself act as an antioxidant but it is an essential component of glutathione peroxidase.

Antioxidants in many foods play an important protective and preservative rôle by neutralising FRs by contributing electrons for the restoration of electron pairs. These foods should be identified and incorporated into the diet. All teas (both black and green) contain high concentrations of polyphenols (see section 5.3.2), which function as powerful antioxidants, and tea is therefore valuable in any healthy diet.

8.4 Experimental Estimation of Antioxidant Activity

8.4.1 Redox Potential Determination

Some substances can exist in an oxidised (or oxidant) form and a reduced (or reductant) form. The two forms are called a redox couple. The oxidant is converted into reductant by accepting electrons, e^-, and the reductant is converted into oxidant by losing electrons.

$$\text{Oxidant} + e^- \longleftrightarrow \text{Reductant}$$

The redox (oxidation-reduction) potential is represented by the electromotive force between one of a pair of electrodes, immersed in a solution containing a mixture of oxidant and reductant, and the other of the pair of electrodes immersed in a H^+ solution under standard conditions. The redox potential is read on a voltmeter connected between the electrodes. A negative reading means that the substance has a lower affinity for electrons than does hydrogen, and a positive reading that it has a higher affinity. If the redox potential of a compound is low, less energy is required for it to donate electrons and therefore it has a higher antioxidant potential.

The redox potential of compounds may be determined using pulse voltammetry with a reference electrode. The redox potentials of flavonoids have been determined in this way, in order to estimate their antioxidant capacity. However, redox potential values do not fully predict the ranking of compounds as antioxidants in biological systems.

The biological activity of an antioxidant in a living system is determined by its bioavailability at the particular site, which is a major factor determining antioxidant activity *in vivo*.

Therefore, apart from redox potential determinations, other methods have been developed to measure the antioxidant activity of compounds in which the *in vivo* conditions are mimicked as far as possible.

8.4.2 Scavenging of Free Radicals

In this method, FRs are generated chemically, and the relative ability of compounds to scavenge or reduce them is measured.

The FR used most commonly, in both aqueous and lipophilic systems, is the 2,2'-azinobis-(3-ethylbenzothiazolin-6-sulphonic acid) (ABTS) radical. The ability of the test compound to scavenge ABTS radicals is compared with the ability of Trolox (a water-soluble analogue of vitamin E) to do so. The antioxidant capacity of the compound is quantified as the TEAC (or Trolox equivalent antioxidant capacity) value. Another FR, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, is also widely used in lipophilic systems.

The results depend on the relative rates of reaction of the test compounds with the FRs and their hydrogen-donating properties. Consequently, different methods do not give the same absolute values, although similar relative rankings are given. The FR could be quantified using spectrophotometry, or electron spin resonance (ESR) or electron paramagnetic resonance (EPR).

A more biologically appropriate approach is to generate FRs that are of pathological significance to living systems. The FR used most often is the peroxyl radical generated using an azo initiator, such as 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) and linoleic acid. Initially, the TRAP (or total radical-trapping antioxidant parameter) method was developed, in which the initial lag phase of the reaction involving antioxidants is measured, and compared with a known antioxidant such as Trolox.

This method has been improved in an assay called ORAC (oxygen radical antioxidant capacity), where the reactions are allowed to go to completion. In this method, the antioxidants in the sample and a less efficient, fluorescent compound, called phycoerythrin, are added to the FRs together, and allowed to react competitively with the FRs. The linear reduction in fluorescence is measured using fluorescent detectors.

In the FRAP (ferric reducing ability) assay, the ability of antioxidants to reduce ferric ions to ferrous ions is determined by allowing the ferrous ions produced to bind to a chromophore measurable by spectrophotometry. This method is often used with biological fluids such as plasma, and is gaining popularity as it is relatively inexpensive and easy.

CHAPTER 9

Tea Antioxidants Against Disease and Ageing

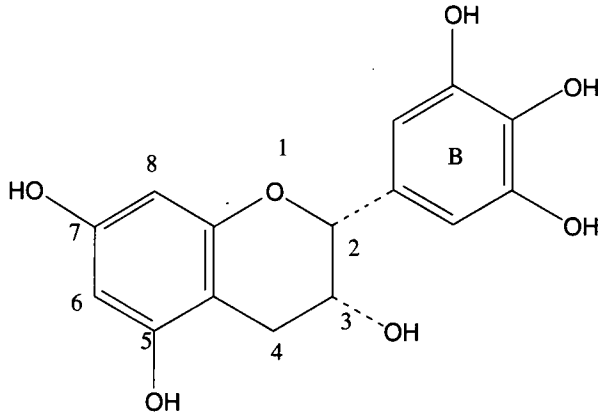
Tea should be, in most parts of the world, the cheapest drink next to water, and is probably therefore the second most consumed drink worldwide. Tea is comparable to red wine (which is of course much more expensive) as a pleasant source of antioxidants.

Tea is an important and cheap source of antioxidants, and much is known on its ability to reduce oxidative stress.

9.1 The General Structure of Tea Polyphenols

Catechins and other flavonoids, which are polyphenols, found in both green and black made tea have a common carbon skeleton. Theaflavins and thearubigins, formed in the oxidative (the so-called 'fermenting') stage of black tea processing, also retain this structure.

FIGURE 7
The Common Skeleton of Tea Flavonoids



Flavonoids are able to act as antioxidants because of the hydrogen-donating capacity of their phenolic groups. In addition, in the flavonoid molecule, hydroxyl groups at positions 5 and 7, and oxygen at position 1, make the carbons at positions 6 and 8 strongly nucleophilic. Thus, the carbons at positions 6 and 8 can form C-O or C-C bonds with reactive oxygen species (ROSs), causing the flavonoid molecules to undergo oxidative polymerisation.

The presence of two adjacent hydroxyl groups on the aromatic ring also makes them strong metal-ion chelators. They can bind with free ferric, ferrous and other metal ions, and thus decrease the free cellular metal ions which are required for the generation of ROSs in cellular reactions (Yang and Wang, 1993).

9.2 Research on Antioxidant Activity of Tea

As already mentioned (Section 1.1), tea *sensu strictu* is made from the terminal shoots of *Camellia sinensis*, although other,

herbal 'teas' are widely available. Du Toit *et al.* (2001) draw attention to the fact that these infusions, from many plants including spices, are often descibed as being "rich in antioxidants", often without scientific support.

In early research on tea and health, many studies were carried out and the antioxidant activity (or free radical scavenging capacity) of *C. sinensis* extracts and individual tea flavonoids *in vitro* were established. In subsequent *in vivo* studies, the antioxidant activity of green, black and oolong teas, and of tea components, was established.

However, as Du Toit *et al.* (2001) caution, *in vitro* results cannot always be extrapolated to conditions in the human body, owing to variations in bioavailability of tea components (Chapter 7).

9.2.1 Phenols and Antioxidant Activity

Karakaya *et al.* (2001) found that the total phenol content and the antioxidant capacity (or FR scavenging ability) of foods, common in Turkey, are significantly correlated.

Total phenols in the foods were determined by the Folin-Ciocalteu method, and the antioxidant capacities by the ability of the foods to scavenge ABTS radicals in the aqueous phase (see Section 8.4.2).

The total phenol content in some of the beverages tested was in the order: black tea > instant coffee > Coca Cola > red wine > Turkish coffee.

9.2.2 Comparing Tea Polyphenols with Vitamins C and E

In an *in vitro* study by Zhao *et al.* (1989), the scavenging ability of tea polyphenols on superoxide anions were found to be comparable with that of vitamins C and E. Superoxide anions

were generated by irradiation of a system containing riboflavin and 5,5-dimethyl-1-pyrroline-1-oxide, and their formation was measured by electron spin resonance. A green-tea polyphenol extract, vitamin C and vitamin E were added separately, and their scavenging rates on the generated superoxide anions measured. The rates were 72 per cent, 96 per cent and 23 per cent for tea polyphenols, vitamin C, and vitamin E, respectively.

9.2.3 Comparing Tea with Vegetables and Fruit

Food from plant sources in general are rich in antioxidants. However, the antioxidant capacity of black and green tea is much higher than that of many vegetables (Cao *et al.*, 1996) (Fig. 8).

Du Toit *et al.* (2001) generated and used FRs, the polyaromatic hydrocarbon, 2,2-diphenyl-1-picrylhydrazyl (DPPH), to measure and compare the radical scavenging capacities of fruits, vegetables, black, green and oolong teas, and herbal teas. The results are given in vitamin C equivalents.

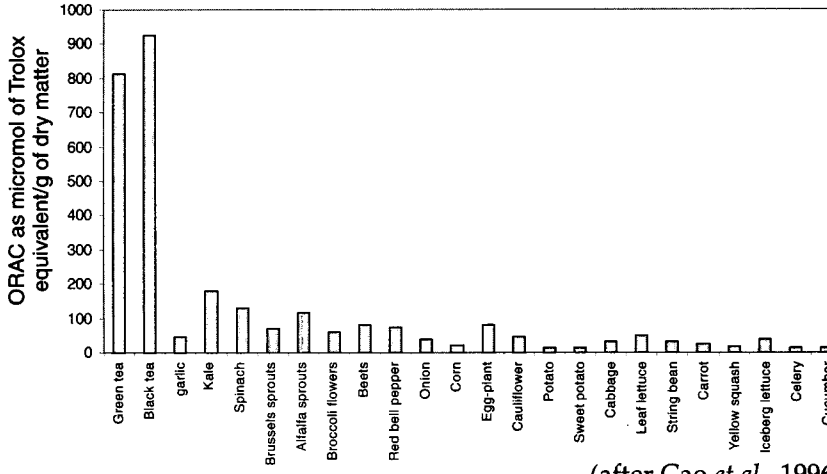
There are no statistically significant differences in the scavenging capacities of black, green and oolong teas, but that of the 'teas' from other plants are significantly lower.

One or two cups of tea are equal in antioxidant capacity to five portions of fruits or vegetables, or 400 mg of vitamin C.

Paganga *et al.* (1999) compared the antioxidant capacities of 100 g (a 'serving') of fresh fruit and vegetables with previous reports on 150 ml servings of different beverages (500 ml in the case of beer), and expressed the results in Trolox equivalents.

The following were found to be equal in their antioxidant activities: one glass of red wine, 12 glasses of white wine, two cups of tea, four apples, five portions of onion, 5.5 portions of

FIGURE 8
Total Antioxidant Capacity of Tea and Common Vegetables



(after Cao *et al.*, 1996)

egg plant, 3.5 glasses of blackcurrant juice, 3.5 glasses of beer, seven glasses of orange juice, 20 glasses of apple juice.

It is significant that, according to these results, drinking just two cups of tea is the equivalent of drinking one glass of red wine. Tea is of course much cheaper than red wine, presumably wherever the two are available together, and tea would therefore be the most affordable source of health-giving antioxidants to the larger part of the world's population.

9.2.4 Comparing Oolong, Green and Black Teas

Yen and Chen (1995) tested oolong, green and black tea extracts for their scavenging activity on FRs *in vitro*.

Activity against peroxidation was determined by incubating tea samples with linoleic acid, and measuring oxidation in the reaction mixtures by spectrometry, after colour generation with

ferric chloride and thiocyanate. Oolong tea showed 73 per cent inhibition of linoleic acid peroxidation, and both green and black tea showed an inhibition of 40 per cent.

Superoxide anions were generated by a non-enzymic system, and their oxidative activity in reaction mixtures containing tea samples determined spectrophotometrically by measuring the reduction of nitro blue tetrazolium. Oolong, green and black teas gave 75 per cent, 58 per cent and 52 per cent inhibition of oxidative activity, respectively.

Hydroxyl radicals were generated by the reaction of hydrogen peroxide with ferrous ions and trapped in 5,5 -dimethyl-pyrroline- N-oxide (DMPO). The DMPO-OH formed, a measure of the oxidative activity of hydroxyl, was determined by electron paramagnetic resonance (EPR) spectrometry. Oolong, green and black teas reduced oxidative activity, as shown by the EPR signal, by 92 per cent, 91 per cent and 77 per cent, respectively.

The carcinogenic effect of polyaromatic hydrocarbons may be due to the *in situ* generation and oxidative activity of their free radicals. The scavenging effect of the tea extracts on the polyaromatic hydrocarbon, DPPH, was measured. Oolong, green and black teas reduced the oxidative activity of DPPH radicals by 54.6 per cent, 59.4 per cent and 49.0 per cent, respectively.

9.2.5 Tea Polyphenols *in Vitro*

Scott *et al.* (1993) found that catechin and epicatechin have a scavenging action on hydroxyl, superoxide, trichloromethyl peroxyl radicals, and hypochlorous acid.

Catechin was found to be the most effective of the tea components in quenching singlet oxygen (Tournaire *et al.*, 1993).

Rice-Evans *et al.* (1995) measured the antioxidative capacity of several plant-derived polyphenols, including quercetin, myricetin, epicatechin and catechin which are found in tea. The total antioxidant activity against aqueous phase radicals were measured and compared with the activity of the water-soluble analogue of tocopherol, Trolox. Results are given as the Trolox equivalent antioxidant capacity (TEAC). The TEAC values for quercetin, myricetin, epicatechin and catechin were 4.7, 3.1, 2.5 and 2.2, respectively. These results indicate that the tea components have a greater antioxidant potential than Trolox.

Theaflavins in black tea were shown to be better scavengers of superoxide than gallocatechins (Jovanovic *et al.*, 2000).

9.2.5.1 Inhibition of Lipid Oxidation by Catechins

Green tea catechins inhibit the oxidation of LDL and the accompanying cholesterol oxidation (Osada *et al.*, 2001).

Katiyar *et al.* (1994) demonstrated inhibition of lipid peroxidation in mouse epidermal microsomes by epicatechin derivatives from green tea.

Epidermal microsomal protein was incubated with ferric ions and ADP. After one hour, the reaction was terminated by the addition of trichloroacetic acid. The production of malondialdehyde (MDA) was used as an indication of lipid peroxidation. MDA levels were measured by spectrophotometry after reaction with thiobarbituric acid.

Doses of 50, 100 and 200 μmol of epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG) and epigallocatechingallate (EGCG) were added separately to reaction mixtures to measure inhibition against a control. The control had

a value of 10.69 nmol of MDA equivalent. For the 50, 100 and 200 μmol doses, EC gave, respectively, nmol MDA equivalents of 10.51, 10.30 and 7.65; EGC 9.55, 8.95 and 6.81; ECG 8.47, 7.11 and 5.10; and EGCG 6.79, 5.25 and 3.15.

In a similar experiment, Hong *et al.* (1994) demonstrated that catechin derivatives obtained from tea inhibit the lipid peroxidation of rat heart mitochondria.

Miura *et al.* (1994) examined the effect of tea polyphenols on lipid peroxidation in LDL. The LDL fraction was separated from porcine serum by ultra centrifugation, and incubated with copper sulphate and ECG, EGCG, EC, C and EGC, in separate reaction mixtures. The antioxidant, dibutyl hydroxy toluene (BHT), was used as standard. Peroxidation was estimated by measuring the thiobarbituric acid reactive substances (TBARS) formed. The TBARS values obtained were ECG 0.95; EGCG 1.03; EC 1.13; C 1.36; EGC 2.74; and BHT 3.09. This indicates that inhibition of LDL lipid peroxidation by tea catechins is better than by BHT.

9.2.5.2 Inhibition of Lipid Oxidation by TFs and TRs

Since most of the catechin derivatives in green leaf are converted to theaflavins (TFs) and thearubigins (TRs) during black tea manufacture, Yoshino *et al.* (1994) studied the ability of TFs and TRs to inhibit lipid peroxidation. Lipid peroxidation in rat liver homogenate was initiated by adding t-butyl hydroperoxide, and peroxidation measured by the thiobarbituric acid reactive substances (TBARS) formed. The inhibition potential of TFs and TRs was more than that of C and EC. On the other hand, ECG, EGC and EGCG had more potential than TFs and TRs. All the tea derivatives were found to be more effective than glutathione (GSH) and α-tocopherol.

In similar experiments, Shiraki *et al.* (1994) showed that TFs isolated from black tea inhibited lipid peroxidation, initiated by t-butyl hydroperoxide, in both rabbit erythrocytic membrane and rat liver microsomes.

9.2.5.3 Inhibition by Tea of Damage to DNA *in Vitro*

Another important area where the effect of tea components has been tested is the prevention of damage to DNA.

Shiraki *et al.* (1994) also investigated the effect of TFs on DNA single-strand cleavage by hydrogen peroxide. A bacteriophage RFI DNA was used for the experiment. RFI DNA is a super coiled double-strand circular DNA. Any single-strand cleavage can be detected by observing the change of the RFI form to the RFII form, which is a relaxed circular form. RFI DNA was treated with hydrogen peroxide, cytochrome c (Fe^{2+}) and diethylenetriaminepentaacetate to induce the cleavage. Reaction mixtures were incubated and electrophoresed on agarose gel to separate and quantify the RFI and RFII forms. Addition of TFs to reaction mixtures reduced the strand cleavage by 60 per cent, while α-tocopherol reduced it by only 30 per cent.

TFs from black tea showed inhibitory effects on H_2O_2-induced and tert-butyl hydroperoxide (tBuOOH)-induced cytotoxicity (evaluated by the tetrazolium bromide reduction), cellular oxidative stress (detected by the oxidation of 2', 7'-dichlorofluorescin), and DNA damage (measured by the amount of 8-OhdG) in rat normal liver epithelium cell RL-34 cell lines (Feng *et al.*, 2002).

9.2.5.4 Limitation by Green Tea of Death of Gut Cells

Cells from human gastric, and mice small intestinal,

epithelium were exposed to the oxidants, DPPH, H_2O_2 and peroxynitrite, and then incubated with vitamin C, or extracts of green tea or the plant called cat's claw, in order to compare their antioxidant action (Miller *et al.*, 2001).

The extracts did not interact with H_2O_2 but did with DPPH (both as measured by spectroscopy), although less effectively than did vitamin C.

In contrast, vitamin C was significantly less effective in protecting the human cells from apoptosis induced by DPPH, H_2O_2 and peroxynitrite. The extracts were equally protective against H_2O_2 and peroxynitrite, but green tea was more effective than cat's claw in reducing DPPH-induced apoptosis.

Necrotic cell death was just evident with H_2O_2 and peroxynitrite, but was attenuated both by green tea and cat's claw.

In the mice cells, vitamin C and the extracts were equally effective against apoptosis.

All three antioxidants, vitamin C and the extracts, were therefore able to limit cell death in the gut epithelium induced by oxidative stress.

9.2.6 Tea Polyphenols *in Vivo*

After it became clear that tea flavonoids could act as antioxidants *in vitro*, the effect of tea components in biological systems within the body, *in vivo*, were investigated. A considerable number of experiments have been done to date, and some of these are described in this section.

Lipid oxidation, especially low-density lipoprotein (LDL) oxidation, facilitates the atherogenic process (the build-up of fatty or cholesterol deposits in the arteries). Therefore, the effect of tea

components to inhibit lipid oxidation have been tested in a number of living systems.

9.2.6.1 Inhibition of Lipid Oxidation by Tea Ingestion

Sano *et al.* (1995) studied the effect of green tea and black tea powder in the diet of rats. The rats were divided into three groups with one group as control. One experimental group received green tea powder, and the other the same weight of black tea powder, in the diet. They were kept on these diets for 50 days, after which lipid peroxidation was induced in incubated liver slices using t-butyl hydroperoxide. The extent of lipid peroxidation was estimated by measuring TBARS.

Green tea and black tea in the diet reduced lipid peroxidation by 23 per cent and 38 per cent, respectively.

However, since powdered green and black tea was used, it can be argued that some of the components in the powder would not necessarily be present in the infusion brewed for drinking, and that therefore no conclusions can be drawn from this experiment on the effects of normal tea drinking.

Hodgson *et al.* (2000), studying the acute effects of ingestion of black and green tea in 20 healthy men, found that black tea had a mild acute effect on *ex vivo* lipoprotein oxidation in serum.

The effect of catechins, isolated from green tea, on serum α-tocopherol levels and lipid peroxidation has been investigated in Wistar rats (Nanjo *et al*, 1993). The rats were divided into four groups. The diet of two groups contained palm oil, and that of the other two perilla oil. (Palm oil is rich in saturated and monounsaturated fatty acids, and perilla oil in unsaturated fatty acids.) One of the palm oil and one of the perilla oil groups received a crude catechin mixture from green tea. The other

dietary ingredients, including α-tocopherol, were similar in all four groups. After one month on these diets, plasma samples were obtained from the rats. Plasma α-tocopherol and TBARS (to measure the level of lipid peroxidation) were measured. The α-tocopherol concentrations in the plasma samples (μg/dl) from the four groups were: palm oil (433), palm oil + catechin (493), perilla oil (55), and perilla oil + catechin (134).

The conclusion is that α-tocopherol in the plasma of the perilla oil rats decreased because it was acting as an antioxidant. Addition of catechins to both palm oil and perilla oil diets had increased the plasma α-tocopherol levels, suggesting that tea catechins may be counteracting the decrease in α-tocopherol by acting as an antioxidant themselves.

The plasma TBARS (nmol/ml) in the four groups were: palm oil (1.18), palm oil + catechin (1.16), perilla oil (3.86), and perilla oil + catechin (3.36). (The differences between the values for perilla oil and for perilla oil + catechin are statistically significant.) The reduction of TBARS in the catechin groups suggest that the catechins inhibit lipid peroxidation.

9.2.6.2 Inhibition by Tea of Damage to DNA *in vivo*

Green tea was found to give protection against benzo[a]pyrene (B[a]P)-induced mutations in the liver of mice (lacI transgenic male mice, C57BL/6 Big Blue) (Jiang *et al.*, 2001). The mice were given a 2 per cent green tea hot-water extract as their sole source of drinking water for 10 weeks. After 7 weeks, they were given B[a]P.

Mice treated with B[a]P gave a two-fold higher lacI mutant frequency than did the untreated controls. B[a]P also increased the frequency of the mice's characteristic mutation (GC →TA

transversions) nearly five-fold. However, in the mice given green tea, the induced B[a]P mutant frequency decreased by 63 per cent, while the GC → TA transversions were reduced by 54 per cent.

Lodovici *et al.* (2000) investigated the effect of black tea polyphenols on 1, 2-dimethylhydrazine (DMH)-induced oxidative DNA damage in rat colon mucosa. Rats (Fischer 344) were treated orally with thearubigins (TRs) or theaflavins (TFs) for 10 days, and then injected intra-peritoneally with DMH or saline. Rats pre-treated with TR had significantly less DMH-induced oxidative DNA damage. A similar, although less marked, effect was observed with TF.

The heterocyclic amine, 2- amino -1- methyl- 6- phenylimidazo [4,5-b]pyridine (PhIP), a carcinogen, is formed during the cooking of proteinaceous animal foods (meat, chicken and fish). Inhibition of PhIP-DNA adduct formation is likely to lead to inhibition of PhIP carcinogenicity.

The chemopreventive properties of green and black tea on PhIP carcinogenesis were evaluated by their effects on PhIP-DNA adduct formation in female F-344 rats (Schut and Yao, 2000). Compared with the rats given regular drinking water, PhIP-DNA adduct formation was inhibited in small intestine, colon, liver, and mammary epithelial cells of rats given green or black tea as the sole source of drinking fluid.

9.3 Green and Black Tea against Disease

Results from the studies mentioned above provide evidence for the antioxidative action of tea components, both under *in vitro* conditions and under biological conditions, *in vivo*, and indicate how tea helps in the prevention of disease. These experiments

show that both green and black tea have preventive effects against oxidant-manifested diseases.

9.4 Tea against Ageing

Ageing is thought to be due to a programming of the genes to shut down after a finite lifespan which is characteristic of the animal species concerned. More specifically, it might be due to deterioration with time of the body's oxidative defence mechanisms because of a genetically-programmed reduction in the synthesis of natural antioxidants in the cells (Wickens, 2001).

The diminution of natural antioxidants would lead to a sharp increase in a host of degenerative diseases, characterised as diseases of old age, which are the result of attack by FRs, both ROSs and reactive nitrogen species. Premature ageing is also the result of injury to metabolic systems brought on by these reactive species (Weisburger, 2000). Tea polyphenols stave off these changes and would, therefore, contribute to the maintenance of health and longevity.

It has been postulated, from work with mice at the Massachusetts Institute of Technology and elsewhere, that ageing is a side effect of the operation of specific proteins, that serve to prevent the uncontrolled cell growth that leads to tumours and cancers.

Means of slowing the rate of depredations by FRs in the aged are being studied. It has been shown, using animal models, that administering antioxidants could increase lifespan. Although in humans, there is growing evidence that diet supplemented with antioxidants may help in preventing a variety of diseases, such as cancer and cardio-vascular diseases, it is not conclusively proven with human subjects that dietary antioxidants could

contribute to longevity. However, antioxidants could limit cell damage occurring during the diseases of old age, and thereby improve the quality of life of the elderly (Venarucci, 1999).

Advances in medical science over the last few centuries, by controlling life-threatening infectious diseases, have largely contributed to an increase in the human lifespan (from 40 years to about 75 years, at least in the developed world), and the proportion of the elderly in the world is therefore increasing.

According to the World Health Organisation (1998), by the year 2020 the world population will be 1000 million, of which 700 million will be in the developing countries. In the developed world, particularly, the ageing population is growing at a rapid rate. People over 60 years of age in 2020, as a percentile of total population, will be: Europe 25, North America 23, East Asia 17, Latin America 12 and South Asia 10. The percentiles in the poorer countries (in Latin America and South Asia) will roughly be what they are now.

As a prelude to its Second World Assembly on Ageing (to be held in Madrid, April 2002), the United Nations reports that in the next 50 years the number of people over 60 years of age in the world will nearly quadruple (from 629 million in 2002 to almost 2 billion in 2050). Today one in every ten is 60 years or over; by 2050 it will be one in every five.

The chronic degenerative diseases of the elderly are the biggest problem faced by medical scientists at the present time. An ever-ageing population will add to this problem, and there will be an increased demand for health-care services for cardiovascular, neurological, cancer and rheumatological diseases, and other physical and mental problems of the old. This will impose bigger burdens on the health services of countries, and consume a greater

part of national incomes. In addition, there would be indirect costs: a loss of productive life, hospitalisation, permanent disabilities, and negative financial and emotional effects on close associates and relatives.

Relatively simple and cheap preventive, or ameliorative, measures would be of huge economic benefit, as well as improving the quality of life of individuals. A balanced and sensible diet would be a major component of these measures, and tea as a part of that diet would certainly be invaluable, according to reports from many sources.

CHAPTER 10

TEA AND CARDIOVASCULAR DISEASES

Cardiovascular diseases (CVDs) affect the heart and the blood vessels (arteries and veins) that circulate blood in the body. The coronary arteries supply the wall of heart with oxygen-containing blood, and this maintains the heart's pumping action.

10.1 Coronary Heart Disease

The most common heart disease is coronary heart disease (CHD), where there is partial blockage of the coronary arteries and restriction of blood flow owing to the thickening of the arterial walls (arteriosclerosis), or complete blockage from a blood clot or thrombus (thrombosis) in the thickened arteries. The build-up of plaques (deposits comprising cholesterol, the waste products of cells, and calcium) on the inner lining of thickened arteries, called atherosclerosis, contributes to blockage.

10.2 Ischemia, Stroke and Atherosclerosis

Deficiency in the blood supply to the heart muscle is called

ischemic heart disease or ischemia, which could result in malfunction or death of heart muscle (called a myocardial infarction or a heart attack).

A similar condition in the brain, cerebrovascular disease, is commonly called a stroke. The damage from a stroke could be either due to ischemia as in the heart, or to hemorrhage owing to rupture of a blood vessel.

Atherosclerosis, which leads to heart disease and stroke, develops in stages. The most notable risk factors are an inherited or family prediposition, high blood-cholesterol levels, high blood pressure, diabetes and the smoking habit.

10.3 Low-Density Lipoproteins and Plaque Formation

The transport medium for nutrients and other molecules in the body is the blood stream. Fat or lipids are transported bound with proteins as lipoproteins. Lipoproteins are broadly divided into high-density lipoproteins (HDL) and low-density lipoproteins (LDL). Cholesterol-carrying LDLs are believed to play an important role in atherogenesis.

LDL itself is not considered atherogenic, but oxidised LDL is. Oxidative stress, or the presence of large amounts of FRs, accelerates LDL oxidation. Oxidised LDL gets entrapped in the artery walls, in their sub-endothelial spaces. Immune cells get attracted to the site, specially monocytes and monocyte-derived macrophages. Macrophages accumulate excessive amounts of oxidised LDL and become foam cells. This lesion, the fatty streak, progresses into a plaque by accumulation of more LDL.

The concentrations of activated immune cells, the macrophages, also result in inflammation reactions. In

inflammation, more FRs are produced by the macrophages and the situation is aggravated. Antioxidants therefore play a major role in preventing initiation and progression of the atheromatous plaque.

In addition to plaque formation which reduces the inner diameter of arteries and restricts blood flow, the artery walls also lose their elasticity and become more rigid. In the advanced stages of atherosclerosis, artery walls get damaged or ulcerated, causing blood clots and complete blocking of the arteries.

10.4 Study Methods

Measurements of antioxidant activity in the body fluids using the methods detailed in Section 9.2, and determinations of blood lipids (cholesterol, LDL and HDL), platelet aggregation and the factors which facilitate blood clotting, are used in correlations with tea drinking, in order to assess tea's effectiveness against cardiovascular diseases.

10.5 Tea against Atherosclerosis

Studies with isolated cells and animal models have demonstrated that tea flavonoids inhibit inflammatory activity, and would therefore be expected to stop or delay the progression of atherosclerosis, which is considered to be a disease with a strong inflammatory component. Although doubts were initially expressed about extrapolating findings in isolated cells and animals to humans (Kenny *et al.*, 1990; Sakagami *et al.*, 1992), it is now accepted, on evidence from clinical studies, that tea flavonoids inhibit inflammatory activity and atherosclerosis in humans too (Libby *et al.*, 2002).

In a study by Vinson *et al.* (2001), foam-cell formation was induced in weanling hamsters. The consumption of both green and black tea significantly inhibited atherosclerosis, with no significant difference between green and black tea at human-equivalent doses. Three mechanisms of operation of the administered tea are reported: antioxidant, hypolipemic (reducing lipid levels), and hypofibrinogenic (reducing fibrinogen, the precursor of fibrin which is involved in blood clotting).

Xuan *et al.* (2001) showed that tea inhibits the adhesion between monocytes and vascular endothelial cells induced by oxidised LDL, thus limiting atherosclerosis.

10.6 Tea and Heart Diseases: Epidemiological Studies

The results from epidemiological studies strongly suggest a protective rôle for tea and flavonols with respect to cardiovascular diseases. Some studies have found that tea consumption has a protective effect against the risk of coronary heart disease (CHD), possibly caused by flavonoids acting as antioxidants (Thelle, 1995).

10.6.1 Flavonols and Flavones against CHD and Stroke

In a 25-year cross-cultural study of 16 cohorts (12,763 men; 40-59 years) in seven European countries, flavonol and flavone intake in the diet was inversely related to mortality from CHD (Hertog *et al.*, 1995).

One of the cohorts of this study, a Dutch cohort of 805 men (65-84 years), were in a 5-year study, the Zutphen Elderly Study (ZES). It was found here that the risk of dying from CHD was significantly lower in men with a high daily intake of tea flavonol and flavone: a reduction in mortality risk of more than 50 per

cent at an average flavonol intake of 42 mg/day (Hertog *et al.*, 1993 a). This effect was independent of the major established risk factors. A follow-up after 10 years confirmed the earlier findings, this time a dose-response relationship between flavonol intake and CHD mortality being established (Hertog *et al.*, 1997 a).

In the ZES, flavonol and flavone intake was also associated with a lower incidence of stroke in 552 men (50-69 years) after the total period of 15 years (Keli *et al.*, 1996). The risk reduction was about 70 per cent at an average intake of over 30 mg/day.

In a 20-year study of a Finnish cohort of 5,133 men and women (30–69 years old), death from CHD was inversely related to flavonol and flavone intake, but not strongly (Knekt *et al.*, 1996). The relative risks between the highest intake (over 5 mg/day) and the lowest intake (less than 2.5 mg/day) were 0.73 and 0.67 for men and women, respectively.

A study, with 34,789 male health professionals (40-75 years) in the USA, found a weak inverse association between intake of flavonol (7–40 mg/day) and flavone, and CHD mortality, but only for those with a history of CHD (Rimm *et al.*, 1996)

An apparently contradictory result to other epidemiological studies (where there was a negative or zero relationship) was given by the Caerphilly Study in Wales on a population of men: flavonol intake (14-43 mg/day) was found to be *positively* related to ischemic heart disease (Hertog *et al.*, 1997 b).

According to the authors, the reason for the contradiction was that heavy tea drinkers in Wales were from a social class characterised by an unhealthy life style (smoking and high fat-intake). In more sophisticated groups, tea drinking is commonly associated with a healthy life style.

The authors suggested a further reason. The men drank their tea with milk, and the milk proteins could have inhibited absorption of flavonoids. However, other results show that milk in tea has no effect on the levels of catechins (Van het Hof *et al.*, 1998) and flavanols (P.C.H. Hollman, unpublished) in human plasma.

10.6.2 Black Tea against Myocardial Infarction

The first indication of the protective effect of a high consumption of black tea on ischaemic heart disease was given by the Boston Collaborative Surveillance Programme in 1972. A non-significant risk reduction for a myocardial infarction of 34 per cent was found for drinkers of more than six cups of tea a day, as against non-tea drinkers.

In the ZES (Hertog *et al.*, 1993 a), the daily flavonoid intake had an inverse relationship to the incidence of myocardial infarction. Sixty-one per cent of the dietary flavonoids came from black tea, as against 13 per cent from onions and 10 per cent from apples, the other major sources. The incidence of myocardial infarction for three levels of flavonoid intake, low (0-19.0 mg/day), medium (19.1-29.9 mg/day) and high (more than 29.9 mg/day) were, per 1000 persons, 16.2, 13.8 and 7.6, respectively.

Daily black tea consumption at three levels, low (0-2 cups), medium (2-4 cups), and high (more than 4 cups) gave an incidence of myocardial infarction of 17.1 per cent, 8.1 per cent and 9.5 per cent, respectively.

A study in Finland using 25,372 smokers has found that intake of flavonols show an inverse relation to myocardial infarction (but only a weak inverse relation to CHD mortality) (Hirvonen *et al.*, 2001).

The association of tea and flavonoid intake with myocardial infarction was examined in the general Dutch population, using the data from the Rotterdam Study of 4,807 men and women, aged 55 years and over, who had no history of myocardial infarction (Geleijnse *et al.*, 2002). The study lasted from 1990-1993 until 31 December 1997. Data were adjusted for age, gender, body mass, smoking, education level, and daily intake of alcohol, coffee, polyunsaturated and saturated fats, fibre, vitamin E and total energy.

During 5.6 years of follow-up, 146 first myocardial infarctions occurred, 30 of which were fatal. The relative risk of myocardial infarction was lower in tea-drinkers, with an intake of over 375 ml per day, than in non tea-drinkers. The inverse relation with tea drinking was stronger for fatal than for non-fatal events.

The authors conclude that an increased intake of tea and flavonoids may contribute to the primary prevention of ischemic heart disease.

Meta-analysis of the data indicates that three cups of black tea per day results in a 11 per cent reduction in risk of myocardial infarction (Balentine, 2001).

10.6.3 Catechins against Ischemia

It was confirmed in the ZES that catechin intake is inversely related to the risk of ischemic heart disease, but not the risk for stroke (Arts *et al.*, 2001).

10.6.4 Regulation of Serum Lipids and Blood Pressure

In a cross-sectional study with 1,371 men in Yoshimi, Japan, increased green tea consumption was associated with decreased

concentrations in the serum of total cholesterol and triglyceride, an increase in HDL cholesterol and a decrease in LDL cholesterol (Imai and Nakachi, 1995). Three levels of tea were consumed: 0-3, 4-9, and > 9 cups. Total cholesterol for these levels were, respectively, 4.85, 4.76 and 4.58 mmol/l; triglyceride 1.65, 1.60 and 1.45 nmol/l; HDL cholesterol 36.4, 36.5 and 37.4 per cent; and LDL cholesterol 62.5, 62.6 and 61.7 per cent.

Although the values are statistically significant and the conclusion is made, from these results, that green tea may protect from cardiovascular disease, it may be argued also that the relatively small changes in the serum values can have no clinical significance.

In a similar study with men in Northern Kyushu, Japan, green tea consumption was found to be inversely related to serum cholesterol levels (Kono *et al.*, 1992). Four levels of daily green tea were consumed: 0-2, 3-5, 6-8 and > 9 cups. Total cholesterol for these levels was 193, 190, 187 and 185 mg/dl, respectively. However no association was found with triglyceride or HDL cholesterol.

A study in Norway, with 9,856 men and 10,233 women, showed that black tea consumption was inversely related to total serum cholesterol and systolic blood pressure (Stensvold *et al.*, 1992). Four levels of daily black tea were consumed: 0-1, 1-2, 3-4 and > 5, cups. In the men, total serum cholesterol for these levels was 6.24, 6.20, 5.96 and 6.19 mmol/l, respectively, and blood pressure was 136.2, 135.0, 135.9 and 133.1 mm, respectively. In the women, total serum cholesterol was 6.11, 5.96, 5.89 and 5.92 mmol/l, respectively, and blood pressure was 131.7, 130.2, 127.9 and 127.2 mm, respectively.

However, a study in Israel, with 3,858 men and 1,511 women, showed no significant association between black tea consumption and serum cholesterol levels (Green and Harari, 1992). However in this study, only a small percentage of subjects consumed tea at the higher levels. The percentiles of men consuming the five levels of tea daily (0, 1-2, 3-4, 5 and > 5 cups) were 23.4, 61.1, 11.9, 1.5 and 1.9, respectively. The corresponding percentiles of women were 27.4, 58.9, 11.3, 1.3 and 0.9, respectively.

The Scottish Heart Health Study (with 10,359 people in 22 Scottish districts consuming a mean of 4 cups of black tea per day) found no relationship between tea consumption and diagnosis of CHD or serum cholesterol levels (Brown *et al*, 1993).

10.6.5 Tea against Blood Clotting

In addition to red- and white blood cells, the blood also contains platelets which are small, detached cell fragments. Platelets, by their aggregation, play a central rôle in blood clot or thrombus formation, and also help to repair breaches in the walls of blood vessels.

Tea polyphenols have been found to inhibit platelet aggregation and blood coagulation, strengthen the walls of blood vessels, and interact with catecholamines in anti-inflammatory activity (Tijburg *et al.*, 1997 a).

In a cross-over epidemiological study in Australia with 22 men, a consumption of five cups of tea per day was compared with hot water consumption for five weeks, and the plasma levels of molecules, called p-selectin, which promote cell adhesion were measured (Hodgson *et al.*, 2001). It was found that tea drinking lowers p-selectin levels, indicating that tea may act as an anti platelet-aggregator and so reduce thrombus formation.

In a similar placebo control, cross-over study in the U.S., six cups of black tea (3.6 g tea solids, 1.2 g tea flavonoids) per day for three weeks reduced platelet function induced by collagen (Balentine, 2001).

10.7 Tea and Changes in Blood: Animal Studies

10.7.1 Serum Lipids

Experiments with animals have given convincing data on the lipid-lowering effects of tea.

Several animal studies have demonstrated the anti-atherogenic and cholesterol-lowering effects of tea components. For example, a significant decrease in serum cholesterol and triglycerides was found when rats were given dried tea or polyphenol-containing black tea fractions.

Consumption of green-tea polyphenols for four months significantly reduced aortic atherosclerosis in hypercholesterolaemic rabbits (that is, those with high serum cholesterol levels). Consumption of mixed catechins decreased serum cholesterol levels in rats fed an atherogenic diet.

Black, oolong and green teas reduced serum cholesterol to normal levels in experimental rats in which cholesterol levels had been elevated by giving a high sucrose diet (Yang *et al.*, 2001).

Wistar rats fed crude catechins (isolated from green tea extract), at levels of one and two per cent of a cholesterol-rich diet, showed significantly decreased total cholesterol concentrations in the serum (Muramatsu *et al.*, 1986). Addition of one per cent cholesterol to the diet significantly increased the cholesterol concentration, when compared with rats not receiving

cholesterol in the diet. However, addition of one per cent tea catechins decreased serum cholesterol to a level slightly above the baseline, while a two per cent addition brought cholesterol levels down to the baseline value.

Other authors have reported similar results (Yamaguchi *et al.*, 1991; Ali *et al.*, 1988).

The observed reduction of serum cholesterol levels may occur owing to the decreased absorption of cholesterol from the gastro-intestinal tract, under the influence of tea polyphenols. Thus, both cholesterol and triglyceride absorption was decreased in the presence of tea catechins. This decreased absorption may be due to decreased micellar solubility of cholesterol (Ikeda *et al.*, 1992). In the same experiment, it was observed that tea polyphenols precipitated cholesterol solubilised in mixed bile-salt micelles.

To find the effect of green tea on the lipid levels in body tissues, female mice were fed diets containing one, two and four per cent green-tea powder for 16 weeks (Sayama *et al.*, 2000). It was found that the concentrations of total cholesterol in the liver, triglycerides in serum and the liver, and non-esterified fatty acids in serum, of mice administered the green tea diets were lower than in the controls.

10.7.2 Platelet Aggregation

Mitane *et al.* (1990) studied the inhibitory effects of tea catechins on rabbit platelet aggregation *in vitro*. It was found that 0.025, 0.05, 0.1 and 0.2 mg/ml of epigallocatechin (EGCG) isolated from green tea inhibited platelet aggregation in a dose-dependent manner. At the 0.2 mg/ml level, aggregation was completely inhibited.

10.8 Tea against Endothelial Dysfunction

Drinking tea restores the normal function of the inner lining of arteries, the endothelium, in people in danger of heart disease and stroke. The endothelium functions by causing the arterial walls to expand to accommodate blood flow. It does this by releasing nitric oxide which relaxes the smooth muscle cells making up the outer lining of the arteries. One feature of coronary artery disease is that the arterial walls are not able to expand sufficiently. The nitric oxide released by the endothelium of the blood vessels is the signal that modulates blood flow in the body.

Endothelial vasomotor dysfunction is related to oxidative stress and is an early phase of atherosclerosis; it occurs in those with atherosclerosis and other risk factors for coronary disease such as hypertension and hypercholesterolemia (Balentine, 2001).

In an elegant, non-invasive study, Duffy *et al.* (2001) quantified endothelial function as 'flow mediated dilatation' (FMD) of the brachial artery in the human arm.

Sixty-six patients with coronary artery disease were given black tea and water in a cross-over design. Short-term effects were examined two hours after the consumption of 450 ml of tea or water, and long-term effects after the consumption of 900 ml of tea or water every day for four weeks. Plasma flavonoids increased after short- and long-term tea consumption.

Vasomotor function of the brachial artery was examined, initially and following each intervention, with vascular ultrasound.

Both short- and long-term tea consumption raised the FMD to about the same extent. Consumption of water had no effect. Acute tea consumption, following on chronic tea consumption,

gave an additional increase in FMD over that from the two types of consumption separately.

Equivalent doses of caffeine had no short-term effect on FMD.

That tea acts directly on the endothelium is shown by the fact that its consumption has no effect on the response to nitroglycerin (which is an endothelium–independent vasodilator), on the size of the resting artery, or on blood pressure or heart rate.

A similar Australian study (Hodgson *et al.*, 2002) showed that five cups of black tea every day for four weeks, compared with hot water consumption, gave significant endothelium–dependent dilatation and endothelium–independent dilatation in the brachial artery. These results indicate that one way in which black tea may reduce cardiovascular risk is by improving vasodilation of conduit arteries.

Flavonoids reportedly accumulate between the endothelium and the smooth muscle cells, and the beneficial effect of tea is probably due to a boosting of nitric oxide metabolism by its flavonoids (Balentine, 2001).

The findings of Duffy *et al.* (2001) and Hodgson *et al* (2002) partly explain the observed association between tea consumption and decreased cardiovascular disease events.

10.9 Conclusion: Tea reduces the Risk of CVD

From the epidemiological studies, it is clear that tea consumption reduces the risk of cardiovascular diseases (Balentine, 2001). Clinical and other studies to date support this conclusion: consumption of tea, including black tea, does promote cardiovascular health, for example through improved vascular function and decreased aggregation of platelets.

CHAPTER 11

TEA AND DIABETES

Diabetes is a degenerative disease which must be managed or kept in check, but which cannot be cured. There are an estimated 135 million diabetics in the world (World Health Report, 1997).

There are two types of diabetes: type 1 or insulin-dependent diabetes mellitus (IDDM), and type 2 or non-insulin-dependent diabetes mellitus (NIDDM). Approximately 90 per cent of diabetics have NIDDM, which manifests in adults.

In Britain, in a population of nearly 59 million, there are at least one million people with NIDDM, most of them over 40 years. According to National Statistics, the total number of diabetics in Britain is predicted to be 1.51 million by 2023.

India has nearly 30 million diabetics in a total population of over one billion. According to the Voluntary Health Service and the National Institute of Epidemiology, Chennai, 99.6 per cent of the diabetics have NIDDM. The proportion of NIDDM

incidence increases with age: 4.16 per cent in those below 20 years of age to 9.25 per cent in those over 40 years.

The World Health Organisation's projection is that the number of NIDDM cases in Asia as a whole will increase by almost 50 per cent by 2010.

11.1 Insulin Production and Glucose Uptake

The hormone, insulin, is secreted by endocrine cells in the pancreas, and functions in carbohydrate utilisation by the body (starting with glucose uptake into metabolising cells), and stimulation of protein and lipid synthesis (or lipogenesis). Lipogenesis occurs in fat cells or adipocytes.

High blood glucose levels or hyperglycaemia, usually after meals, lead to increased secretion of insulin into the blood. Within minutes, the insulin increase stimulates liver and muscle cells to take up and metabolise more glucose, and at a higher rate. This causes blood glucose levels and the rate of insulin secretion to decrease, and the rate of glucose uptake by the cells returns to the basal level. Thus, in individuals with normal metabolism, blood glucose would be maintained at a constant concentration of 3.6-6.7 mM/l (or 65-120 mg/dl).

11.2 Insulin Receptors

Insulin molecules in the blood bind to specific proteins, located on the outer surface of cell membranes, called insulin receptors. The insulin-receptor complex then enters the cell through its outer membrane, thus temporarily diminishing the concentration of insulin receptors on the cell surface.

Molecules other than insulin, namely specific antibodies, can

bind to the insulin receptors, enter the metabolising cells, and there mimic the effects of insulin.

11.3 Diabetes: Types 1 and 2

Type 1 diabetes or IDDM is defined as the condition in which the pancreas does not produce enough insulin. Type 2 diabetes or NIDDM is when the body cells cannot utilise the insulin that is produced.

Chronically high blood glucose levels lead to persistently elevated insulin levels in the blood. Owing to the constant stimulation by insulin, cells normally responsive to insulin could become relatively insensitive to it, because of the entry of a high proportion of the insulin receptors, bound to insulin, into the cells with a resultant drop in receptor concentration on the outer surface. In such an event, normal individuals would secrete more insulin to compensate. However, in type 1 diabetics with impaired insulin production, this is not possible and high blood glucose levels persist.

High blood glucose in type 2 diabetics is because, although sufficient amounts of insulin are produced by the pancreas, the cells progressively stop utilising the insulin and glucose uptake is reduced.

11.4 Insulin-like Activity of Tea and Other Extracts

Broadhurst *et al.* (2000) tested extracts from 49 different plants for insulin-dependent utilisation of glucose oxidation. Adipocytes isolated from fat 'pads' taken from the rat epididymis, the duct carrying sperm to the vas deferens, are a suitable means of bio-assay in such studies.

Cinnamon was found to be the most effective in directly stimulating glucose metabolism by the cells, with witch hazel, green and black teas coming next, in that order. The bioactivity of these highly effective, anti-diabetic plant extracts, but not of some of the others, seems to depend on their phenolic structure.

The flavonol, myricetin, present in black tea and some other plants was found to mimic insulin in its stimulation of lipogenesis and glucose uptake through cell membranes, in rat adipocytes *in vitro* (Ong and Khoo, 1996). The myricetin molecules probably function by becoming interposed into the membrane bilayer, and therefore may have therapeutic value, in managing non-insulin-dependent diabetes (NIDDM), by stimulating glucose uptake in the absence of fully functional insulin receptors.

Humans with NIDDM exhibit lowered activity of the enzyme, acetylcholinesterase (AChE), and this has been linked to certain brain defects. Since AChE in the brain and AChE bound to the membrane of red blood cells, or erythrocytes, are reported to be similar, Rizvi and Zaid (2001) studied the *in vitro* effect of both insulin and epicatechin from tea on erythrocytic AChE in normal and diabetic subjects. The AChE activity was significantly lower in the diabetics than in the normal controls. However, both insulin and epicatechin restored AChE activity to normal levels in the diabetics, indicating that epicatechin is able to mimic insulin.

11.5 Tea against Diabetic Hyperglycaemia and Cataracts

The enzyme, α-amylase, catalyses the hydrolysis of starch to glucose. Catechins in tea are known to inhibit α-amylase activity in the human small intestine (Hara, 1997), and by this means could serve to reduce glucose levels in the blood.

Black tea significantly reduced blood glucose levels in rats

in which diabetes had been induced by streptozotocin (STZ) (Gomes *et al.*, 1995). The tea had both preventive and curative effects on the diabetic rats. (Green tea is also known to be effective.)

Extracts of oolong tea fed to STZ-diabetic mice and KK-A^y diabetic mice lowered their blood glucose levels, but did not increase the blood insulin levels in the STZ-mice (Sugihara *et al.*, 2001). This indicates that the lowering of blood glucose by tea extracts was not through insulin release.

In another study, rats were injected with STZ to induce diabetes, one week before their diet was supplemented with green or black teas for three months (Vinson *et al.*, 2001). Diabetes, indicated by high blood glucose and diabetic cataracts, were significantly inhibited by both teas. In addition, green tea significantly decreased blood lipids.

Tea, which in this case happened to be grown with organic inputs only ('bio-tea'), was administered to normal albino mice, where it caused a significant decrease in blood glucose, or hypoglycaemia, after 30 minutes, with the minimum level at two hours (Shenoy, 2000). Normal blood glucose was restored after eight hours.

The tea-treated mice showed a significant decrease in blood glucose in a glucose tolerance test, one hour after they were given glucose.

In the same study, diabetes was induced in the albino mice by alloxan, and they were then given five doses of the tea for three days. A continuous decrease in blood glucose occurred four hours after administration of the last dose, and the hypoglycaemic effect persisted thereafter.

A polysaccharide-protein complex obtained from so-called 'coarse' tea was injected into normal mice and model mice with high blood glucose (Wang *et al.*, 2001). Blood glucose decreased significantly in both types of mice.

It is important to note that diabetes is traditionally treated with 'coarse' tea in China and Japan.

Cataracts were induced in rat models by subcutaneous injection of selenite (Thiagarajan *et al.*, 2001). The models were injected every day with the human equivalent of six cups of green or black tea for 12 days. This treatment retarded the progresion of lens opacity, and after six weeks the cataracts were less advanced than in controls.

Although not well established, anthocyanins found in tea may decrease the risk or severity of diabetic retinopathy and other retinal pathologies (Scharrer and Ober, 1981). (Anthocyanins are charged flavonoids, usually with attached sugar moieties.)

11.6 Control of Diabetes by Tea in Rodent Models

A diet containing large amounts of the sugar, fructose, induces a condition resembling human NIDDM in rats. The condition is characterised by non-responsiveness to insulin, high triglyceride levels in the blood, and hypertension.

Wu *et al.* (2001) studied Sprague-Dawley rats in which diabetes had been induced by high fructose. They divided the rats into three groups: the first group, the control, was given their usual diet which included water; the second a fructose-rich diet also including water; and the third a fructose-rich diet and green tea instead of water. The rats were fed these diets for 12 weeks.

Unlike the control group, the fructose group, as expected, developed fasting hyperglycaemia, high triglyceride and high insulin levels in the blood, and systolic hypertension. There was decreased insulin binding to adipocyte cell membranes in the epididymal bio-assay, resulting in decreased glucose uptake.

However, the third group whose diet included green tea, showed none of these metabolic defects.

The conclusion is that tea in the diet controls diabetes.

Hwang *et al.* (2001) found that green tea may improve glucose tolerance and insulin responsiveness in Sprague-Dawley rats.

The rats were divided into two groups: one given water as the dietary fluid, and the other green tea, for 12 weeks. Green tea consumption improved both glucose tolerance and insulin sensitivity, and significantly reduced fasting plasma glucose, insulin, free fatty acids and triglycerides, when compared with water consumption. There was increased insulin binding and glucose uptake in the group given tea.

11.7 Risk of Diabetes in Children from Coffee and Tea

Diabetes developed during childhood is type 1. Virtanen *et al.* (1994) examined the possibility of children developing type 1 diabetes when they consumed coffee or tea before they were diagnosed as having diabetes, or when the parents did so at the time of conception or during pregnancy.

Six hundred newly-diagnosed diabetic children (diagnosed in 1986-1989), and 536 randomly selected children, all under 15 years of age, and their parents, participated in this nationwide study in Finland.

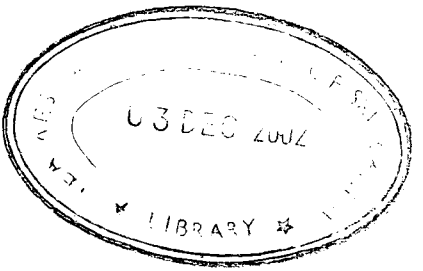
The conclusion was that the risk of type 1 diabetes increased in children who consumed at least two cups of coffee, or one or two cups of tea, per day. Adjustments were duly made for the mother's level of education and the child's age and gender.

Parental consumption of coffee or tea were found not to affect the risk of diabetes in the children.

11.8 Conclusion: Tea is of Benefit in Diabetes

Studies, both with human subjects and animal models, indicate that tea consumption can be highly beneficial for diabetics, and can even retard the development of the pathologies that they are ultimately prone to.

However, one study suggests that both tea and coffee consumption is best avoided by children.



CHAPTER 12

TEA AND CANCER

12.1 Carcinogenesis

Normally body cells remain localised in particular tissues or organs, growing and reproducing relatively slowly and restricted by controlling factors, at a rate just sufficient to replace those cells that die by natural or other means. The new cells keep the tissue or organ rejuvenated and constant in size.

In normal tissues, the optimum size is retained by adjustments in the controlling processes according to needs. For example, when cells are tightly packed in a tissue or organ and unable to move, they reproduce slowly. If the cell density is reduced, the reproduction rate increases and the cell density returns to normal. Thus, when a part of the liver is surgically removed, the surrounding cells multiply rapidly and the lost tissue is regenerated. Wound healing occurs similarly.

Cells become cancerous when restrictions on their reproduction are removed and they multiply unceasingly to

become tumours. Unlike normal cells, cancer cells frequently move into neighbouring tissues, where they initiate new tumours. Some enter the blood stream and reach more remote tissues to multiply there. This is called metastasis.

All cells contain genetic material or DNA (deoxyribonucleic acid), which control all their activities, and their growth and reproduction. In the initiation stage of a cancer, DNA is damaged, or undergoes a change or mutation, which alters the functions of the cells it controls and may initiate their transformation into cancer cells. However, in most cases, the altered cells merely die. Alterations in DNA expression cause the rapid growth and multiplication of the initiated cells, and additional mutations are necessary for an initiated cell to become cancerous. These constitute the promotional and progression stages of carcinogenesis.

Agents that cause mutations are called mutagens, and those that cause cancers are called carcinogens.

Although all mutations do not progress to cancer, any agent that can damage DNA is a potential carcinogen. Certain chemicals, radiation, smoke and viruses act as carcinogens.

Radiation, such as β-rays produced in nuclear reactors, causes mutations. Ultra violet (UV) radiation is a major cause of skin cancer.

Smoking has been for long a well-established cause of human carcinogenesis, as well as of cardiovascular disease. Cigarette smoke induces ROSs *in vitro* (Church *et al.*, 1985; Pryor, 1997). It also induces DNA single strand breaks in blood cells (Piperakis *et al.*, 1998), probably through oxidative damage to DNA (Howard *et al.*, 1998; Asami *et al.*, 1997).

12.2 Study Methods

Various methods are used to measure the effectiveness of tea consumption and tea components on the initiation, promotion and progression stages of carcinogenesis.

The mutagenicity or anti-mutagenicity of substances may be determined by the Ames test, initially developed by Bruce Ames.

In this method, use is made of a mutated bacterial species, *Salmonella typhimurium*, that carries a defective gene which prevents it synthesising the amino acid, histidine. The species cannot therefore survive in a medium that does not contain histidine. This mutation can however be reversed ('back mutation'), and 'revertants' (or *S. typhimurium* in which the mutation has been reversed) can survive in a medium lacking histidine. A mutagen will increase the number of bacteria surviving in a histidine-free medium, and conversely an anti-mutagen will decrease the number. This allows a quantitative estimation of mutagenicity or anti-mutagenicity.

S. typhimurium is a prokaryotic organism, and therefore not by any means a perfect model of the human organism. Consequently, many other, rapid methods are now being developed using the same principle. In these improved methods, eukaryotic cells such as yeast or mammalian cells are used together with modern, recombinant DNA technology.

The carcinogenicity or anti-carcinogenicity of substances are widely tested using animal models. Cancer is induced in various body organs of the models by chemical carcinogens or radiation, and the effects of anti-carcinogens are measured by the size or the numbers of tumours formed. The models are used to monitor the effects of test substances on the promotion and progression stages.

Transgenic mice, used in this kind of studies, provide easy and rapid methods of quantification. For example, hairless mice are used in skin cancer studies.

12.3 Inhibition of Carcinogenesis by Tea

The identification of EGCG in the late 1980s opened up a vista of cancer prevention in humans, arising from its success in inhibiting carcinogenesis in rodent models (Fujiki *et al.*, 1997) and in epidemiological studies with human cohorts (Imai *et al.*, 1997).

Research, both *in vivo* and *in vitro*, had indicated that tea and tea polyphenols interfere with or inhibit carcinogensis at initiation or during the further stages, by different mechanisms. Further, tea consumption decreases the growth rate of tumour cells and prevents metastasis and the formation of large tumours (Weisburger, 2001 a).

FRs and ROSs are implicated in the initial damage to DNA (mutagenesis) and in the further stages of carcinogensis. It is now well known that the antioxidant properties of tea flavonoids play a major role in cancer prevention (Weisburger, 2001 b). Over the years, many experiments have shown that tea extracts and their polyphenolic fractions reduce the mutagenic activity of chemical carcinogens and radiation.

Many studies carried out *in vitro*, and at many sites in animal models (skin, lung, stomach, colon, oesophagus, mammary glands, kidney, liver, pancreas and uterus), show that the components of both black tea and green tea have the ability to reduce the initiation, promotion and progression of cancer. In addition to the antioxidant activity of the tea components, other mechanisms also seem to be responsible.

Thus, tea flavonoids are easily oxidised to the corresponding quinones in a reversible reaction. Flavonoids can therefore act as both hydrogen acceptors and donors. This makes it possible for them to interact with the active form of most chemical carcinogens and convert them into inactive forms (Mukhtar *et al.*, 1994; Yang and Wang, 1993).

Most chemical carcinogens entering the body are not water-soluble and are therefore not in the active form. The cytochrome P-450 enzymes in the electron-transport chain, found in the liver within microsomes, bring about the hydroxylation of foreign compounds in general (called xenobiotics), making them more soluble and thereby facilitating their excretion from the body.

However, this beneficial action of the P-450 enzymes system, belonging to the so-called Phase I enzymes, has its downside. In the case of xenobiotics which are potential carcinogens, an increase in their water-solubility converts most of them into active carcinogens. In addition, in this process, ROSs and other FRs are formed. However, so-called Phase II enzymes, which are detoxifying enzymes such as those of the glutathione enzyme system and catalase, and others such as glucuronyl transferases and quinone reductases, act to convert them to innocuous forms that are excreted from the body.

Apart from neutralising ROSs and other FRs produced by the P-450 system, tea components inhibit some enzymes in the P-450 system thereby reducing the bioactivation of carcinogens. Flavanoids can react with active forms of carcinogens converting them into innocuous, neutral forms.

Of the Phase II enzymes, tea consumption increases the activity of glucuronyl transferases alone, and their increased levels bring about a lower carcinogenecity of many carcinogens (Weisburger, 1999 b).

Recent research has shown that, in addition, tea components interfere with the other mechanisms of cancer promotion. Thus, they alter the expression of certain genes. Down-regulation (or a diminution in expression) of four genes, NF-kappaB-inducing kinase, death-associated protein kinase 1, rhoB, and tyrosine-protein kinase, and up-regulation (or an increase in expression) of one gene, retinoic acid receptor alpha 1, were effected by tea components (Okabe *et al.*, 2001). Other studies have shown attenuation of the activation of the gene, kappaB, and resulting production of polypeptides called cytokines, certain of which act as growth promoters in some tumours (Levites, 2002; Surh *et al.*, 2001; Amarakoon *et al.* 1995).

Cell-to-cell signal transduction is also important in the development of cancer. Tea components are involved in the inhibition of these signal transduction pathways (Yang *et al.*, 2002; Wiseman *et al.*, 2001).

For the growth of cancer cells, one important requirement is an adequate blood supply. Therefore, generation of new blood vessels (angiogenesis) to the cancer tissue is essential. In the treatment of cancer, some drugs are targeted at preventing angiogenesis (angioprevention). Tea components also show angioprevention activity (Tosetti *et al.*, 2002; Bertolini *et al.*, 2000).

Receptors for vascular endothelial growth factor (VEGF) play a major part in angiogenesis. Physiological concentrations of tea catechins, EGCG, CG and ECG, induce rapid blocking of these receptors, suggesting that the anticancer properties of tea extracts may be due to their inhibition of VEGF-dependent angiogenesis (Lamy *et al.*, 2002).

Induction of programmed cell death, or apoptosis, could help to reduce the progression of cancer. Recent studies have shown

that tea can induce apoptosis (Zhang *et al.*, 2000, Hayakawa *et al.*, 2001).

Potential carcinogens in food, specially in cooked meat, are the heterocyclic aromatic amines (HAA). Tea components reduce the mutagenicity of HAA (Stavric, 1996).

Tea components are effective against radiation injury in general. They have been found to give protection against DNA scission induced by β-rays (Yoshioka *et al.*, 1997), and UV radiation (Record and Dreosti, 1998; Lou *et al.*; 1999; Nomura *et al.*, 2000).

Tea components are able to reduce carcinogenicity from smoking. Of the numerous carcinogens in cigarette smoke, the most potent appears to be 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK). Many studies have shown that tea components could inhibit tumour formation by NNK (Chung, 1999).

12.4 Animal Studies on Tea and Carcinogenesis

There are several reports indicating that both green and black tea extracts give protection against the development of cancers, and have anticarcinogenic effects at different sites in animal models. These findings are especially significant because the positive effects in animals are produced by tea at concentrations usually consumed by humans (Dreosti *et al.*, 1997).

A large number of experiments with animal models has been conducted to find the effect of both black and green tea on cancer in different body organs, such as the stomach, colon, lung, liver and skin. Both black and green tea extracts inhibit carcinogenesis induced by a wide range of chemical agents and radiation, such as UV radiation.

Tumours in male Sprague-Dawley rats, normally induced by subcutaneous injection with N-nitrosomethylbenzylamine (NMBzA), were inhibited by green tea and black tea extracts given orally (Wang *et al.*, 1995).

Sixty-five per cent of rats treated with NMBzA for five weeks developed oesophageal tumours, 39 weeks after the treatment commenced. Either decaffeinated green tea or decaffeinated black tea, provided as the only fluid for drinking, during the five weeks of treatment with NMBzA, reduced tumour incidence by 70 per cent. When the tea preparations were given after the NMBzA treatment period, the tumour incidence was reduced by 50 per cent.

This study shows that the reduction in tumour formation is not by tea components reacting directly on the carcinogen, since tea and NMBzA were administered by two different routes, orally and subcutaneously, respectively. It is suggested that the tea inhibits carcinogen activation, or prevents oxidative damage to DNA by ROSs produced during carcinogen metabolism.

Ohishi *et al.* (2002) found that epigalocatechin gallate (EGCG) could reduce colon carcinogenesis induced in rats by the carcinogen, asoxymethane.

12.5 Epidemiological Studies on Tea and Carcinogenesis

Epidemiological studies on the effect of tea on human cancers have given mixed results (Blot *et al.*, 1997). There have been a range of such studies: on cancers of the mouth, pharynx, oesophagus, stomach, colon, rectum, pancreas, lung, mammary glands, bladder and the kidney

Alhough dose-related effects have not been demonstrated,

several studies suggest a lowered risk of digestive tract cancers among tea drinkers, especially those consuming green tea (Blot *et al.*, 1996; Blot *et al.*, 1997).

A study in the Netherlands using 3,692 people has shown that black tea consumption is inversly related to the incidence of bladder cancer (Zeegers *et al.*, 2001). A population-based study in the US, with 1,452 bladder-cancer subjects, 406 kidney-cancer subjects and 2,434 control subjects, also found an inverse relationship between tea consumption and bladder cancer (Bianchi *et al.*, 2000). However, there was no association with kidney cancer.

Another study of a population in the south-western United States has shown that hot black tea consumption is associated with a lower incidence of squamous cell carcinoma in the skin (Hakim *et al.*, 2000).

In a population in China, it was found that green tea consumption reduced lung cancer in non-smokers, but not in smokers (Zhong *et al.*, 2001).

With 1,160 hospital patients in Japan, followed from 1988 to 1999, it was found that drinking green tea lowered the risk of recurrence of breast cancer (Inoue *et al.*, 2001).

However, in other epidemiological studies on breast cancer, no association was found. Thus, in a large population-based prospective cohort study in Sweden (the Mammography Screening Cohort), comprising 59,036 women, aged 40-76 years, no association was found between tea consumption and breast cancer (Michels *et al.*, 2002). Similarly, no association was found between green tea-drinking and cancer of the stomach, colon, rectum, liver, gall bladder, pancreas, lung, breast and bladder,

in a prospective study in Hiroshima, Japan, where 38,540 people were followed from 1979 to 1994 (Nagano *et al.*, 2001).

12.6 Does Tea cause Oesophageal Cancer?

A high incidence of oesophageal cancer is observed in a geographical belt extending from the region of the Himalayas to northern China, and the consumption of large amounts of tea by the populations in these areas was implicated as a contributory factor (Dhar *et al.*, 1993).

The tea is commonly drunk boiling hot and salted, and the constant drinking of fluid at excessive temperatures may be the major contributory factor, rather than any of the tea components. Kumar *et al.* (1992) have suggested that the chronic irritation of the oesophagus by heat is responsible.

Another contributory factor is likely to be the high dietary intake of amines and nitrates in these areas, which results in cancer-causing nitrosation reactions (Siddiqi *et al.*, 1992).

According to Weisburger (1999 b), tea has no adverse effects when consumed at moderate temperatures or as a cold beverage, but only if taken excessively hot when it can cause cancer of the oesophagus.

12.7 Conclusion: Tea reduces the Risk of Cancer

Although animal-model experiments have demonstrated anticarcinogenic activity in tea, epidemiological data from case-control and cohort studies have not given conclusive evidence of this. To a large extent, this is the result of non-comparability between epidemiological studies, for example in the number of subjects, the amounts of tea drunk, and the presence of

confounding variables that have not been resolved. Such reasons could explain why, although the amounts of tea consumed in different countries vary so much, differences in the incidence of cancer are small.

An overall appraisal of the results from epidemiological and animal studies, along with the by now well-established antioxidant activity of tea components, indicates that it is prudent to conclude that tea drinking may reduce the risk of cancer.

CHAPTER 13

TEA IN THE IMMUNE SYSTEM AND IN ANTIBIOSIS

13.1 Inflammatory and Immune Responses

A mixture of saponins extracted from tea leaves was found to have anti-inflammatory and anti-microbial activity (Sagesaka *et al.*, 1996).

The tea saponins inhibited rat paw oedema induced by carrageenan (a mixture of seaweed polysaccharides) in a dose-dependent manner. The saponins also prevented the activation of hyaluronidase, an enzyme involved in inflammatory reactions, and antagonised the action of leukotrien D 4, one of the chemical mediators of these reactions.

No toxic symptoms resulted in mice following oral administration of the tea saponins at 2 g/kg.

Leucocytes or white blood cells play an important rôle in the inflammatory process, during which invading micro-organisms are repulsed. The leucocytes migrate from the intravascular lumen into the tissues to attack the micro-organisms.

In order to study this process of migration, Hofbauer *et al.* (1999) cultured endothelial cells from the human umbilical vein on microporous membranes to obtain a monolayer. The number of neutrophils (polymorphonuclear leucocytes), freshly isolated from healthy subjects, migrating through untreated monolayers was used as a baseline (taken as 100 per cent migration). Neutrophils and/or monolayers were then treated with EGCG from green tea extracts at different concentrations. Treating neutrophils or monolayers, or both, led to significant reductions in neutrophil migration through the monolayers, showing the efficacy of EGCG in inhibiting the inflammatory process. The treatment of both cell types gave an additive effect.

In the study of Wang *et al.* (2001) (see Section11.5), the injection of the polysaccharide-protein complex from 'coarse' tea into mice caused an increase in antibody concentration of 45 per cent, suggesting that tea could be beneficial in the immune response.

There was also an effect of the polysaccharide-protein complex on interleukin production. Interleukins are factors secreted by some helper T cells of the immune system in response to antigens. Interleukins serve to activate various white blood cells.

The treatment with the polysaccharide-protein complex was found to be beneficial for arthritic rats, in that it inhibited the production of too much of one of these interleukins, interleukin 1, in the spleen of these rats. Interleukin 1 causes macrophage cells in the blood to accumulate near activated T cells and become more efficient in phagocytosis.

The treatment, on the other hand, stimulated the production of another interleukin, interleukin 2, in the spleen of these arthritic rats. Interleukin 2 binds to the surface of activated T cells causing them to proliferate and thereby generate antigen-specific T cell lines.

13.2 Tea against Pathogenic Micro-organisms

Whole aqueous extracts of tea and fractions inhibit the growth of a wide variety of micro-organisms, gram-positive and gram–negative bacteria, fungi, yeasts, viruses, mycoplasmas and protozoa, as well as inactivating their toxins and enzymes (Hamilton-Miller and Taylor, 2001). The main active principle is EGCG.

Constant consumption of tea, which allows tea components to be chronically present in the intestinal tract, brings about the elimination there of undesirable micro-organisms, and their replacement by beneficial forms (Weisburger, 2001 a). The anti-viral action of tea may also contribute to increased human longevity (Weisburger, 1999 a).

13.2.1 Bacteria

The earliest report of the antibacterial action of tea was in 1906. This documents the finding of McNaught, a British army surgeon, that tea kills *Salmonella typhi* which causes typhoid fever and *Brucella melitensis* which causes brucellosis.

Tea extracts have since been shown to inhibit common pathogenic bacteria such as strains of *Staphylococcus aureus, Staphylococcus epidermidis, Bordetella, Clostridia, Salmonella, Shigella, Vibrio* and *Pseudomonas* and the fungi *Tricophyton spp., Candida albicans* and *Cryptococcus neoformans* (Toda *et al.*, 1989; Hamilton-Miller, 1995).

Escherichia coli, a pathogen found in the gut, was susceptible to green tea extracts at concentrations used for human consumption (Toda *et al.*, 1989).

Ikigai *et al.* (1993) investigated the bactericidal action of tea catechins. EGCG has a strong bactericidal action and EC a much weaker action. EGCG was found to cause leakage of 5,6-carboxyfluorescein from phosphatidylcholine liposomes while EC, on the other hand, causes little damage to the membrane of the liposomes. This indicates that the mode of bactericidal action of the catechins is primarily owing to damage to the lipid bilayer of the bacterial membranes.

Gram-negative bacteria are more resistant to bactericidal catechins than gram-positive bacteria, perhaps in part because they contain negatively-charged lipopolysaccharide molecules.

In calves, green tea extracts improved the balance of intestinal microflora, prevented digestive and respiratory diseases and thereby reduced the number of deaths during the nursing period (Ishihara *et al.*, 2001). Tea extracts would therefore be probably useful in feed for livestock.

Ishihara *et al.* (2001) found that feed with green tea extracts gave high faecal counts of *Bifidobacterium* spp. and *Lactobacillus* spp., and decreased the counts of *Clostridium perfringens*. The improved microflora balance decreased the frequency of diarrhoea in the calves.

They also found that tea extracts inhibit the growth of pathogenic bacteria, including seven strains of *Staphylococcus* spp., seven strains of *Streptococcus* spp., one strain of *Corynebacterium suis*, 19 strains of *Escherichia coli*, and 26 strains of *Salmonella* spp. It is suggested that the ester-linked galloyl moiety of the polyphenols in the extracts may be mainly responsible for the inhibition of bacterial growth.

13.2.1.1 *Staphylococcus aureus* and *Vibrio cholerae*

Among the catechins tested, EGC, ECG, and EGCG inhibited the growth of *Staphylococcus aureus* and strains of *Vibrio cholerae*, with *S. aureus* being more sensitive to inhibition (Toda *et al.*, 1990). EGCG had a bactericidal action against a *V. cholera* strain.

Pyrogallol had stronger antibacterial activity against these two bacteria than tannic and gallic acids. Rutein or caffeine had no effect on them. ECG and EGCG had the most potent anti-haemolysin activity against toxins and haemolysins produced by these bacteria. The findings suggest that the catechol and pyrogallol groups are effective in the antibacterial and bactericidal activity, and that the conformation of the catechins have an influential rôle in the anti-haemolysin activity.

The bactericidal action of EGCG and theaflavin digallate from tea extracts, against methicillin-resistant *Staphylococcus aureus* (MRSA), and strains of *S. aureus* that cause food poisoning, were also investigated by Toda *et al.* (1990). The growth of both types of *S. aureus* was inhibited by the addition of tea extract, and EGCG and theaflavin digallate. Tea extract inhibited MRSA even at the same concentration as in ordinarily brewed tea.

These findings indicate that tea and catechin can be used in prophylaxis against MRSA infection.

Tea catechin has bactericidal activity against various bacteria, including MRSA, the catechin damaging the lipid bilayer of the bacterial cell membranes (Takahashi *et al.*, 1995). The antibiotic, oxacillin, is effective against MRSA, below the minimum inhibitory concentration, only if catechin is present. Some other antibiotics were also found to be effective in the presence of catechin.

Tea extracts of different types and from various sources inhibited a wide range of pathogenic bacteria including MRSA and *Yersinia enterocolitica*, at well below the concentrations found in ordinary cups of tea (Yam *et al.*, 1997). In green tea, the antibacterial activity resided in EGC, EGCG and ECG. In black tea, theaflavin and its gallates are additional bactericides.

In studies with isolates of MRSA, ECG was found to markedly lower the minimum inhibitory concentration of the antibiotics, oxacillin and other β-lactams (Shiota *et al.*, 1999) and, although a combination of two antibiotics, ampicillin and sulbactam, was not effective, these antibiotics were effective when further combined with EGCG (Hu *et al.*, 2001).

Extracts of tea can reverse methicillin resistance, and also penicillin resistance to some extent, in *S. aureus* (Yam *et al.*, 1998).

In a study by Sharquie *et al.* (2000), 35 patients with impetigo contagiosa, a bacterial skin condition, were found to be infected with 33 isolates of *Staphylococcus aureus* (94.3 per cent), and two isolates of *S. aureus* and *Streptococcus pyogenes* in combination (5.7 per cent). The use of tea liquor *in vitro* proved highly effective against *S. aureus*.

In the same study, another 64 patients with the same condition were treated with tea liquor and ointment. Tea ointment gave a cure rate of 81.3 per cent. Control groups treated with antibacterial ointment, and with an oral antibiotic, gave cure rates of 72.2 and 78.6 per cent, respectively.

13.2.1.2 *Salmonella*

Extracts of black tea, green tea and coffee inhibited the growth of various bacteria which cause diarrhoeal diseases (Shetty *et al.*,

1994). Tea and coffee also had bactericidal activity against *Salmonella typhi, S. typhimurium* and *Vibrio cholerae.*

Black tea extracted in alcohol was assayed for activity against *Salmonella* serotypes causing enteric fever, namely *S. typhi* and *S. paratyphi* (Ciraj *et al.*, 2001).

All the strains of *S. paratyphi* were found to be inhibited by the tea extract, but only 42 per cent of the *S. typhi* strains were. The inhibition against *S. paratyphi* also occurred at lower concentrations of the extract than were effective against *S. typhi.*

13.2.1.3 *Helicobacter pylori*

Chronic atrophic gastritis (CAG) can lead to stomach cancer, and the bacterium, *Helicobacter pylori,* is known to increase the risk of CAG.

A cross-sectional, epidemiological study was made on 636 inhabitants of a farming village in Japan on the relationship between green tea consumption, CAG and *H. pylori* infection (Shibata *et al.*, 2000).

Lifestyle factors that may affect CAG and the infection were investigated concurrently: smoking, alcohol consumption, consumption of four beverages including green tea, and five foods.

H. pylori-IgG (immunoglobulin G) antibodies were used in determining *H. pylori* infection, and serum pepsinogens in defining CAG.

H. pylori infection was positively related to the risk of CAG. High green tea consumption (more than 10 cups per day) was negatively related to the risk of CAG, even after adjustment for the lifestyle factors.

The conclusion is that high green tea consumption prevents CAG.

In an epidemiological study in a town in an area of Japan, where *H. pylori* is endemic, the effect of a range of factors including food, on *H. pylori*-IgG prevalence, was carried out by Toyonaga *et al.* (2000).

The daily intake of (green) tea, as well as algae, was higher in those who did not have antibodies for *H. pylori.*, in a randomly selected group of 365 adults (20 years and over).

Yee and Koo (2000) found that a Chinese tea (Lung Chen), in the concentration that would be given by its daily consumption, and two of its constituents, namely epigallocatechin gallate (EGCG) and epicatechin, inhibited the *in vitro* growth of *H. pylori.*

EGCG is probably the most active against the infection.

13.2.2 Fungi

In the study of Sagesaka *et al.* (1996) (see Section 14.1), tea saponins were shown to have high activity against pathogenic dermal fungi such as *Microsporum audouinii.*

13.2.3 Viruses

Nineteen commercially-available juices and beverages were tested for their activity against poliovirus type 1 (Konowalchuk and Speirs, 1978). Grape and apple juices and tea were particularly effective.

Tea extracts have been shown to prevent rotaviruses and enteroviruses from infecting monkey kidney cells in tissue culture, and black tea inhibits the infectivity of influenza A and B viruses (Mukoyama *et al.*, 1991).

Specifically, EGCG from green tea, and theaflavin digallate from black tea, inhibited infections of cultured monkey kidney cells with rotaviruses and enteroviruses (Mukoyama *et al.*, 1991). The suggestion is that the antiviral activity is caused by interference with virus adsorption onto the cells.

Nakayama *et al.* (1993) showed that EGCG and theaflavin digallate inhibit influenza A and B viruses in canine kidney cells *in vitro*. Electron microscope studies showed that, like antibodies, they bring about agglutination of the viruses and prevent their adsorption onto the kidney cells.

In this study, EGCG and theaflavin digallate also inhibited haemagglutination by influenza viruses, suggesting that they bind to the viral haemagglutinin and block infectivity.

Human retroviruses are a major cause of potentially fatal diarrhoea in young children. Gu *et al.* (2000) have found that tea extracts (as well as cacao and pine seed extracts) inhibit the adsorption of rotavirus onto cells.

Catechin derivatives, including ECG, EGCG, EGC, and green tea extract inhibited cloned human immunodeficiency virus type-1 reverse transcriptase (HIV-1 RT), and different viruses and DNA polymerases including viruses causing hepatitis B and herpes simplex (Tao, 1992). EGCG and ECG were found to be powerful inhibitors of HIV-1 RT.

Yamaguchi *et al.* (2002) also examined the possibility of EGCG from tea inhibiting the human immunodeficiency virus type-1 (HIV-1). Apart from destroying the viral particles, they found that EGCG affects each step of the HIV life cycle: viral attachment to cells, post-adsorption entry into cells, reverse transcription, viral production from chronically-infected cells, and the level of expression of viral mRNA.

Crude theaflavin extracted from black tea, and five HPLC fractions obtained from it, were tested individually and in combination for activity against bovine rotavirus (Clark *et al.*, 1998). The combination of four of the fractions was more active than the sum of their individual activities, indicating synergy.

Only the crude extract was tested against bovine coronavirus. It was found to be effective.

13.2.4 Protozoan Pathogens

The trophozoites of a strain of *Toxoplasma gondii* was obtained from the peritoneal exudate of infected mice, and treated with tea at different concentrations and time periods (Ryu, 1982). The trophozoites were then inoculated into the mice intraperitoneally.

Oolong and green teas were more strongly protozoacidal than black tea. The lowest tea concentration to kill *T. gondii* was 0.5 per cent.

CHAPTER 14

Oral Health and Antibiotic Effects of Tea

14.1 Fluoridisation of Teeth and Dietary Fluoride

The bony tissues of a tooth consist of a hard substance, calcium apatite, demarcated into an inner core called dentine, and an outer covering layer called enamel.

When teeth are being formed, fluoride reaching them in the blood replaces some of the hydroxyl groups in the calcium apatite to form fluoroapatite. Fluoroapatite has a closely packed structure and is therefore stronger and more acid resistant than calcium apatite itself. This mineralisation with fluoride, or fluoridisation, thus makes teeth more resistant to decay.

$$\underset{\text{apatite}}{Ca_{10}(PO_4)_6(OH)_2} + 2F^- \longrightarrow \underset{\text{fluoroapatite}}{Ca_{10}(PO_4)_6F_2} + 2\,OH^-$$

Fluoridisation can also occur when the surfaces of functional teeth come into contact with solutions, such as tea, which contain high concentrations of fluoride.

The ability of fluorides to bind to the enamel surfaces *in vitro* was found to be considerable (Simpson *et al.*, 2001). The bonds were only partially dissociated by solutions with ionic strengths far greater than than that of saliva.

Tea is a major source of dietary fluoride, a cup of tea (170 ml) containing 0.14-0.34 mg of fluoride. Dietary surveys in Britain have revealed that tea alone provides 0.04-2.7 mg of fluoride per person per day (Duckworth and Duckworth, 1978). Studies in India indicate that it could be 0.3-1.9 mg per day (Gulati *et al.*, 1993). Consumption of 4–6 cups of Sri Lankan tea could provide 1.3–2.0 mg of fluoride per day (Udagama and Amarakoon, 2001).

The main chemical form of fluoride in tea brew is the F^- ion, which has the highest bioavailability among various fluoride compounds (Horie *et al.*, 1992). Tea is therefore a significant fluoride source in the human diet, and could also serve to reduce the incidence of tooth decay.

It was found that 34 per cent of the fluoride in tea is retained in the oral cavity following rinsing with tea solutions (Simpson *et al.*, 2001). To test fluoride accumulation in the human oral cavity, three groups of subjects were given low-fluoride tea, high-fluoride tea, and no tea (Yue *et al.*, 1998). The groups drinking tea for three weeks accumulated more fluoride in saliva and dental plaque than the groups drinking tea for two weeks.

14.2 Fluorosis from Tea

There is adequate evidence that intake of fluoride from tea improves oral health (Rosen *et al.*, 1984; Onisi *et al.*, 1980; Mann *et al.*, 1985).

However, although under normal conditions, the ingestion of dangerous levels of fluoride from tea is very unlikely (Jenkins,

1991; Pang *et al.*, 1992), in rare instances where the fluoride levels in drinking water is high (as in some regions of China and Tibet), tea drinking could contribute to dental fluorosis and then fluoride toxicity at other sites in the body. Fluorosis has been observed, for instance, among drinkers of 'brick' tea in Tibet (Cao *et al.*, 1996). The symptoms of fluorosis are first manifested in the teeth, but may spread to the bone joints, muscles and nervous system.

Unlike in the processing of conventional tea, where only tender shoots are used, mature leaves are also used in the production of brick tea. The macerated wet leaves are moulded into brick shapes and dried. Since the mature parts of the tea shoots contain more fluoride than the tender parts, brick tea drinking can lead to fluorosis (Wang and Huang, 1995; Han *et al.*, 1995). In mature leaves, the fluoride concentration is several times that in the tender leaves (A.M.T. Amarakoon and M.D. Kumarasiri, 2001; unpublished). Liquors prepared from Sri Lankan black tea, made separately from the bud, 1st leaf, 2nd leaf, tender stem and mature leaf, were analysed for fluoride. The values obtained (in mg/l) were: bud 0.1, 1st leaf 0.3, 2nd leaf 0.5, tender stem 0.1, and mature leaf 3.8.

14.3 Dental Caries and Plaque Formation

When food particles are allowed to remain in the oral cavity, especially in the gaps between the teeth, owing to inadequate brushing and cleaning, they become foci for the growth and proliferation of bacteria. Bacterial enzymes catalyse the conversion of carbohydrates and sugar in the food particles into acids, which chronically dissolve the hard parts of a tooth, producing a hole that enlarges and, if untreated, brings about loss of the tooth. This is called tooth decay or caries. The causative acid is mostly lactic acid produced by lactic acid bacteria. Gum

disease or gingivitis which leads ultimately to loss of teeth is also a result of bacterial action.

Dental plaque is formed of deposits of bacteria clumped together, and stuck to the surface of teeth, by means of a gluey matrix formed from sugars under the action of glucosyltransferase. It has been established that tea components reduce dental plaque formation (Ooshima *et al.*, 1994), and that these components inhibit the synthesis of the water-soluble glucan from sucrose by glucosyltransferase in *Streptococcus mutans* (Hattori *et al.*, 1990; Kashket, 1985; Nakahara *et al.*, 1993), thus effectively reducing attachment to teeth surfaces.

Apart from fluoride in tea which makes teeth stronger and more resistant to decay, Yu *et al.* (1995) have shown that the organic components of tea also possess the ability to increase the acid resistance of human tooth enamel.

Tea polyphenols reduce tooth decay by inhibiting the growth of cariogenic bacteria notably the most dangerous of them all, *Streptococcus mutans*. That tea components reduce populations of cariogenic bacteria has been observed in several studies (Kitamura *et al.*, 1990; Sakanaka *et al.*, 1992). Reduction of caries fissures by tea flavonoids has also been observed in experimental animals (Kempler *et al.*, 1977; Sakanaka *et al.*, 1992).

The development and progression of caries were attenuated in cariogenic rats given black tea to drink for two weeks (Touzy *et al.*, 2001). In a study, reported by Professor C.D. Wu at the American Society for Microbiology in May 2001, ten volunteers rinsed their mouths for one minute, five or ten times a day for one week, with either black tea or water. Rinsing with tea, ten times a day, produced less plaque, and less cariogenic bacteria and less acid in the plaque.

14.4 Tea against Oral Cancer

Drinking green tea, and a mixture of tea polyphenols, tea pigments and whole tea extracts, by animal models (Syrian golden hamsters) reduced the incidence of chemically-induced oral cancer by 42.6 and 67.2 per cent, respectively (Li *et al.*, 1998).

A pre-cancerous lesion of oral cancer called leukoplakia has proved ideal for investigating the effects of antioxidants in preventing oral cancer. Li *et al.* (1999) reported the effects of the same tea mixture on 59 leukoplakia patients, randomly divided into a treated group (ingestion and topical treatment with the mixture) and a placebo group. After six months, the oral lesion decreased in 37.9 per cent of the 29 treated patients and increased in 3.4 per cent. In the other 30, the placebo group, the lesion decreased in 10.0 per cent and increased in 6.7 per cent. Cell proliferation decreased in the treated patients, and other indicators also gave direct evidence of the preventive effects of tea on oral cancer.

The retention of tea flavonoids in the oral cavity and saliva would be an important factor in reducing the risk of oral cancer. Microgram quantities of catechins from green tea remain in the saliva for 60 minutes after rinsing with green tea (Tsuchiya, 1997). Catechin levels were found to increase in the mouth after rinsing with green tea solutions (Yang *et al.*, 1999). The oral and salivary catechin levels after drinking 2-3 cups of the tea were EGC 11-43, EGCG 4.8-22, and EC 1.8-7.5 μg/ml. The half-life of salivary catechins was 10-20 minutes.

14.5 Tea against Bacteria from Infected Root Canals

Extracts of four kinds of Japanese green tea was examined against 24 bacterial strains from infected root canals, and all were

found to have bactericidal action (Horiba *et al.*, 1991).

14.6 Tea against Candidiasis

A study was done to find the effect of tea on the growth of the yeast-like fungus *Candida albicans,* the extensive growth of which causes candidiasis or thrush in the oral cavity. It was found that black tea extracts inhibit the growth of *C. albicans* by 30 per cent (Udagama *et al.*, 2000).

CHAPTER 15

Must Tea Drinking Cause Iron Deficiency?

15.1 Iron-deficiency Anaemia

Iron-deficiency anaemia is a common problem in developing countries. It is particularly dangerous during pregnancy because it also affects the newborn.

The World Health Organisation mounts educational programmes to deal with the problem in member countries. A public-awareness advertisement in Sri Lankan newspapers in May 2000, issued jointly by the Sri Lankan Ministries of Health and Indigenous Medicine, and of Plan Implementation and Parliamentary Affairs, and bearing the UNICEF logo, carried the message that iron is essential for life. It said also that drinking tea or coffee within two hours of a meal can greatly reduce the absorption of dietary iron by the body, and it recommended waiting a while after a meal "before you reach for your favourite drink".

15.2 Iron: Requirement and Sources

The element, iron, is required in the diet because it is literally the central component of the haem molecule which, joined to a protein called globin, forms haemoglobin. Haemoglobin is the red respiratory pigment found in the red blood cells that carries oxygen from the lungs to all parts of the body.

The main dietary sources of iron are meats and fish (the muscle of vertebrate animals), and mainly pulses and vegetables, particularly leafy green vegetables. The iron in meats come from myoglobin (a smaller part, a quartile, of the haemoglobin molecule and therefore also red in colour), which is meant for storing oxygen in muscle.

15.3 The Absorption of Iron and the Effect of Tea

The iron from meats and fish ('haem' iron) is more easily absorbed from the gastrointestinal tract than iron from vegetables, etc. ('non-haem' iron); 15-35 per cent of the former as against 2-20 per cent of the latter. People who are vegetarian or predominantly vegetarian, by choice or by circumstance, are therefore at a greater risk of iron deficiency than those who normally include meat and fish in their diet.

It first appeared that tea had an inhibitory effect on iron absorption in the 1970s. Thus, a 60 per cent decrease in iron absorption was recorded when food was consumed with a large quantity of tea (200–250 ml) (Disler *et al.*, 1975). Iron absorption from a Western breakfast was reduced by 56 per cent when taken with 150 ml of tea (2.5 g of made tea) (Rossander *et al.*, 1979).

Tea reduced the iron absorbed from ingested bread by 35 per cent in a study carried out with nine adult Chilean woman

(Pena *et al.*, 1991), and by 36-61 per cent from a typical Tunisian 'couscous' meal (Hamdaoui *et al.*, 1994). Meal-time tea drinking has been implicated in iron-deficiency anaemia which is widespread in Tunisia.

The levels of serum ferritin (the iron-storage protein) were found to have a negative relationship with meal-time tea consumption in a study with 15 women in the U.K. (Razagui *et al.*, 1991).

The inhibitory effect of tea on iron absorption is proportional to the quantity consumed with meals.

In infants fed with tea, the concentration of haemoglobin was significantly lower than those in non tea-fed infants (Merhav *et al.*, 1985).

However, iron absorption is facilitated by factors present in meat and fish and by vitamin C (ascorbic acid). Those who take a balanced diet, that is a diet that includes also different kinds of vegetables and fruit, and so contains vitamin C and other factors that help iron absorption, will not normally suffer from a lack of iron.

15.4 Inhibition of Iron Absorption by Polyphenols

Polyphenols are widespread in foods and animal feed derived from plants. They are the major chemical component of tea shoots (Chapter 3) and, being water-soluble, polyphenols are present in tea brew (Section 5.3.2).

Polyphenols, such as tea flavonoids, have the property of binding to or forming strong, insoluble complexes (called chelates) with metal ions, which include ferrous and ferric ions. As a result, when present in the gastrointestinal tract, polyphenols bind to

elemental iron ingested in the food and inhibit iron absorption through the gut wall, thereby reducing its availability for metabolism in the body.

However, this is the case only with non-haem iron and not with haem iron.

In a similar manner, polyphenols reduce the bioavailability of dietary vitamin B_{12} (the only vitamin containing a metal, cobalt), which coincidentally is also essential for the formation of red blood cells. Thus, not only tea, but all foods containing polyphenols, can cause deficiencies leading to anaemia if they are not a part of a balanced diet, which is necessary whether one is vegetarian or not.

15.5 Drinking Tea with Meals, or In Between?

Drinking tea with Western-type meals will not significantly affect body-iron status, but tea with meals is contra-indicated when the meals are mainly from plant sources.

Thus, people in the U.S., in Europe and other parts of the first world, who generally take a varied, meat-based diet with an adequate iron content, can drink as much tea with meals as they like and not be at risk from anaemia. However, in populations, usually in the third world, where iron-deficiency anaemia is virtually endemic, or in groups or individuals having lowered body-iron, combining tea with the main meals is best avoided.

Although a depletion of body-iron causes more iron to be absorbed from the food in the gut, this is not adequate to compensate when the diet does not contain sufficient iron itself.

As advocated in the Sri Lankan Ministries' newspaper advertisement, tea is best taken between meals (that is, two hours

after one meal), but this is necessary only if the meals are basically vegetarian and likely to be low in iron, or if there is some risk of anaemia as in pregnancy.

It has to be emphasised that tea is quite safe when taken together with haem-iron diets.

15.6 Tea in a Balanced Diet

Tea is best consumed as part of a balanced diet, that is one that includes different kinds of vegetables and fruit. Such diets are likely to contain polyphenol-degrading enzymes, and iron-absorption enhancers such as vitamin C, which would serve to overcome the inhibition of iron absorption and maintain an adequate iron status in the body (Zijp *et al*, 2000), even if large amounts of tea are consumed with meals.

These enzymes and iron-absorption enhancers would increase the bioavailability of iron in the body, and also, incidentally, the bioavailability of calcium, another element that is commonly deficient in the body.

Lime or lemon juice, *nelli* and other fruit juices rich in vitamin C, would help to circumvent iron and calcium deficiency which tea drinking might cause.

Like vitamin C, milk is also found to increase the absorption of non-haem iron, and so tea with added milk should be suitable for vegetarian meals.

15.7 Tea for treating Iron "Overload"?

While those prone to iron-deficiency anaemia, such as pregnant women, should avoid excessive amounts of tea, tea drinking could, arguably, be beneficial to those on Western-type diets, provided of course that they are not pregnant.

Western-type diets, which have a high meat or fish content, provide up to 85 per cent of iron in the haem form. Although this is not the case in healthy individuals, those with problems in metabolism and taking only Western-type meals may develop iron "overload" in the body, which could lead to oxidative stress (that is, a preponderance of oxidants over antioxidants; Section 8.3.2).

It has been suggested that tea, with its inhibitory effect on iron absorption, might be used to treat patients suffering from diseases and conditions characterised by iron overload. The possible therapeutic use of tea in these cases has been the subject of research, although it has to be emphasised that tea-drinking must not be regarded as therapy for specific diseases, but only as part of a healthy lifestyle.

In healthy humans there is no risk of iron overload, the iron balance in the body being regulated through interlinked iron absorption, storage and excretion (Hallberg *et al.*, 1994).

15.8 Tea for treating Anaemia?

As an intriguing and contrary aside to the question of tea and anaemia, mention can be made of a suggestion arising from studies carried out in India in the early 1960s. The suggestion was that tea may actually be useful in treating anaemic conditions since, in addition to iron and other minerals, it contains copper, which is essential for the utilisation of iron in the biosynthesis of haemoglobin.

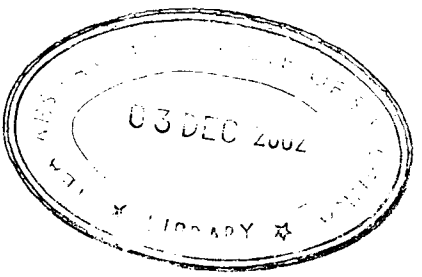
However, this finding must be treated with caution. Even five to six cups of tea per day are computed as contributing only 7 per cent of the body's requirement for copper, and 0.2 per cent of that for iron.

The amounts of minerals in tea are so low, compared to that in other foods in the diet, that tea by itself would not meet the body's daily requirements for minerals, although it is certainly a valuable additional source, particularly of manganese and flouride (Section 5.4.2).

15.9 Promoting "Safe" Tea

Given the undoubted antioxidant and nutritional health benefits of tea, and its cheapness and ready accessibility to all social strata, it is clearly counterproductive in terms of food security, to stress a negative feature, the possibility of its reducing iron absorption, when this is so easily addressed.

One way of doing so is to encourage the drinking of tea with added milk. Another is to initiate a serious campaign to specifically promote warm or cold tea, liberally spiked with lime juice or containing slices of lime, at and outside of meal times.



CHAPTER 16

TEA, COFFEE AND CAFFEINE

16.1 Caffeine in Tea

Caffeine is the major methylxanthine alkaloid found in tea, being present in the tender shoots to an extent of 3–5 per cent of dry weight.

The concentration of caffeine in black tea is 1–5 per cent, of which 80 per cent is extracted into the brew. Thus the theoretical lowest attained in black tea brew is 0.8 per cent, although the European Union limit for caffeine content in tea brew is 0.5 per cent. In many countries, the caffeine limit for decaffeinated teas is set at 0.1 per cent.

A cup or serving of tea (180 ml) is said to contain approximately 60 mg of caffeine (as against 100 mg in 180 ml of coffee) (Ashihara *et al.*, 1997), although the range in a cup of tea could vary from 30 to 90 mg. A normal daily consumption of 5–6 cups per day would therefore give an intake of 150-540 mg of caffeine.

This is below the level of 600 mg advocated for those with ischaemic heart disease and hypercholesterolaemia, and in pregnancy (Ashton, 1987). However one has to consider intake from other sources as well: coffee, cola beverages, chocolate, over-the-counter stimulants, analgesics and cold preparations, all of which contain considerable amounts of caffeine (Leonard *et al.*, 1987).

Only the populations in three of 44 countries, in which studies were undertaken, had an average intake of over 300 mg per capita per day (Phelps and Phelps, 1988). Britain, a nation of heavy tea drinkers, also had the same intake: around 300 mg per day.

In a recent study by Khokhar and Magnusdottir (2002), the caffeine content of teas commonly consumed in the U.K. was measured. Black teas contain significantly higher caffeine (22-28 mg/g of dry matter) than in less fermented green teas (11-20 mg/g of dry matter). The caffeine intake from tea, calculated on the basis of an average consumption of three cups of tea (200 ml per cup, 1 per cent tea leaves w/v), ranged between 92 and 146 mg/day.

16.2 Caffeine Metabolism

In adults, more than 90 per cent of the caffeine ingested in tea, or by some other means, is absorbed rapidly by the gastrointestinal tract and distributed to the tissues and organs. Peak plasma levels are reached within 15-45 minutes after ingestion. The half-life of caffeine in the plasma varies between individuals and ranges from 2.5 to 7.5 hours.

The metabolism of caffeine could be influenced by the subject's lifestyle, particularly smoking and alcohol consumption.

Caffeine and its metabolites do not accumulate in the body, but are demethylated, oxidised and then excreted (Hollman *et al.*, 1997).

16.3 The Beneficial Effects of Caffeine in Tea

The beneficial effects of caffeine on the various body systems can be realised through the drinking of reasonable amounts of tea, say 5–6 cups per day, the equivalent of 150-540 mg of caffeine.

Caffeine acts as a stimulant to the central nervous system, including stimulation of the respiratory, vagal and vasomotor centres in the medulla. This is due to inhibition of intracellular phosphodiesterase and the blocking of adenosine receptors (Hamilton-Miller and Taylor, 2001).

As a result, there is:

mood enhancement;

a sharpening of mental clarity and vigilance, and an enhancement of the higher functions of the brain (such as those involved in mental arithmetic), as well as the delaying of mental fatigue;

increased alertness, and a shortening of the subject's reaction time by increasing the motor effects of conditioned reflexes and psychomotor coordination;

an increase in the rate of respiration and in the amount of work which can be performed by the voluntary (or skeletal) muscles, together with an increase in the time period for the onset of physical fatigue;

and a vasodilatory and stimulatory effect on cardiac (or heart) muscle.

All of these advantageous effects are produced, to varying degrees, merely by the consumption of tea.

Tea has the advantage over other caffeine-containing beverages, and caffeine in the pure form, in that its stimulatory effect is not associated with a subsequent depression or "hangover". Excess caffeine causes insomnia, muscular tremor, and finally anxiety states.

Leonard *et al.* (1987) have reviewed the effect of caffeine on various body systems.

The caffeine in tea

increases the secretion of gastric acid and pepsin, and the rate of emptying of the gastrointestinal tract;

exercises a diuretic effect on the kidneys causing an increased output of urine and increased excretion of calcium; and

has a bronchodilatory effect.

It enhances the drug- and xenobiotic- metabolising activity of the liver (Curotolo *et al.*, 1983; Reynolds *et al.*, 1993).

Tea enhances the effects of catecholamines which are involved in anti-inflammatory reactions.

Although in excess, pure caffeine causes anxiety and unpleasant abdominal sensations, tea itself does not produce these side-effects. Rather, it inhances one's performance level, reaction time and mental alertness, elevates mood and improves memory. Its regular comsumption in the day helps reduce physical and mental fatigue.

There is recent evidence that tea gives protection against neurological damage by oxidative agents such as ferrous ions.

Until the discovery of more potent and specific modern drugs, tea was used as a dietary constituent for treatimg ischaemic heart disease, bronchial asthma (owing to tea's bronchodilatory action) and cardiac oedema (owing to its diuretic action).

16.4 The Adverse Effects of Caffeine

Caffeine is known to increase blood pressure which increases the risk of stroke (Pincomb *et al.*, 1985). It is also believed to have an atherogenic effect.

A study with rats has shown that caffeine added to the diet induces hypercholesterolemia (Yokogoshi *et al.*, 1983). However, since polyphenols in tea acts in the opposite direction, the overall effect of tea ingestion was found to be hypocholesterolemic.

Most studies on the metabolic effects of caffeine are done in animal models, using pure caffeine in doses exceeding any normal intake by humans. Therefore, extrapolation of these results to humans is questionable.

Epidemiological studies have not found any adverse effects when caffeine is consumed in moderation. On the other hand, high caffeine intake could increase heart rate and cause arrhythmia in some individuals. Therefore, a moderate caffeine intake is advised for people with cardiovascular diseases.

However, it must be remembered that, with tea, the polyphenols present reduce the risk of these diseases. When present together in optimal amounts, as in tea, polyphenols and caffeine have a protective effect on the heart and cardiovascular system.

Caffeine passes through the placental barrier, and extremely high doses of caffeine administered to pregnant animal models

caused malformations (or teratogenic effects) in the foetus. The foetus at an initial stage has not as yet developed the enzyme systems capable of metabolising and excreting caffeine.

On the other hand, epidemiological studies have not found any relationship between caffeine intake and abnormalities in human foetuses. However, it is advisable to reduce caffeine intake during pregnancy.

16.5 A Comparison of Tea and Coffee

Tea has the same antioxidant levels as coffee, but tea is better as a beverage since the effects of caffeine in coffee are not mitigated by the presence of flavonoids.

An *in vitro* study by Richelle *et al.* (2001) compared the antioxidant activity in tea, coffee and cocoa, all of which contain polyphenols, using the LDL oxidation method and measuring the time (the 'lag time') during which antioxidants are consumed. According to the lag time (minutes), coffee had the highest antioxidant activity: coffee 292-948, cocoa 217-444, green tea 186-338, black tea 67-277 and herbal tea 6-78.

Coffee contains the polyphenolic antioxidants known as chlorogenic acids (not flavonoids as in tea), about which almost nothing is known regarding their action in the body, either from epidemiological or *in vivo* research. Coffee preparations contain much more solids than tea brew and, if only *in vitro* assessments are made, coffee would be expected to show higher antioxidant activity than tea. However, what is important is the bioavailability of the respective antioxidants, chlorogenic acids and flavonoids, at the appropriate sites in the human body where antioxidant activity is desirable.

Apart from the obvious, large variation in the polyphenol content of the four green teas and five black teas examined by Richelle *et al.*, an adequate extraction of tea polyphenols into the liquor depends on the use of boiling water, and not hot water, as used by these authors.

16.5.1 Cardiovascular Disease

A marked positive, dose-response correlation was found between coffee drinking and the concentration of total homocysteine (tHcy) in plasma, in an epidemiological study by Nygard *et al.* (1997). tHcy is a risk factor for cardiovascular disease and for an adverse outcome from pregnancy.

The population studied consisted of 7,589 men and 8,585 women, 40-67 years of age with no history of hypertension, diabetes, ischaemic heart disease, or cerebrovascular disease, from a particular county in Norway in 1992-1993. The group included 89.1 per cent who used coffee every day.

In the 40-42 years age range, the mean tHcy for men was 10.1 μmol/l for non coffee-drinkers and 12.0 μmol/l for those who drank nine cups of coffee or more per day, and for women 8.2 and 10.5 μmol/l, respectively.

Coffee drinking was associated with smoking and a low intake of fruit, vegetables and vitamin supplements but, when these variables were statistically adjusted for, the coffee-tHcy relation was still only moderately reduced. Both smoking and high coffee intake gave particularly high tHcy concentrations.

On the other hand, there was a strong inverse relation between tea intake and tHcy concentration. This inverse relation was substantially attenuated after smoking and coffee intake were adjusted for.

16.5.2 Myocardial Infarction

This Section should be considered in conjunction with Section 10.6.2.

From a study of 101,774 people in a Californian hospital in 1978-1986, Klatsky *et al.* (1990) found that coffee drinkers were at a higher risk of acute myocardial infarction in comparison to non-drinkers of coffee. The relation was also significant when controlled for blood cholesterol, blood glucose, blood pressure and adiposity, singly or combined.

However, tea use was found to be unrelated to myocardial infarction. Both coffee and tea use was unrelated to other coronary diagnoses. The authors advise a limit of four cups of coffee per day for persons at risk of myocardial infarction.

From a similar study in California of the relations of 128,934 people to 4,501 subsequent deaths, Klatsky *et al.* (1993) found a slightly increased risk from acute myocardial infarction among those who drank four or more cups of coffee per day, as against non-drinkers. Apart from this, the study suggested that coffee and tea are not linked to major causes of death.

In a hospitals-based, case-control study conducted in 1980-1983 in the eastern U. S., 1,873 men under 55 years of age with first non-fatal myocardial infarctions were compared with 1,161 controls admitted for conditions unrelated to coffee intake (Rosenberg *et al.*, 1988). The relative risk estimate for myocardial infarction increased with increasing, recent daily intake of caffeine-containing coffee relative to zero coffee intake. The association was found in each age group, and both in smokers and non-smokers.

The conclusion is that men who drink five cups of coffee, or

more, per day increase their risk of myocardial infarction by at least two-fold.

A more recent epidemiological study has confirmed that tea lowers the risk of myocardial infarction, but that coffee does not (Sesso *et al.*, 1999).

16.6 Tea and Coffee in Moderation

Even though coffee consumption in the USA has fallen precipitously over the last 25 years or so, it is now picking up because of the introduction of new forms and images of coffee. The typical U.S. consumer takes more coffee than tea, health effects notwithstanding.

The same projection trends are evident in the UK, with the number of coffee bars expected to grow strongly in the next few years. A 2002 report (by Allegra Strategies) gives the cups of up-market coffee (espresso, cappuccino, etc.) drunk in 2001 as 4.4 million per week, as against 3.2 million in 2000.

In 1984, the American Medical Association Council on Scientific Affairs, proclaimed that moderate tea or coffee drinkers should probably have no concern for their health, with regard to their caffeine consumption, provided other lifestyle habits are moderate as well.

CHAPTER 17

Conclusions and Summary

17.1 Tea against Degenerative Diseases

Weisburger (1999 b, 2001 b) has succinctly articulated the conceptual development behind the scientific recommendation of tea, with its constituent polyphenols, as a cheap but significant part of the dietary armoury against the chronic and debilitating diseases of our modern times.

Today the main diseases afflicting the people of the western world, and those in the developing world who are able to copy western lifestyles, are cardiovascular diseases (coronary heart disease, hypertension and stroke), neoplastic diseases or cancers, and adult-onset diabetes (NIDDM). These diseases, caused at least in part by improper and unbalanced nutrition, are responsible for 50-70 per cent of premature mortality.

Up to about 15 years ago, research into aspects of palliative nutrition was centred on solid foods as macro- and micronutrients. Since then, there has been a shift to liquid or

fluid intake as a modulating factor for chronic diseases: clean, potable water, but also tea as the next most consumed beverage worldwide. There is much to be said for the drinking of tea in regions where water supplies are contaminated with micro-organisms, since making tea necessarily involves the use of boiling, and therefore sterilised, water.

From a myriad of scientific investigations carried out, using *in vitro* laboratory studies, experiments with animal models, and epidemiological surveys, the antioxidant activity of its polyphenols emerges as the most important factor in tea that reduces the risk of degenerative diseases. It is the general polyphenolic chemical structure that has effective antioxidant action against damaging free radicals, and not specific polyphenol molecules.

17.2 The Equivalence of Green and Black Teas as Antioxidants

It is used to be thought, until comparatively recently, that green tea was the most, or only, effective antioxidant-containing tea, and that green-tea catechins alone were the antioxidants giving tea its health-giving attributes. It is now well-known that the theaflavins and thearubigins, produced by the polymerisation of oxidised catechins, during the 'fermentation' stage in the manufacture of black tea, are equally effective antioxidants (e.g. Leung *et al.*, 2001).

17.3 The Modes of Action of Tea

Weisburger (1999 b, 2001 b) has focused the mode of action of tea and its polyphenols down to four distinct mechanisms.

17.3.1 Tea antioxidants against cardiovascular diseases, cancer, immune-system diseases and ageing

Over the last few years, it has been found that many types of body cells can produce free radicals (FRs) under certain conditions (Weisburger, 2001 a), which can be both internal and environmental. These are reactive oxygen species (ROSs), reactive nitrogen species, and peroxides.

Unless the formation of FRs are prevented, or FRs are destroyed, by antioxidants such as those from tea, FRs oxidise LDL-cholesterol, and the risk of atherosclerosis and heart and vascular diseases increases. Regular tea-drinkers have a lower risk of developing these diseases than non tea-drinkers.

In recent years, it has also been found that carcinogens and radiation from the environment generate FRs within the body which cause oxidative changes to DNA (the genetic material present in all cells), and which damage cell proteins. The major change in DNA is the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG). These changes to DNA carry the risk of cancers, and the production of abnormal proteins carry the risk of diseases such as immune-system diseases. Both disease types can be inhibited by tea antioxidants.

The impairment of cell function by changes to DNA and proteins causes premature ageing. The habit of tea consumption, adopted early enough in an individual's life, can hopefully prevent these changes and improve the quality of old age.

17.3.2 Tea in the production of anti-carcinogenic enzymes

It is now known that tea consumption induces the increased production of specific, detoxifying enzymes of the cytochrome

P-450 system, particularly glucuronyl transferases. Apparently increased levels of glucuronyl transferases lower the carcinogenecity of many molecules by converting them into detoxified glucuronides. (One class of these carcinogens are the polycyclic aromatic hydrocarbons which form, incidentally, in teas which are processed in leaking, 'smokey' driers.) The detoxified glucuronides are voided harmlessly in the urine.

17.3.3 Tea in the reduction of cancer-cell duplication

Both *in vivo* and *in vitro* research has shown that tea consumption decreases the rate of general cell cycling, and the growth rate of tumour cells. The unrestricted growth of tumour cells leads to metastasis (the dispersion of cancer cells from their points of origin to secondary sites).

DNA, altered by carcinogens through mutation, acts as a template for the proliferation of more of the altered DNA. The altered DNA can be repaired by specific enzyme systems. However, this repair is difficult if the rate of duplication of the cells containing the altered DNA is too high. Tea, working mainly through its antioxidant polyphenols, helps by slowing cell duplication rates.

Decaffeinated tea was found to be less active than regular tea, and caffeine was subsequently shown to be antimutagenic like the other antioxidants.

17.3.4 Tea in the improvement of intestinal bacteria

A variety of enzymes from microflora in the intestines metabolises compounds entering in the food, as well as in the bile from the liver. Consumption of tea and its polyphenols, either chronically or over a period of weeks, reduces unpleasant

Enterobacteriaceae and increases Lactobacilli and Bifidobacteria that produce beneficial metabolites.

The gradual replacement in the intestine of less desirable bacteria, by bacteria that constitute a useful metabolic system, is one of the beneficial consequences of tea drinking.

17.4 The Prudent Conclusion: Tea Protects

In vitro and *in vivo* studies have given overwhelming evidence that the polyphenolic and caffeine components of tea have powerful antioxidant activity, and therefore would be invaluable in mitigating and protecting against a surprising variety of diseases and conditions, including cardiovascular diseases and cancers.

However, from the epidemiological studies, an unequivocal conclusion cannot be drawn that tea will prevent heart disease or cancer in individual cases. Some studies have found that tea drinking could reduce the risk of heart disease and cancer, while others have not.

This apparent contradiction should not be allowed to undermine the real value of epidemiological studies, properly carried out, to give meaningful results. By their very nature, these studies are confounded by non-comparability; for example in the presence of histories, behaviours and idiosyncracies in individuals in test populations that have not been taken into account, perhaps because they are not known.

However, when an overall, holistic approach is made to the evidence from the three experimental and observational sources (the *in vitro, in vivo* and epidemiological studies), the conclusion is quite clear: it is wise and prudent to assume that tea drinking

will in fact, at least, reduce the risk of cardiovascular diseases, cancers and some other conditions.

While present research goes on apace all over the world, it is likely that an incontrovertible case will soon be made that molecules extracted from tea do have therapeutic value.

Some of the strongest evidence so far is that tea works against two of the most dreaded human scourges: cancer and heart disease. For cancer prevention the evidence is so overwhelming, that the Chemoprevention Branch of the National Cancer Institute in the United States is involved in the development and use of compounds from tea in human anti-cancer trials.

As has already been pointed out (Section 2.3), Weisburger (1999 a) believes that adequate knowledge already exists, on the beneficial action of tea components, to allow their use in public health promotion.

17.5 Tea in a Sensible Lifestyle

Although tea has undoubted health benefits, no specific health claims are being made for it as yet because acceptable pharmaceutical and clinical trials would take years to complete. The tea trade cannot pre-empt the results of any of such trials and make a premature strap line, for example, on tea packets to the effect that tea has medicinal value.

Tea is to be regarded as an inexpensive, pleasant beverage that confers definite psycho-physical benefits, and not as a specific medicine for certain ailments. It goes with a healthy, modern lifestyle, for young and old, being a natural food needing no additives or preservatives.

The refreshing and relaxing drink of tea must be treated as

precisely that, and drunk and enjoyed as widely as possible by all types and conditions of people. The facts, revealed by modern science, that it has several beneficial effects on health and well-being are an additional bonus to the drinker of tea.

The virtual consensus of nutritionists the world over that tea, like other foods rich in antioxidants, should be a component of most people's daily diet is sensible advice. It is the cheapest beverage next to water, it is easily available almost everywhere, and it is a natural food manufactured and prepared without additives. It has been time-tested by populations and civilisations over millenia, and in general nothing but good is said about it, including these days by reputable scientists on tea's therapeutic value, based on hard evidence from research findings.

Tea components are now being suggested as 'nutraceuticals' and functional foods.

In discussing lifestyle, one must recognise the dichotomy between the two "Worlds", the First and the Third. In the former, quality of life is pursued, with a nutritional emphasis on rational foods and beverages that give health. In the latter, it is an increased standard of living that is desired, with food that gives essential nutrients and calories for the daily grind. For both, tea in the diet seems eminently desirable.

17.6 How, and How Much Tea, to Drink?

The amount of black or green tea to be consumed every day, anywhere in the world, has been fixed by Weisburger (2001 a) as 5 to 10 cups (one cup may be taken as containing 160-180 ml). However, larger amounts, 10 or more cups, may be taken by individuals to their advantage and without any untoward effects.

As Weisburger points out, no toxic or adverse effects have been shown by tea or its polyphenols in the course of many, properly carried out scientific studies.

Care should be exercised only in respect of infants, young children at risk of type 1 diabetes, pregnant women, those who have already suffered from kidney and bladder stones, and those whose diets are nutrionally unbalanced. Recently another group has been added to this list by Dr D.K. Ganguly (Emeritus Scientist, CSIR and now with the Tea Research Association, Calcutta): those on psychoactive drugs whose effects could be negated by tea, and epileptics in whom tea may enhance convulsions.

Although boiling water gives the best brew, Weisburger (2001 a) cautions against drinking tea boiling hot, since any food taken very hot can damage the oesophagus. A temperature below 60-70°C (just hot enough to hold) is recommended.

As to brewing time, catechins including EGCG, EGC and ECG, are present in tea extracts within 1-2 minutes (Dashwood *et al.*, 1999). Other polyphenols ("tannins" which give bitterness) and caffeine are released in larger amounts after brewing for 3-10 minutes.

Warm, cold or iced tea, at a concentration of 1-2 per cent (that is, as normallly made, without an unpleasant taste) bestow identical benefits.

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ACRONYMS

AAPH - 2,2'-azobis (2-amidinopropane) dihydrochloride
ABTS - 2,2'-azinobis-(3-ethylbenzothiazolin-6-sulphonic acid)
AChE - acetylcholinesterase
ADP – adenosine diphosphate
BHT - dibutyl hydroxy toluene
B[a]P - benzo[a]pyrene
C - catechin
CAG - chronic atrophic gastritis
CHD - coronary heart disease
DMH - 1,2-dimethylhydrazine
DMPO - 5,5-dimethyl-pyrroline-N-oxide
DNA - deoxyribonucleic acid
DPPH - 2,2-diphenyl-1-picrylhydrazyl
EC – epicatechin
ECG - epicatechingallate
EGC – epigallocatechin
EGCG - epigallocatechingallate
EPR - electron paramagnetic resonance
ESR - electron spin resonance
FMD - flow mediated dilatation
FRAP - ferric reducing ability
FRs - free radicals
GC – guanine-cytosine
GSH - glutathione
HDL - high-density lipoproteins
HAA - heterocyclic aromatic amines
IDDM - insulin dependent diabetes melltius
IgG - immunoglobulin G antibodies
LDL – low-density lipoprotein
MDA – malondialdehyde
MRSA - methicillin-resistant *Staphylococcus aureus*
NADP – nicotinamide adenine dinucleotide phosphate
NADPH – NADP combined with one hydrogen (H) ion
NIDDM - non-insulin-dependent diabetes mellitus

NMBzA - N-nitrosomethylbenzylamine
NNK - 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone
8-OhdG - 8-hydroxy-2'-deoxyguanosine
ORAC - oxygen radical antioxidant capacity
PhIP - 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
RF – relaxed form
ROSs - reactive oxygen species
STZ - streptozotocin
TA – thymine-adenine
TBARS - thiobarbituric acid reactive substances
tBuOOH - tert-butyl hydroperoxide
TEAC - Trolox equivalent antioxidant capacity
TFs – theaflavins
tHcy - total homocysteine
TRs - thearubigins
TRAP - total radical-trapping antioxidant parameter
VEGF - vascular endothelial growth factor
ZES - Zutphen Elderly Study