AN INVESTIGATION OF THE INTERACTIONS BETWEEN MILK PROTEINS AND TEA POLYPHENOLS

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INTRODUCTION

The astringent taste of a black tea infusion has been attributed to the theaflavins[1] and the addition of milk to an infusion results in a reduction of the astringent taste. The present investigation was carried out to determine whether the reduction in astringency might be attributed to some interaction between milk proteins and the black tea polyphenols.

A tea infusion contains a complex mixture of phenolic compounds. ROBERTS[2] has published a review of the substances present in green tea and the changes they undergo during the manufacture of black tea. He has detected a series of polyphenolic substances characteristic of black tea which he has called A, B, C, D, P, Q, R, S_1, S_{1a}, S_{11}, X, Y and Z, and suggested that they are all derived from oxidation of *l*-epigallocatechin and its gallate. BHATIA AND ULLAH[3] have produced evidence that *l*-epicatechin gallate also takes part in the fermentation process. A, B and C are colourless compounds and are bisflavanols. P, Q and R are coloured compounds present in trace amounts. The most important polyphenols of black tea are the theaflavins (ROBERTS' compounds X and Y), and the thearubigins (ROBERTS' fractions S_1, S_{1a}, and S_{11}), which account for practically all the colour of a tea infusion[4]. The theaflavins are bright orange substances with very weak acidic properties which account for up to 3 % of the total dry weight of the tea. ROBERTS AND MYERS[5] have proposed structures for the theaflavins, and ROBERTS[6] and TEDESHVILI[7] have suggested different reaction mechanisms for further oxidation to the thearubigins, which are considered to be dimeric. VUATAZ AND BRANDENBERGER[8], however, have found evidence for the presence of nitrogen in part of the thearubigin complex, and suggest that thearubigins may be partly composed of substances akin to humic acids.

The major part of the analytical work on tea has been based on two-dimensional chromatography in butanol–acetic acid–water (4:1:2.2) and 2 % acetic acid[9]. For the simple colourless polyphenols this gives a good separation, but for the coloured compounds the separation is poor. Of the latter compounds only the theaflavins move in the second direction, and even these are not completely separated from the bulk of the coloured material which gives a long streak in the butanol–acetic acid–water direction. It is desirable that a better method of separation of the coloured compounds should be effected, and to this end we have investigated the use of paper electrophoresis.

Starch grain column electrophoresis has been used in this laboratory to achieve a

certain separation of the components of the protein system present in skim milk, and electrophoresis on cellulose acetate membranes has been used for the identification of the milk proteins in the resulting fractions. These methods were applied to the study of possible interactions between milk proteins and tea polyphenols.

EXPERIMENTAL

The teas used in the present work were an Assam tea, Rupai Pekoe Fannings, and a Ceylon tea which was used for the preparation of fractions S_1, S_{1a} and S_{11} by the method of ROBERTS *et al.*[9] The milk was reconstituted from a skim milk powder prepared from a milk which had been heated to 170°F for 15 sec before evaporating and spray drying.

100 ml of boiling water were added to 10 g of tea in a lagged vessel, the mixture was allowed to stand for 10 min and then filtered through glass wool to give a tea infusion containing *ca.* 3.5 % tea solids. A 1 : 1 mixture of this infusion with 20 % skim milk powder solution was used for the starch column electrophoresis. This mixture has the same proportion of tea to milk solids as that present in a cup of tea with milk at normal drinking strength, but the overall concentration is 7 times greater in the mixture than in the cup of tea.

1. *Starch column electrophoresis*

The apparatus was essentially as described by FLODIN AND PORATH[10]. Columns 2.4 cm × 50 cm were packed with potato starch in 0.05 *M* phosphate buffer pH 6.7. 2 ml of sample, not previously dialysed, were applied to the top of the column and subjected to electrophoresis with a current of 10 mA and an applied voltage of 300 V for 48–50 h at 6°C. The column was eluted over a fraction collector and 1.6 ml aliquots were collected. The optical densities of these aliquots at 280 mμ were measured and plotted against tube numbers.

2. *Membrane filter electrophoresis*

Electrophoresis on cellulose acetate strips was performed in a Shandon micro-electrophoresis tank at room temperature, essentially as described by KOHN[11]. Two 10 μl samples were applied to each 5 cm wide strip and electrophoresis was carried out in 0.05 *M* phosphate buffer pH 6.7 with a current of 2 mA per 5 cm width, and 6 V/cm length of strip for 4 h. Proteins were detected by staining with 0.005 % Nigrosine in 2 % acetic acid. β-Lactoglobulin (Light and Company) and α-lactalbumin (kindly presented by Dr. R. ASCHAFFENBURG*) were used as standards for the identification of protein bands.

3. *Paper electrophoresis*

Experiments were carried out in an LKB electrophoresis tank using Whatman 3 MM paper. Four 30 μl samples were usually applied across the 17.5 cm width of paper. Current was supplied from a constant current power pack. Proteins were detected by staining with 0.2% Lissamine Green in 2% acetic acid, and polyphenols with 1% $FeCl_3$–1 % $K_3Fe(CN)_6$ (1:1). Papers were also examined in U.V. light for the location

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of the polyphenols. Borate (pH 8.5) and molybdate (pH 4.9) buffers were used. The borate buffer was prepared by mixing 0.1 *M* H_3BO_3 and 0.025 *M* $Na_2B_4O_7$ (1:1). The molybdate buffer was made by dissolving 25 g hydrated sodium molybdate in 2,400 ml water and adjusting to pH 4.9 by addition of approx. 35 ml 5 *N* H_2SO_4.

RESULTS AND DISCUSSION

1. *The effect of tea on skim milk*

The elution diagram obtained after starch column electrophoresis of 10 % skim milk powder solution is shown in Fig. 1a. On the basis of relative mobility values[12], and further analysis of the fractions using paper, starch gel and membrane filter electrophoresis, peak I is considered to be due to the α-casein complex as defined by BRUNNER *et al.*[13], peak II to the major whey protein β-lactoglobulin and certain minor whey proteins such as blood serum albumin and proteose peptone, and peak III to β-casein and α-lactalbumin. Peak IV, which is considerably reduced in height when the sample is dialysed before electrophoresis, is considered to be mainly non-protein.

During the electrophoresis of milk to which tea infusion had been added the majority of the brown tea components were seen to move down the column as a sharp band and were eluted with the α-casein fraction. The elution diagram is shown in Fig. 1b. The absorbance of the eluate in tubes 1–20 was measured at 280 mμ where both protein and polyphenols exhibit absorption maxima, and also at 400 mμ in order to estimate the distribution of the coloured material present. Some coloured material remained at the starting zone and could not be eluted from the column.

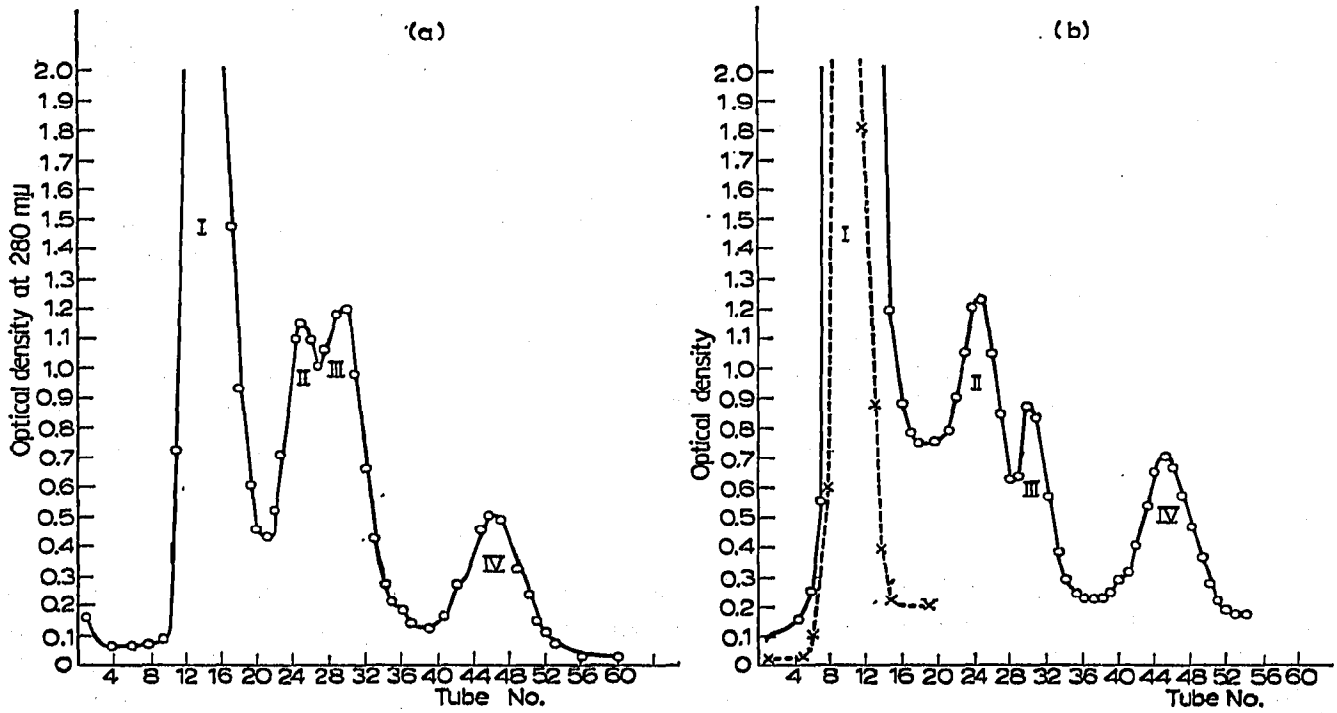

Fig. 1. (a) Elution diagram obtained from starch column electrophoresis of 2 ml 10 % skim milk powder solution. (b) Elution diagram obtained from starch column electrophoresis of a mixture (50:50) of 20 % skim milk powder solution and a strong tea infusion. O—O Optical density at 280 mμ; ×---× optical density at 400 mμ.

The concentration of milk proteins in the experiments represented by Figs. 1a and 1b was the same so that the elution patterns should be directly comparable with respect to protein distribution. Comparison of these two figures shows that:

(a) The majority of the brown tea polyphenols move with the α-casein complex represented by peak I.

(b) Peak II produced mainly by β-lactoglobulin is essentially unaltered by the presence of the tea polyphenols in the sample.

(c) Peak III due mainly to β-casein and α-lactalbumin is considerably reduced in height when the sample contains tea polyphenols.

(d) Peak IV is slightly increased in height when the sample contains tea polyphenols.

Membrane filter electrophoresis of the contents of certain tubes gave the protein patterns shown in Fig. 2. The α-casein complex isolated from milk by starch column electrophoresis gives a poorly defined protein pattern on membrane filters (Fig. 2a).

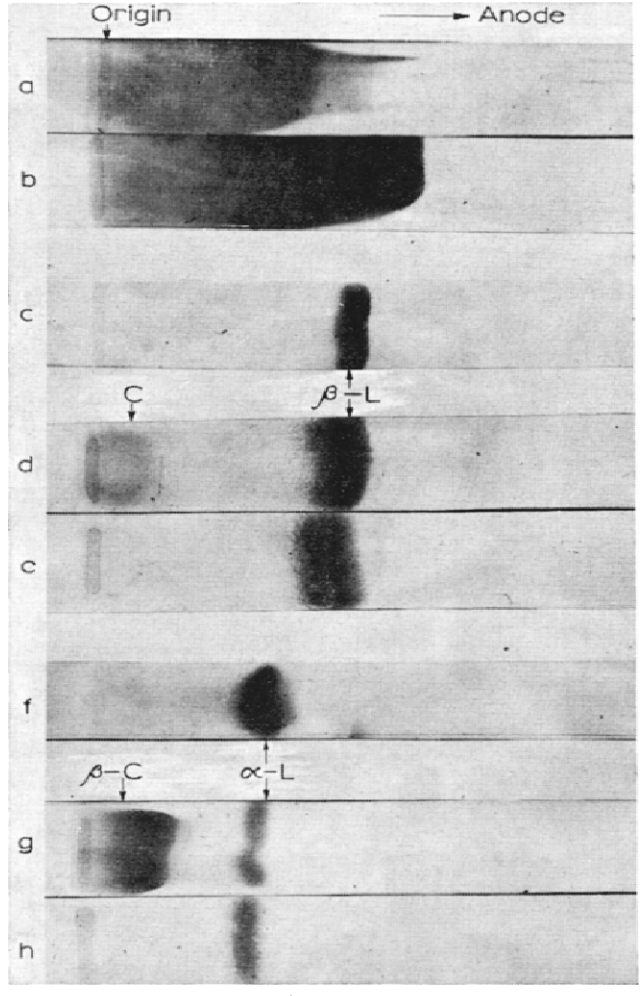

Fig. 2. Membrane filter electrophoretic patterns of milk proteins isolated from milk and milk + tea infusion samples by starch column electrophoresis. (a) α-Casein complex. Tube 14, milk sample. (b) α-Casein complex. Tube 10, milk + tea infusion sample. (c) 0.15% solution of crystalline β-lactoglobulin (β–L). (d) Tube 25, milk sample. (e) Tube 25, milk + tea infusion sample. (f) 0.1% solution of α-lactalbumin (α–L). (g) Tube 30, milk sample. (h) Tube 30, milk + tea infusion sample. C = casein; β–C = β-casein.

The α-casein complex isolated in a similar manner from the sample containing tea polyphenols gave a membrane filter protein pattern (Fig. 2b) which showed that the protein had migrated further along the strip than in pattern Fig. 2a, and had not been adsorbed to the same extent. A large part of the brown tea components was found to move with this protein band.

Fig. 2c and f show the membrane filter patterns given by solutions of crystalline β-lactoglobulin and of α-lactalbumin respectively. By comparison with these standard patterns membrane filter patterns of the fractions showed that tube 25 from the milk sample contained β-lactoglobulin and a little casein (Fig. 2d), whereas tube 25 from the sample of milk + tea infusion contained β-lactoglobulin but no contaminating casein (Fig. 2e). Similarly tube 30 from the milk sample contained α-lactalbumin and β-casein (Fig. 2g), whereas tube 30 from the tea infusion + milk sample contained α-lactalbumin only (Fig. 2h).

The membrane filter patterns shown in Fig. 3 further demonstrate the effects of the tea polyphenols on the milk proteins. When a strong tea infusion (*ca.* 3.5 % solids) is added in the proportions 1:2 to the α-casein complex isolated from milk by starch

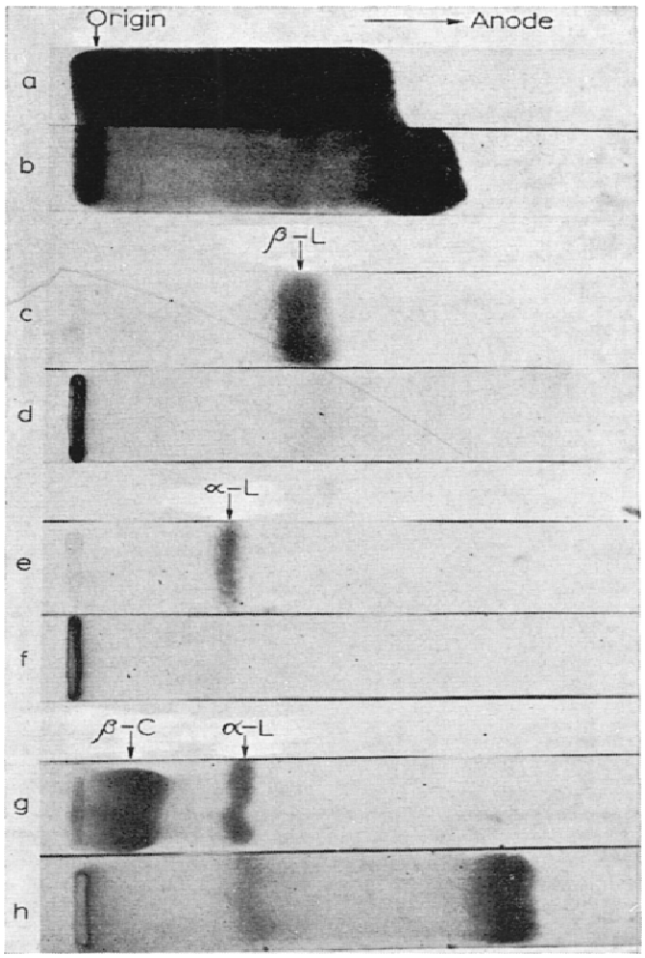

Fig. 3. Membrane filter electrophoretic patterns of milk proteins with and without added tea polyphenols. (a) α-Casein complex. Tube 14, milk sample. (b) α-Casein complex + tea infusion (2:1). (c) β-Lactoglobulin. (d) β-Lactoglobulin + tea infusion (2:1). (e) α-Lactalbumin. (f) α-Lactalbumin + tea infusion (2:1). (g) α-Lactalbumin + β-casein. Tube 30, milk sample. (h) α-Lactalbumin + β-casein + tea infusion.

column electrophoresis, and the resulting solution is subjected to membrane filter electrophoresis, the protein is found to move further along the strip in the presence of the tea polyphenols, and a large part of the brown tea components move with the protein (*cf.* Fig. 3a and b).

Solutions from tubes 25 and 30 obtained by starch column electrophoresis of milk + tea infusion, and which have been shown to contain β-lactoglobulin and α-lactalbumin respectively uncontaminated by casein (Fig. 2e and h) were treated with tea infusion in a similar manner to the α-casein complex. The membrane filter patterns showed that both the β-lactoglobulin and α-lactalbumin had been precipitated as shown by the immobilization of the protein at the starting zone, (*cf.* patterns (c) and (d), (e) and (f), Fig. 3). The formation of these precipitates was shown to be concentration dependent in the following manner. When a dilute (*ca.* 0.5 % solids) tea infusion was added to an 0.3 % solution of β-lactoglobulin in 0.05 *M* phosphate buffer pH 6.7, both solutions being at 70° to prevent creaming out of the tea, no precipitate was formed up to a protein: tea ratio of 2:1 in the resulting solution of pH 6.7, either at 70° or on cooling. When the experiment was repeated using a strong (*ca.* 3.5 % solids) tea infusion, no precipitate was formed in the resulting solution of pH 6.45, either at 70° or in the cold, until the amount of tea infusion added was such that the infusion to protein solution ratio was 1:1. A dark brown precipitate was then formed at 70°. The pH of the solution was 6.25. It would therefore appear that the formation of a precipitate, possibly an insoluble β-lactoglobulin–polyphenol complex, depends on the relative amounts of β-lactoglobulin and polyphenol present. Similarly, an 0.05 % solution of α-lactalbumin mixed with a strong tea infusion in proportions $> 7:1$ did not form a precipitate, but as the proportion of tea infusion present was increased up to and beyond this value a brown precipitate was formed.

A solution containing both α-lactalbumin and β-casein, obtained by starch column electrophoresis of the milk sample (tube 30), when mixed in proportion 2:1 with a strong tea infusion, on membrane filter electrophoresis gave the protein pattern shown in Fig. 3h. Comparison with pattern Fig. 3g shows that in the presence of the tea polyphenols the mobility of the β-casein was markedly increased, and that some of the α-lactalbumin had been precipitated. The β-casein band was accompanied by coloured tea polyphenols.

The foregoing experiments therefore indicate that on adding a tea infusion to milk both the α-casein and β-casein form soluble casein–polyphenol complexes, whereas the whey proteins, β-lactoglobulin and α-lactalbumin, in the presence of casein in the concentration used in these experiments, appear to be unaffected. In the absence of casein the whey proteins will form either soluble or insoluble protein–polyphenol complexes depending on the relative proportions of protein to polyphenol present.

2. *Paper chromatography*

Starch column and membrane filter electrophoresis of mixtures of tea infusions and milk proteins have shown that some of the coloured polyphenols, *i.e.* theaflavins and/or thearubigins, interact with the milk proteins. It is possible that some of the colourless phenolic substances also interact with the proteins.

Two-dimensional ascending paper chromatography of α-casein and β-casein respectively with tea infusions was carried out on Whatman No. 1 paper using

butanol–acetic acid–water (4:1:2.2) as solvent in the first dimension and 2 % acetic acid in water for the second dimension. The chromatograms were sprayed with ammoniacal silver nitrate solution and the phenolic spots identified by comparison of their positions with those given in the literature by ROBERTS *et al.*[9] and VUATAZ AND BRANDENBERGER[8]. Not all of the phenolic compounds listed by ROBERTS *et al.* were identified, but those colourless compounds of black tea which gave the strongest spots, *viz.* gallic acid, theogallin and the bisflavanols B and C, as well as other strong spots which were not correlated with ROBERTS' findings, were unaffected by the presence of the proteins. A larger part of the coloured polyphenols was immobilized at the origin in the presence of the proteins, but some coloured material did move in the butanol–acetic acid–water direction. There was a significant decrease in the theaflavin area in the presence of the proteins.

3. *Paper electrophoresis*

In view of the rather unsatisfactory nature of the separation of coloured tea compounds by paper chromatography, the use of paper electrophoresis was investigated. Strong tea infusions containing about 3.5 % solids were used.

Low voltage (*ca.* 6 V/cm) paper electrophoresis in 0.1 *M* acetate buffer pH 5.1, 0.05 *M* phosphate buffer pH 6.7 and 0.1 *M* borate buffer pH 6.8 gave essentially the same results, with no satisfactory movement of the coloured compounds.

However, paper electrophoresis of tea infusions in borate buffer at pH 8.5, with an applied voltage of 13.5 V/cm and a current of 9 mA for one hour, resulted in the movement of a large part of the brown coloured material towards the anode, and the separation of two yellow bands which moved more slowly. Inspection of the paper in U.V. light revealed other components which fluoresced strongly. The full pattern of bands is described in Table I. While this method is unsatisfactory for investigating the nature of the coloured components in tea because the colours of the borate complexes formed at this pH probably bear no relation to the colours of the free tea components, it is of value for the comparison of polyphenol fractions isolated from tea.

Paper electrophoresis was also carried out in molybdate buffer at pH 4.9. Again the method is open to the objection that the molybdate complexes probably differ in colour from the original polyphenols. However, this method has the advantage that electrophoresis is performed at the pH of a tea infusion. The pattern obtained in molybdate buffer consisted of a general brown background up to about 6 cm from the origin. Bands could sometimes be seen superimposed on the background, but the ease of detecting these bands depended on the amount of sample applied. If too much sample is applied the background is too strong to show the superimposed bands. Coloured bands at 12.0, 9.4, 7.5, and 6.0 cm from the origin could be clearly distinguished (Table II). In U.V. light none of the bands showed the bright coloured fluorescence observed on the borate patterns.

Group separation of the coloured polyphenols was undertaken in an attempt to identify some of the coloured bands obtained by paper electrophoresis.

The methods of ROBERTS *et al.*[9] and of VUATAZ AND BRANDENBERGER[8] were used. The paper electrophoretic patterns of ROBERTS' fractions S_1, S_{1a} and S_{11} are given for borate and molybdate buffers in Tables I and II respectively: those for VUATAZ AND BRANDENBERGER's polyphenol fractions 1, 2 and 3 are for molybdate buffer in Table II. Reference to these tables shows that in borate buffer ROBERTS' fractions

TABLE I

PAPER ELECTROPHORETIC PATTERNS OF TEA INFUSIONS AND POLYPHENOL FRACTIONS IN BORATE BUFFER pH 8.5

Appearance of band		*Distance moved in cm towards the anode*				
Visible light	*U.V. light*	*Assam tea*	*Ceylon tea*	*Fractions from Ceylon tea*		
				S_1	S_{1a}	S_{11}
Purple	None	10.2	10.0			
None	Blue F	9.5	9.5			
Pale brown	Yellow F	8.4	8.4			
Brown	Dark brown	7.7	7.8	7.7 w	7.8	7.7
Pale brown	Strong blue F	7.1	7.1	7.1 w	7.1	
Brown	Brown	6.5	6.5	6.6	6.5	6.5 w
Pale brown	Orange F		6.1	6.1 w		
Reddish	Brown		5.8			5.8
None	Blue F		5.4			
Yellow	Yellow F	4.7	4.9	4.9	4.8	4.9
None	Blue F	4.1	4.2			
Yellow	Dark brown	3.2 vw	3.2	3.2 w	3.2 w	3.2 w
None	Blue F	2.2				
None	Bluish F	1.5	1.8			
Orange-pink	None	1.2				
Purplish red	None		1.3	1.1		
None	Yellow F	0.6	0.4			

F = Fluorescence; w = weak; vw = very weak.

give more or less the same patterns, variations in the intensities of the bands being the only differences. In molybdate buffer the major part of the patterns for S_1, S_{1a} and S_{11}, up to 6.0 cm is once again the same. These patterns were rather diffuse and did not give very clear cut bands. The polyphenol fractions prepared by the method of VUATAZ AND BRANDENBERGER gave better defined patterns, but were still mixtures and it was not possible to ascribe any definite bands to the theaflavins or individual thearubigins. The sensitivity of the colour of these polyphenols to pH changes and, probably, to complex formation, makes simple identification of theaflavins as orange-yellow substances unwise. However, in this experiment the bulk of the theaflavins seemed to be extracted in fractions 1 and 2, probably because of incomplete removal of ethanol from the initial extract, and there were indications, from the electrophoretic patterns, that the fastest moving bands corresponded to the theaflavins.

Light white soluble casein (BDH nutritional casein) a readily available source of whole casein in the form of soluble sodium caseinates, was used for investigating the interaction between casein and the tea polyphenol fractions. The addition of this casein to a tea infusion altered the electrophoretic pattern of the latter in both borate and molybdate buffers as follows.

In borate buffer the brown band at 7.7 cm (Table I) was reduced in intensity and that at 6.5 cm disappeared. A new brown band appeared at 5.0 cm and this also stained for protein. Light white soluble casein gives a band at 5.2 cm. The α-casein–polyphenol complex isolated from skim milk + tea infusion by starch column electrophoresis gave a very weak brown band at 6.3 cm, and a strong band at 5.0 cm which gave a positive protein reaction with Lissamine Green.

In molybdate buffer the brown band at 7.5 cm (Table II) in tea infusion, fraction

TABLE II

PAPER ELECTROPHORETIC PATTERNS OF TEA INFUSIONS AND POLYPHENOL FRACTIONS IN MOLYBDATE BUFFER pH 4.9

Appearance of band	*Distance moved in cm towards the anode*							
	Assam tea	*1*	*2*	*3*	*Ceylon tea*	S_1	S_{1a}	S_{11}
Colourless, brown in U.V.	12.0				12.1			
Orange-brown	9.4		9.4		9.5		9.2	
Orange-brown		8.0						8.3
Green					7.6			
Orange-brown	7.5		7.5				7.5	
Red-brown	6.1	6.0	6.5	6.1	6.0	5.8	6.2	
		4.9	5.0					
Brown	3.9	4.0					4.5	4.0
					3.5			
Yellow						2.7	3.0	3.1
Green					1.7			
Purple	1.0							
Orange							0.9	0.9
Brown	o w	o w	o w	o s	o w	o s	o s	o s

1, 2 and 3 are polyphenol fractions prepared from Assam tea by the method of VUATAZ AND BRANDENBERGER[9]; S_1, S_{1a} and S_{11} were prepared from Ceylon tea by the method of ROBERTS *et al.*[9]; w = weak; s = strong.

2 and S_{1a} disappears in the presence of casein. The reddish-brown band at 6.0 cm is considerably reduced in intensity but the orange-brown band at 9.4 cm is unaffected. The slower moving coloured bands cannot be easily distinguished but the intensity of colour in this region is not greatly reduced. More brown colour remains at the origin in the presence of the casein. The protein is also immobilized at the origin.

These results suggest that casein reacts selectively with the coloured tea polyphenols in the presence of molybdate buffer, because two of the easily distinguished bands disappear, whilst the third remains unaffected.

The fact that casein does interact with the coloured tea polyphenols was further demonstrated by the use of Sephadex (G.25). The application of this cross-linked dextran to the separation of complex mixtures of peptides, proteins and amino acids has been described by PORATH[14]. Attempts to separate the coloured tea polyphenols in water on a Sephadex column were not successful. All the coloured material remained in a band at the top of the column and was only eluted on addition of *N*/10 NaOH, and then much trailing occurred. Adsorption of aromatic and heterocyclic compounds on Sephadex has been reported in the literature[15]. Casein washes straight through a column of Sephadex because the molecules are too large to enter the pores of the gel. When light white soluble casein was added to the tea infusion (1:1), and this mixture (pH 5.3) applied to a Sephadex column, a large part of the brown colour (*ca.* 90%) passed straight through the column and was eluted as a reddish-brown liquid. Some orange and pink substances remained at the top of the column and were very slowly eluted as trailing bands.

4. *Investigation of the nature of the polyphenol–casein interaction*

The preceding experiments indicate that some coloured polyphenols and the casein of the milk proteins interact in solution to form soluble complexes.

CHAPON *et al.*[16] have studied a similar system, *viz.* beer polyphenols and proteins. These polyphenols have been shown to be condensation products of anthocyanins which are very similar, structurally, to the simple flavanols in tea. The authors concluded that beer polyphenols complex with proteins by the formation of hydrogen bonds. In view of the similarity of the tea polyphenol/milk protein system we investigated the possibility of hydrogen bond formation by performing electrophoresis on membrane filters in the presence of urea which is a well known agent for breaking hydrogen bonds. The absence of complex formation in the presence of urea would suggest a mechanism involving hydrogen bonds.

Membrane filter electrophoresis was carried out as before using 0.05 *M* phosphate buffer pH 6.7 and 7 *M* with respect to urea, on samples of tea infusions and tea infusion + α-casein, β-casein, α-lactalbumin and β-lactoglobulin, as well as on the milk proteins alone. The results are shown in Fig. 4, from which it can be seen that the protein patterns are unchanged by the presence of the tea polyphenols. In the presence of the urea there was no precipitation of α-lactalbumin or β-lactoglobulin on addition of the tea infusion, and in all cases none of the brown colour was seen to move with the protein.

When tea alone is examined by membrane filter electrophoresis in phosphate

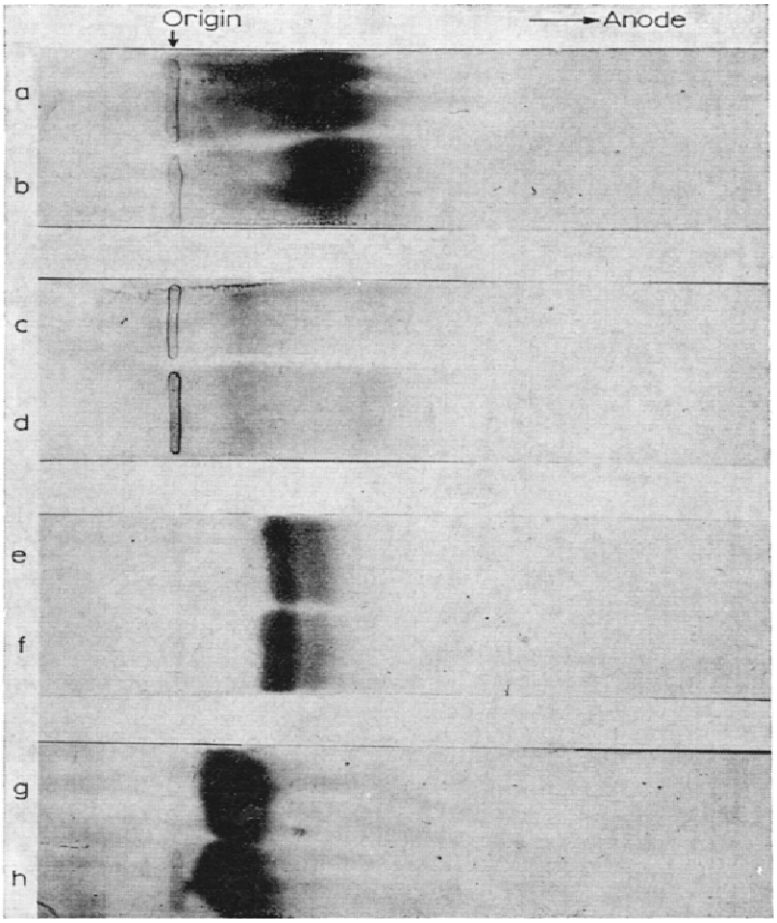

Fig. 4. Membrane filter electrophoretic patterns of milk proteins with and without added tea polyphenols in the presence of urea. (a) α-Casein complex. (b) α-Casein complex + tea infusion. (c) β-Casein. (d) β-Casein + tea infusion. (e) α-Lactalbumin. (f) α-Lactalbumin + tea infusion. (g) β-Lactoglobulin. (h) β-Lactoglobulin + tea infusion.

buffer 7 *M* with respect to urea the coloured material remains at the origin. A very faint yellow band moves to a position just ahead of that reached by the β-lactoglobulin used as standard. This tea pattern is not altered by mixing the tea with milk proteins.

The indications are that 7 *M* urea inhibits the interactions between milk proteins and the coloured tea polyphenols which suggests that these interactions are due, at least initially, to the formation of hydrogen bonds.

SUMMARY

Electrophoretic methods have been used to investigate the interactions in solution between coloured tea polyphenols and milk proteins. It has been found that:

1. When a tea infusion is mixed with milk in the proportions but not the overall concentration obtaining in a cup of tea with milk, the coloured tea polyphenols interact mainly with the α-casein complex and the β-casein of the milk to form soluble casein–polyphenol complexes. β-Lactoglobulin and α-lactalbumin, the main whey proteins, appear to be unaffected by the polyphenols in the presence of the casein at the concentrations used in this investigation.

2. In the absence of the casein both α-lactalbumin and β-lactoglobulin interact with the coloured tea polyphenols, forming either soluble or insoluble protein–polyphenol complexes, the type formed depending on the relative and overall proportions of protein and polyphenol present in the mixture.

3. Paper electrophoresis of tea infusion in borate buffer (pH 8.5) and in molybdate buffer (pH 4.9) is a useful method for the comparison of tea infusions and of polyphenol fractions isolated from tea.

4. Paper electrophoresis of tea infusion with added casein in molybdate buffer indicates that the casein interacts selectively with some of the coloured tea polyphenols in the presence of the molybdate complexing agent.

5. Membrane filter electrophoresis in phosphate buffer (pH 6.7) and 7 *M* with respect to urea indicates that the milk protein/tea polyphenol interactions are at least initiated by the formation of hydrogen bonds.

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