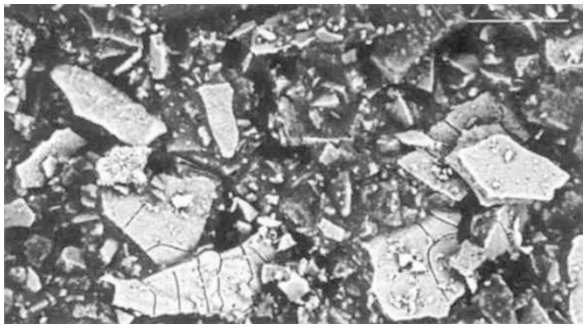
What causes scum on tea?

SIR — When tea is brewed in hard water, an unsightly film or scum develops on the surface of the cup and in the teapot. To study the scum quantitatively, we have devised a method for collecting it from the surface of a large beaker of tea infusion with an aluminium scoop. Examination of washed, dried scum in the scanning electron microscope (see figure) shows scum particles with small white patches on the surface. We found these to be $CaCO_3$. Chemical microanalysis shows that all the calcium, plus small amounts of magnesium, manganese and other metals introduced partly from London mains water and partly from the tea leaves, can be removed by treating the scum with dilute hydrochloric acid. The remaining organic matrix is insoluble in all the solvents tried except highly concentrated alkali. Matrix-assisted ultraviolet-laser desorption/ionization mass spectrometry (MALDI; ref. 1) revealed a wide range of masses with a broad maximum around 1,000.



Scanning electron micrograph of tea scum. Scale bar, 100 μm. The small white patches on the surfaces are calcium carbonate.

We determined the rate of formation of scum by infusing teabags of a popular black tea blend (Typhoo) in thermostated London mains water at 80 °C for fixed periods, removing the teabag and any foam on the surface, and allowing the scum to develop for various 'standing time' periods at constant temperature, usually 80 °C, before collecting it.

We found that scum continued to be formed over many hours, rising approximately linearly with time during the first hour. After 4 h a thick layer of scum had formed on the surface whose mass was proportional to the surface area of the tea infusion. The rate of scum formation depended on the ambient atmosphere. More scum formed in an oxygen atmosphere than in air, and much less under nitrogen. The scum was therefore produced by the oxidation of tea solubles such as certain polyphenolic components. Its formation was not affected by the teabag material.

We saw no scum on tea infused in distilled water or in distilled water containing calcium chloride. Scum developed only in water containing both calcium (or magnesium) and bicarbonate ions. No scum formed if the calcium ions were complexed with agents such as EDTA. Addition of acid decreased the amount of scum. No scum formed in lemon tea, partly because of the low pH and partly because of calcium complexation by citrate[2,3]. The more teabags we used, the less the amount of scum formed. The main reason for this unexpected result was the fall in pH produced by the tea polyphenols. Addition of milk greatly increased the amount of scum formed. Scum formation increased with increasing temperature of the infusion during the standing period. The resulting activation energy of 34 kJ mol^{-1}, together with other evidence, showed that the rate-controlling step was chemical rather than diffusion to the surface.

Thus tea scum is a complex organic material derived from the oxidation of tea solubles mediated by calcium salts and accompanied by calcium carbonate. The process of scum formation shows significant similarities to the enzyme-catalysed oxidation or 'fermentation' by which macerated green tea is converted to black tea. Further details will be published elsewhere.

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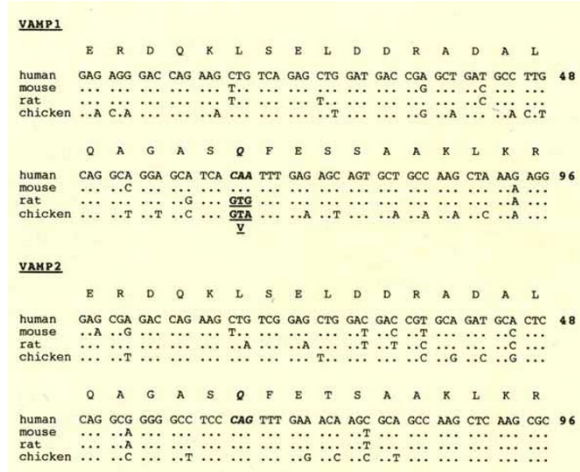
Neurotransmission and secretion

SIR — VAMP (vesicle associated membrane protein; ref. 1) has been implicated in the docking of cell vesicles to their appropriate target compartment[2,3]. In nervous tissue, specific recognition between VAMP, localized on neurotransmitter-containing vesicles, and syntaxin, an integral protein of the presynaptic membrane, is ensured by a set of cytosolic proteins (*N*-ethylmaleimide-sensitive fusion protein (NSF) and soluble NSF attachment proteins (SNAPs))[2]. Two VAMP isoforms (VAMP-1 and VAMP-2) are present in the nervous tissue, and their relative proportion varies in different parts of the central nervous system[4]: VAMP-1 accounts for about two-thirds of spinal-cord VAMPs.

We have found that rat brain VAMP-2 is the specific target of the zinc-dependent proteolytic activity of tetanus and botulinum B neurotoxins[5], two powerful toxins that block neuroexocytosis. Rat brain VAMP-1 is resistant to both toxins because of a Val for Gln substitution at the site of VAMP-2 proteolysis. At variance, human brain VAMPs have identical cleavage sites[6] and hence both of them are expected to be cleaved by the two neurotoxins.

These findings can be related to the different toxin sensitivity of humans and rats. Although humans are very sensitive to tetanus and botulinum B neurotoxins, thousand times higher doses are required to kill rats[7]. Variations of toxin potency among animal species have been attributed to variations in binding to the neuromuscular junction. However, the above-mentioned findings suggest the possibility that amino-acid variations at the unique site of neurotoxin proteolysis may also contribute to the different susceptibility to tetanus or botulism B.

To test this possibility, we determined



Nucleotide and amino-acid derived sequences of human, mouse, rat and chicken neuronal VAMP-1 and VAMP-2 regions containing the site of proteolytic cleavage of tetanus and botulinum B neurotoxins. Bold letters indicate the P1 proteolytic site; the codon change conferring toxin resistance is underlined.

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