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The composition of tea infusions examined in relation to the association between mortality and water hardness

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SUMMARY

Recent epidemiological studies have shown that death-rates from certain chronic diseases are higher in areas with soft than in areas with hard drinking-water. In the striking negative correlation found in the county boroughs of England and Wales between cardiovascular mortality and water hardness the important underlying factor is apparently the water calcium. Interest is therefore focused on the dietary significance of calcium present in drinking-water. In relation to that interest, the present report gives a quantitative account of the composition of tea infusions prepared with waters containing different amounts of calcium. It is shown that a substantial part of water calcium is taken up by the tea leaf during the preparation of infusions. The analysis of the infusions covers a wide range of individual components, including trace metals and polyphenolic substances. It appears that the principal change caused in infusion composition by the presence of calcium in the water is a substantial reduction in the relatively high oxalate content. The question is raised whether there may be some connexion between the 'water factor' in cardiovascular disease and the absorption of oxalates from foods.

INTRODUCTION

Attention has been directed in reports from several countries to striking correlations which show that in areas supplied with soft drinking-water the death-rates from cardiovascular disease are generally higher than those in areas supplied with hard water (Kobayashi, 1957; Schroeder, 1960; Morris, Crawford & Heady, 1961, 1962; Biörck, Boström & Widström, 1965). Evidence has also been reported (Turner, 1962) of similar negative correlations between water hardness and mortality from both gastric cancer and primary bone tumours in England and Wales, although with fuller data the association in the case of gastric cancer was later found to have only low statistical significance (Anderson, 1964). More recently Morris and his colleagues (Crawford, Gardner & Morris, 1968) in a thorough epidemiological study covering the period 1958–64 have confirmed that for the county boroughs of England and Wales the association between water hardness and cardiovascular mortality is particularly marked and apparently unrelated to

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social or other environmental factors. These workers conclude that there is an urgent need for further detailed investigation of this problem.

In attempting to relate these associations between mortality and water hardness to chemical entities present in drinking-water, several authors have pointed to trace metals in drinking-water as possibly relevant. It appears, however, that for the associations found in England and Wales the important underlying factor is the water calcium. In particular, it has been shown that cardiovascular mortality is even more highly associated with water calcium (the lower the calcium content the higher the mortality) than with water hardness, and there is no evidence of significant interrelation with any other water component studied (Hollins, 1965; Crawford *et al.* 1968). The important question raised is how might the calcium present in drinking-water perform a protective role.

In hard-water areas of England and Wales the calcium contribution from drinking-water still forms only about a tenth of the average dietary calcium intake (Hollingsworth, 1956). Calcium deficiency is not ordinarily encountered in man (Bronner, 1964) so that it seems unlikely that the small contribution of calcium from drinking-water functions as an essential supplement of dietary calcium for any substantial part of the population in these countries. Attention is therefore turned to the possible ways in which calcium from drinking-water may either modify the composition or influence the absorption of certain dietary components. In this connexion, since a major part of the culinary use of water is associated with the cooking of vegetable materials and particularly with the preparation of beverages such as tea and coffee, information on the interactions of water calcium and the components of these materials is of prime interest. Further interest is directed to the composition of tea infusions by the demonstration, in studies with laboratory animals, that several vegetable tannin extracts possess carcinogenic properties (Kirby, 1960; Korpassy, 1961) and also that tea extracts possess co-carcinogenic properties (Kaiser, 1967).

As a contribution to this field, we have examined quantitatively the interactions which occur between calcium in water and the components of the tea leaf during the preparation of infusions, with attention given both to the movements of calcium between the extracting water and the leaf, and to the influence of water calcium on the composition of the infusions. In the present communication we summarize the results of these studies.

MATERIALS AND METHODS

Preparation of tea infusions

The six samples of black tea used in the present studies were obtained in 1 kg. quantities from a tea broker, and comprised two blends of teas from Ceylon (A, B), two blends of teas from India (C, D), an Assam tea (E), and a China tea (F). An oolong tea from Formosa (G) and a green ('gunpowder') tea from China (H) were also included in some of the work. The samples, which had moisture contents ranging from 3·7 to 5·2 %, were stored in air-tight glass jars.

The relative amounts of leaf and water used in ordinary domestic practice by 10 individuals for preparing tea infusions were measured and found to vary between

8 and 21 g. dry leaf/l. water. The mean value of 15 g. leaf per litre of water was selected for use throughout the present studies.* A standard infusion period of 6 min. was used since, with the exception of the pectinic acids which dissolved slowly, the extraction of all the components studied was essentially complete within this period. The infusions were prepared in tared glass beakers by mixing the leaf with boiling water and maintaining the mixture at 95° C for 6 min. Small additions of distilled water were made as required to offset evaporation losses. Control tests showed that neither the amount of calcium taken up by the leaf, nor the extraction of the principal components from the leaf, was measurably affected by gentle stirring, and, for convenience in sampling for the study of uptake and extraction rates, the infusions were generally stirred gently during the extraction period. For the oxalate studies, the procedure used conformed to ordinary domestic practice with brief stirring only at the start and finish of extraction. After extraction, the infusions were separated from the leaves by pouring through either a nylon tea strainer or a pad of glass wool.

Analytical procedures

A.R. grade reagents were used throughout.

Inorganic components

Extensive use was made of emission flame spectrophotometry for element determination, employing a specially constructed flame spectrophotometer, based on a Zeiss total consumption burner, a 'Uvispek' monochromator, and an E.M.I. 13-stage photomultiplier (Hollins, 1965). The methods used were capable of detecting more than forty metals at a level of 0·5 mg. metal/15 g. tea leaf, these metals including the majority of the Group I and Group II metals, the rare earths, and the transition elements of period IV of the periodic table. The calibration curves of the elements analysed were linear over the range of concentrations used and the standard addition technique (Chow & Thompson, 1955) was used to standardize measurements on all solutions. Measurements were made either directly on suitable dilutions of the infusions or on samples of infusion or leaf which had been wet-oxidized with HNO_3 at 'low-temperature' (Middleton & Stuckey, 1954).

The determination of the uptake of calcium by the leaf was based on measurements both of the reduction of Ca concentration in solution and of the increase in Ca content of the leaves. Close agreement was found between Ca loss from solution and Ca gain by leaves, and there was no evidence of measurable loss of Ca by retention on the surfaces of any apparatus used. These results were confirmed by radioactive assay using Ca-47 as a tracer. In preparing infusions with natural waters, the amounts of Ca lost by precipitation as carbonate were negligible when the waters were heated rapidly to boiling point and used immediately.

* The average consumption of tea in the United Kingdom between 1956 and 1965 was 11·3 g. per person per day (National Food Survey Committee, 1967). Since the present studies provide information on the amounts of material extracted from 15 g. leaf, a useful guide to the average daily intake of the different tea components may be obtained by multiplying the amounts reported here by a factor of 0·75.

The pure metal and alloy plates used in testing the corrosive power of infusions were obtained from various metalware manufacturers. Before use, the plates were rubbed down first with 240 grade and then with 400 grade emery paper, and washed thoroughly in a solution of detergent. The area of metal surface immersed during the preparation of 1 litre of infusion was approximately 500 cm^2.

Aluminium determinations were made colorimetrically using the aurintricarboxylate method (Sandell, 1959). Phosphate was determined by the molybdenum-blue colorimetric method, and total nitrogen by the Kjeldahl method, following, in both cases, the procedures described by Pearson (1962).

Organic components

Polyphenol fractions were obtained by ethyl acetate extraction and lead salt precipitation (Roberts, Cartwright & Oldschool, 1957; Vuataz & Brandenburger, 1961), and were analysed by two-dimensional chromatography (Roberts *et al.* 1957). 'Tannin' was determined by the Löwenthal method (Jacobs, 1958), caffeine spectrophotometrically (Polzella, 1961), and phytic acid by the method of McCance & Widdowson (1935). The pectinic acids were converted to pectic acid which was determined gravimetrically as the Ca salt (Ca content approx. 7·5%), following closely the procedure recommended by Kertesz (1951). The method used for oxalate followed the procedure described by Baker (1952) except that calcium salt precipitation from the infusion, which led to persistent contamination of the oxalate, was replaced by ether extraction. Thus, after protein removal, the infusions were acidified to 1 N strength with HCl and continuously extracted with ether for 40 hr. in subdued lighting. The extraction efficiency, which was monitored by radioactive assay of added C-14 labelled oxalic acid, was between 85 and 90%.

RESULTS

The uptake of calcium by tea leaf

Measurements of the calcium content of all eight tea samples gave a mean value of 55 (range 38 – 75) mg. Ca/15 g. dry leaf. Distilled water infusions of these teas contained between 4·2 and 5·8 mg. Ca/l. showing an extraction of only 6·5 – 12·0% of the leaf calcium. With infusions prepared with calcium chloride solutions containing 100 mg. Ca/l. it was found that in each case there was a net uptake of calcium by the leaf amounting to between 16 and 39 (mean 30) mg. Ca/15 g. dry leaf, the uptake value being characteristic of the tea sample. Evidence that these net uptake figures were not substantially smaller than the total calcium uptake values was obtained by activity measurements of Ca-47, added to the calcium solutions prior to preparing the infusions. Thus, it was found that the fractional loss of Ca-47 activity from the solutions was closely similar in magnitude to the fractional loss of stable calcium. More detailed studies of the pattern of calcium uptake were made with four tea types (A, C, E, H) using solutions with calcium contents ranging from 0 to 150 mg. Ca/l. In each it was found that the uptake curve rose steeply over the lower half of the range and flattened out when the solution calcium was at about 100 mg. Ca/l. A typical set of results is shown in Fig. 1.

Tests with solutions of calcium at 100 mg. Ca/l. as sulphate and nitrate showed calcium uptake values which were not significantly different from the corresponding values obtained with calcium chloride solutions. Calcium uptake measurements were also made using samples of three natural drinking-waters in which the bicarbonate content was roughly equal in equivalents to the calcium content (Ion content of waters, mg/l.: (1) Ca, 15; Mg, 3·3; Na, 34; HCO_3, 41: (2) Ca, 66; Mg, 3·4; Na, 20; HCO_3, 222: (3) Ca, 83; Mg, 54; Na, 60; HCO_3, 240). For each water it was found that the uptake was in good agreement with the value expected on the basis of the results for the calcium chloride solutions. The pH values of the infusions

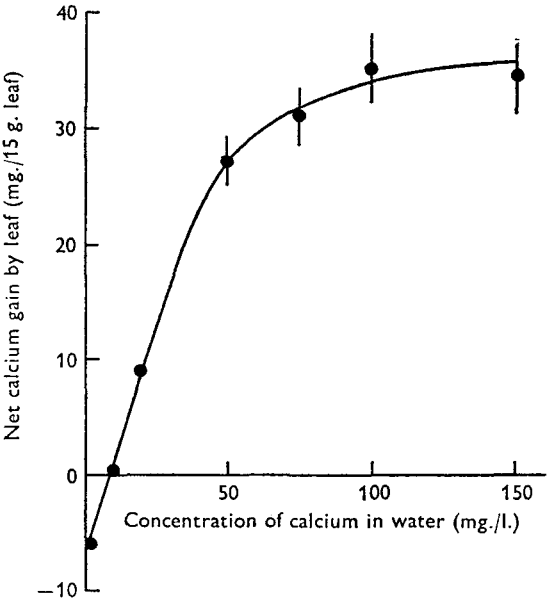

Fig. 1. The relationship between the net amount of calcium taken up by tea leaf during the preparation of infusions and the initial concentration of calcium in the extracting water (tea sample A). The major part of the analytical error in calcium determination was due to the random error of the flame photometric method used; errors shown (2σ) are the total photometric errors introduced into the calculated calcium uptake values.

prepared with the neutral salt solutions were essentially determined by the buffering action of the components extracted from the leaf, and fell within the range pH 4·4–4·9. The pH values of the infusions with natural water samples 2 and 3 were close to 5·3. Further tests were carried out using acetate and collidine buffers (at 0·05 M) to give infusions in which the pH reached 6·5 but no significant effect of pH on the calcium uptake was observed. Thus, in general it appeared that the pattern of calcium uptake (Fig. 1) was not significantly affected by the presence of the other principal ions commonly present in drinking-waters (Taylor, 1958), or by the differences in pH normally encountered in tea infusions.

Studies of infusions prepared with sodium chloride and magnesium chloride solutions showed that there was no analogous uptake of sodium or magnesium by

the leaf. In fact the presence of Mg ions in the starting solution caused an additional release of the magnesium present in the leaf. Thus, solutions of magnesium chloride at 50 mg. Mg/l. extracted about 80 % of the leaf magnesium compared with about 60 % extracted in distilled water infusions.

Further experiments with calcium chloride solutions showed that the calcium uptake process was fairly rapid and essentially complete within 2 min. Tests were also made on leaves recovered from infusions after a 6 min. period. Thus, in experiments in which leaves, separated from infusions with calcium chloride solutions, were subjected to several additional extractions with distilled water, it was found that only a small percentage of the calcium taken up by the leaf was extracted in each successive infusion with distilled water. It was also found that leaves which had been separated from infusions with distilled water showed no appreciable uptake of calcium in subsequent infusions with calcium chloride solutions. Thus, it appeared that the leaf component responsible for the uptake and binding of calcium was either altered or removed during the preparation of a distilled water infusion. Comparative studies were therefore made of the extraction of various components of the tea leaf by waters of different calcium content.

The composition of tea infusions

Preliminary measurements of the total solids present in tea infusions showed that about one-third of the leaf material had been extracted (Table 1), and that there was no large difference between the amounts extracted in distilled water and

Table 1. *General composition of tea infusions*

Component	Average concentration in infusions of 15 g. leaf per litre of distilled water (g./l.) (Figures in brackets represent number of samples studied)	
	Present studies	Previous studies
Total solids	5·10 (8)	—
Inorganic cations	0·23 (8)	0·28 (5)[a]
Polyphenols	2·30 (2)	2·50 (1)[b]
Caffeine	0·53 (3)	0·51 (4)[c]
Protein and amino acids[d]	0·85 (3)	1·00 (5)[a]
Miscellaneous component consisting mainly of sugars (by difference)	1·15 (2)	—

a, McCance & Widdowson, 1956; *b*, Roberts *et al.* 1957; *c*, Smith & Rees, 1963; *d*, Kjeldahl N (corrected for caffeine N) × 6·25.

calcium-loaded infusions. A more detailed set of measurements (Samples A, C) indicated that the presence of calcium in the starting solution (100 mg. Ca/l.) reduced the amount of material extracted by between 2 and 3 %. The results of studies of individual components are now summarized.

Metal ions

Measurements were made of the metal ion content of all eight tea samples, and also of the amounts of these ions extracted in distilled water infusions. The results of these measurements are summarized in Table 2. Attention is drawn to the relatively high levels of aluminium, manganese and zinc found in the samples. Analyses of infusions prepared with solutions of calcium at 100 mg. Ca/l. showed that, except for an increase by about 25 % in the amount of magnesium extracted, the extraction of the metals listed in Table 2 was unaffected by the presence of calcium in the starting solution. As noted above, the extraction of leaf magnesium was also increased to a similar extent by the presence of Mg ions in the starting

Table 2. *Metal ions detected in tea leaf and in distilled water infusions (average of eight tea samples)*

	Amount in dry leaf (mg./15 g.)			Amount in infusion (mg./l.)
Element	Min.	Max.	Mean	Mean
Na	5	10	8·0	7·2
Mg	36	51	44	25
Al[a]	3	14	9·8	2·8
K	135	380	225	182
Ca	38	75	55	5·1
Mn	5	23	18	5·4
Fe	2	5	3·5	< 0·25
Zn	0·5	15	4·0	2·6
Sr	0·18	0·65	0·37	0·06

a, Determinations of Al were made on only 5 tea samples.

solution. A more detailed study, using various ionic concentrations of calcium or magnesium in the starting solutions, showed that the additional release of leaf magnesium caused by Ca ions was closely similar in magnitude to that caused by the same ionic concentrations of Mg ions. It therefore appeared likely that the influence of Ca ions on magnesium extraction was due to an ionic concentration effect on the solubility of the magnesium component present in the leaf, although the possibility that some calcium uptake occurs by ion exchange with leaf magnesium was not fully excluded.

To obtain information on the degree to which the trace metal content of tea infusions may be increased by contamination from metalware currently or formerly used in their preparation, a series of measurements was made of the metal content of distilled water infusions prepared in the presence of various pure metal or alloy plates. No evidence was found of metal contamination at levels above 1 mg. metal/l. infusion from samples of aluminium, stainless steel, modern pewter, silver, or chromium-plated nickel-plated copper. Metal pick-up by infusions was observed from samples of copper (8 mg. Cu/l. infusion), nickel (20 mg. Ni/l.), nickel-silver ([1 mg. Zn + 2 mg. Ni + 7 mg. Cu]/l.), and brass ([3 mg. Zn + 3 mg. Cu]/l.). Infusions were also prepared with calcium-loaded solutions in the presence of nickel and

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copper plates but no evidence was found of any marked influence of the calcium on the corrosive power of the infusions. Furthermore, the uptake of calcium by the leaves was unaffected by the presence of the metal plates.

Tests on distilled water infusions prepared in an old, lead-containing, pewter teapot (6 % Pb) showed the persistent occurrence of a comparatively high pick-up of Pb (2–4 mg. Pb/l. infusion) with little tendency for a protective coating to form inside the pot. Infusions prepared with calcium-loaded solutions tended to leave a film on the metal surface and in these infusions the lead content was reduced to about 1 mg. Pb/l.

Polyphenols

A preliminary investigation of the 'tannin' component (reducing polyphenols) of infusions by the Löwenthal method indicated that the presence of calcium in the extracting liquid had no influence on the extraction of this component. The polyphenolic fractions present in distilled water and calcium-loaded infusions of two tea samples (A, C) were separated and analysed by two-dimensional paper chromatography. Detailed examination of the bands and spots on the chromatograms, which displayed the major thearubigin and theaflavin components together with a wide range of flavonoid and other polyphenols, gave no evidence that calcium in the extracting liquid had a marked effect on the extraction of any of these compounds. The total weights of polyphenolic substances isolated from the infusions were 2·10 g. (Sample A) and 2·50 g. (Sample C).

Total nitrogen and caffeine

Measurements of the total N content of the solids recovered from infusions prepared both with distilled water and calcium chloride solutions (100 mg. Ca/l.) indicated that the presence of calcium had no appreciable influence on the extraction of N-containing components (mainly protein, amino acids and caffeine). This indication was confirmed directly in respect of the caffeine content of infusions of three teas (caffeine content of infusions of Samples A, C, E: 0·54, 0·58 and 0·44 g./l.).

Pectinic acids

In preliminary tests on the solubility of the pectic material present in tea, hot extracts of two teas (A, C) were made by stirring samples in distilled water on the boiling water bath for an 8-hr. period. The weights of pectic acid recovered from these extracts amounted to 1·6 and 2·6 % of the dry leaf weight. When similar extracts were made at room temperature there was a tenfold reduction in the amounts of pectic material extracted.

The determination of the pectic material present in normal 6 min. infusions with distilled water gave values for three teas (A, C, E) of 33, 34 and 18 mg. pectic acid/l. When the infusions were prepared with solutions containing calcium at 100 mg. Ca/l., the pectic content was substantially reduced to values of between 5 and 10 mg. pectic acid/l. Control tests showed that the presence of calcium in the infusion did not interfere with these determinations. It appeared therefore that

the precipitation of low-ester calcium pectinates within the leaf would account for some of the calcium uptake from solution, but the quantity of calcium involved in this process would amount to only 1 – 2 mg. Ca/15 g. dry leaf.

Oxalic acid

Determinations of the oxalate content of distilled water infusions of six teas (A, B, C, D, E, F) gave values ranging from 62 to 98 (mean 82·5) mg. oxalate/l. The corresponding values for infusions with calcium chloride solutions (100 mg. Ca/l.) ranged from 39 to 48 (mean 43·5) mg. oxalate/l. Analysis of the results showed

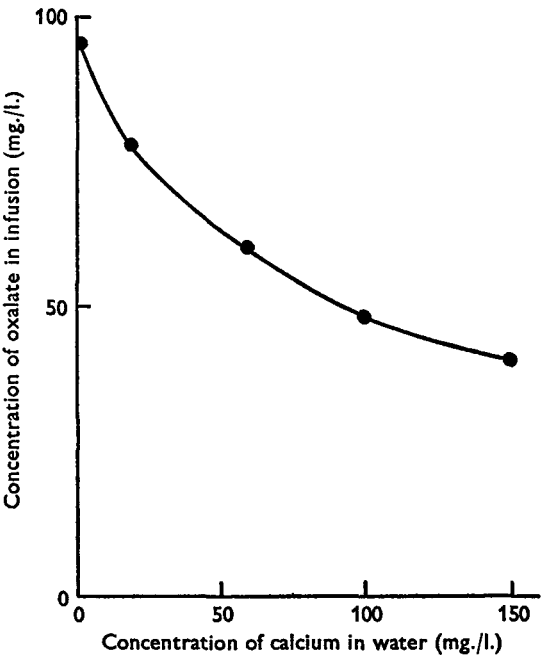

Fig. 2. The relationship between the amount of oxalate ion in tea infusions and the initial concentration of calcium in the extracting water (tea sample A). The overall analytical errors in the oxalate values are estimated to be less than ± 5 % of the plotted values.

that on the average the oxalate content of the calcium-loaded infusions was 53·5 % that of the distilled water infusions. Control tests showed that the presence of calcium in the infusions did not interfere with the oxalate determinations and gave no evidence that calcium oxalate was precipitated from calcium-loaded infusions onto the surfaces of any apparatus used. Further analysis of the results in relation to the values found for calcium uptake by the leaf showed that the deposition of calcium oxalate in the leaf would account for between 42 and 82 (mean 61) % of the calcium uptake values. A series of measurements of the oxalate in infusions prepared with different amounts of calcium in the extracting liquid showed that there was a progressive decrease in the amount of oxalate extracted as the calcium concentration was increased from 0 to 150 mg. Ca/l. (Fig. 2).

Various tests were carried out to discover the fate of the oxalate when milk was admixed with both distilled water infusions and calcium-loaded infusions in the ratio of milk : infusion = 1:5. Following domestic practice, the mixtures were prepared either by pouring the infusions onto the milk, or vice versa, and stirring briefly. The mixtures were then allowed to stand for 10 min. Determination of the oxalate content of the mixtures showed that more than 90 % of the oxalate remained within the body of the mixture. Further checks on the oxalate content both of the undisturbed bottom layers of mixtures held in tea-cups for 10 min., and also of any material deposited on the surfaces of the cups, confirmed that less than 10 % of total oxalate had settled out of the mixtures.

Phytic acid and phosphate

Using a method capable of detecting phytate if present at concentrations above 5 mg./15 g. leaf, no measurable phytate was found in 0·5 N-HCl extracts of four teas (A, C, E, G). This result indicates that, in the process of calcium uptake by the leaf in calcium-loaded infusions, deposition of calcium phytate would not account for more than 1 mg. Ca/15 g. dry leaf. Determinations of the phosphate content of distilled water and calcium-loaded infusions carried out for one tea sample (A) showed that the presence of calcium was without influence on the total phosphate extracted (phosphate content of infusion: 25·0 mg. P/l.).

DISCUSSION

The 'hardness' of drinking waters is due to their content of calcium and magnesium ions. In the majority of waters in the United Kingdom calcium is the principal component of 'hardness', the calcium content ranging from less than 1 mg. Ca per litre in very soft waters to about 150 mg. Ca per litre in the hardest of waters normally supplied (Skeat, 1961). An important fraction of the calcium in most waters is present as calcium bicarbonate ('temporary hardness'), and during the boiling of waters for the preparation of tea infusions some loss of calcium from solution, by deposition as carbonate, will generally occur for waters containing more than about 10 mg. calcium per litre. An indication of the extent of this loss is provided by Hollins (1965) who found that, when solutions containing equivalent amounts of calcium and bicarbonate ions were heated to boiling over a 5 min. period and boiled for a further 3 min., between 10 and 20 % of the calcium was deposited from solutions which had starting concentrations of 50 and 100 mg. calcium per litre. The present studies show that a further substantial loss of calcium occurs from drinking-waters during the preparation of tea infusions due to calcium uptake by the tea leaf. The general pattern of this uptake over the range of calcium concentration normally found in drinking-waters is indicated by the curve in Fig. 1. As outlined in the Introduction, our primary concern here is with the influence of the calcium on the composition of the infusions, and for the present purpose it will suffice to note that calcium uptake was shown in similar degree by all the teas examined and that the uptake process was essentially unaffected by the presence of any of the other principal ions normally found in drinking-waters.

The results found on the composition of the infusions will now be discussed, particularly in relation to the problem of the association between water hardness and mortality.

Several authors (Morris *et al.* 1961; Schroeder, 1966; Davies, 1962) have suggested that the 'water factor' in cardiovascular disease might be related to the trace element content of drinking-waters but no firm evidence has yet been produced to substantiate this view in respect of any single element. Interest in the intake of trace elements from drinking-waters is weakened by the fact that the contribution of these elements from water is generally a small fraction of the total dietary intake. The present results underline this fact specifically for manganese, zinc, and aluminium since the amounts of these metals extracted from the tea leaf into the infusion are higher by an order of magnitude than the corresponding amounts most frequently found in drinking-waters (Schroeder, Balassa & Tipton, 1966; Hollins, 1965; Campbell, Cass, Cholak & Kehoe, 1957). The results of our tests on metal pick-up by infusions in contact with various metallic surfaces indicate that the metalware most commonly used at the present time for the preparation of tea infusions does not give rise to any appreciable contamination of the infusion, although pick-up of copper, nickel, zinc and lead to a level of several milligrams per litre may be expected to occur in infusions prepared in certain types of teapots manufactured at earlier times. Of these various trace metals, lead can be singled out as one carrying a health hazard at a comparatively low intake level (Monier-Williams, 1949), so that any continual use of lead-pewter vessels for preparing tea infusions should be discouraged. In this connexion it should be noted that the spun pewter used in present-day cooking utensils is essentially lead-free. With regard to the other trace metals, it is uncertain (Campbell *et al.* 1957; Underwood, 1962) whether intakes of manganese, zinc, aluminium, copper or nickel have any significant physiological influence at the amounts found for tea infusions although it seems possible that the zinc and manganese contributions may be useful supplements to essential dietary requirements. There is, however, no evidence from the present results of any striking relation between the trace metal composition of the infusions and the hardness of the extracting water. Hard waters tend to extract more magnesium from tea than do soft waters, but the increment involved (about 10 mg. Mg/l. infusion) appears trivial when compared with the normal daily intake of magnesium (about 300 mg.) from other dietary sources (Wacker & Vallee, 1964).

It is also clear from our studies that the calcium in drinking-waters does not have any notable influence on the extraction of the other major tea components listed in Table 1. Special attention was given to the complex polyphenolic flavonoid material present in tea infusions in view of the fact that the chemical units of which this material is mainly composed are closely related in structure to the polyphenolic units present in several carcinogenic vegetable tannin extracts (Kirby, 1960). The analysis of this material, which took account both of the major polyphenolic components in a quantitative way and of the many minor components semi-quantitatively, showed that the presence of calcium in water used to prepare tea infusions has no marked effect on the extraction of any of the polyphenolic

substances studied. There is therefore no evidence from the present study of any interesting link between the supposed water factor in the aetiology of certain forms of malignant and cardiovascular disease, on the one hand, and the polyphenolic substances present in tea infusions, on the other. It may be noted from Table 1 that these polyphenolic flavonoid substances constitute approximately half the total amount of solid material present in the infusions and that the average daily intake of tea polyphenols in the United Kingdom is about 2 g. The recent reports (Korpassy, 1961; Kaiser, 1967) which raise the question whether the continual ingestion of these substances in tea might present either a carcinogenic or co-carcinogenic risk to man should encourage further investigation of their biological activity.

Of the other tea components examined, it will be noted that the presence of calcium in water represses the extraction of both oxalic acid and pectinic acid. It may also be noted that a major part of the calcium uptake process (Fig. 1) is accountable in terms of calcium salt deposition of these two substances in the leaf. It is probable that the remainder of the calcium uptake is due in part to calcium binding by insoluble leaf components, and some uptake by exchange with leaf magnesium may also be involved. The information on the pectinic acid fraction was sought mainly to determine the extent to which this fraction was responsible for calcium uptake, and not by reason of any special pharmacological interest. It is of general interest to note that, in food-canning practice, the process of 'firming' of certain types of fruit and vegetables (e.g. apples and tomatoes) by calcium salt treatment has been ascribed to a calcium uptake related to the deposition of low-ester calcium pectinates within the vegetable tissue (Kertesz, 1951). Evidently a similar type of interaction of water calcium with pectinic acid occurs in tea during infusion preparation but, in the case of tea, deposition of calcium as oxalate forms a more important part of the uptake process. The results found on the oxalate content of the infusions merit further discussion.

Comparatively little information has been published on the oxalate content of tea infusions. Measurements by Bau (1920) indicate the occurrence of about 500 mg. of soluble oxalate per 100 g. tea leaf and Hoover & Karunairatnam (1945) report the presence of about 135 mg. oxalate per litre in a tea infusion. The present values found for six tea samples, showing a range of 62 – 98 mg. oxalate per litre of infusion, are in broad agreement with these earlier results and confirm the general occurrence of appreciable amounts of soluble oxalate in black tea. It is also clear from Fig. 2 that the extraction of oxalate during the preparation of ordinary tea infusions is progressively repressed as the concentration of calcium in the extracting water is increased. It should be noted that in distilled water infusions the oxalate content is in large excess of the calcium content. In infusions prepared with water containing more than about 50 mg. calcium per litre, or in infusions which have been admixed with milk in the ordinary way, the calcium exceeds the oxalate in equivalents. It appears, however, that the oxalate is not readily precipitated from the infusion under ordinary conditions and remains within the body of the liquid after milk addition for periods of at least 10 min. The values found for oxalate in the tea infusions therefore provide a rough guide to oxalate intake from tea. The

results indicate that the average daily intake of oxalate from tea in different parts of the United Kingdom varies inversely with the degree of hardness of the local water supplies, and amounts to some 60 mg. in soft water areas and to half that value in areas supplied with water containing more than about 100 mg. calcium per litre.

Most of the published information on the oxalate content of foods relates to its occurrence in vegetables and fruit. Using the oxalate data of Kohman (1939) together with information on food consumption in the United Kingdom for the period 1956–65 (National Food Survey Committee, 1967) we estimate that the average daily intake of oxalate from vegetables and fruit amounts to some 65 mg., of which about one-third is contributed by potatoes. The figures available for other foods (Jeghers & Murphy, 1945) suggest that cereals, meat, milk, coffee and cocoa, taken as a whole, might contribute a further 50 mg. of oxalate to the average daily intake. These rough estimates indicate that tea infusions are the richest single source of oxalate in the United Kingdom diet. What is not clear is which of the various oxalate sources are the more important ones in terms of the contributions they make to the oxalate level in the blood, for little direct information is available on the complex question of the absorption of these comparatively small amounts of oxalate from different foods.

Various aspects of the metabolism and of the physiological action of oxalates absorbed from food are also problematic. Oxalaemia and oxaluria have frequently been reported as features of a wide variety of diseases, with low-oxalate diets recommended as therapy, although, except in the formation of oxalate calculi and the occurrence of severe renal colic, the relation between the disease states and the tissue oxalate concentrations has generally been indefinite (Jeghers & Murphy, 1945; Nordin & Hodgkinson, 1967). Against this background, it seems appropriate to recommend that, in further research on the significance of dietary oxalates, attention should be given to the oxalate contribution from tea infusions and also to the influence of calcium from drinking-water on both the intake and the absorption of oxalates from foods. An examination of the possibility that there may be some connexion between the 'water factor' in cardiovascular disease and the absorption of oxalates from foods appears to be a valid line for further inquiry.

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