Beneath the dominant, and almost dismissive idea that the roles for proteins in the tears are antimicrobial and immunological, has been a persistent undercurrent from stalwarts investigating the physical contribution of proteins to tears. It is here that we enter the realms of surface tension and viscoelasticity and the many publications of Frank Holly, Anthony Bron and John Tiffany. A low surface tension and a high non-Newtonian viscosity are essential for tear film stability and proper lubrication of the ocular surface, and it appears that proteins are imperative for these properties.

Of the many hundreds of proteins now known to be present in human tears (1), tear lipocalin (~18kD) has attracted special attention for its possible role in affecting surface tension and viscoelasticity. This is because of its relative abundance (15-33% total tear protein), its relatively poor antimicrobial activity, and its ability to bind lipids and hence interact with the lipid layer at the outer surface of the tear film. By comparing changes in surface tension of lipid depleted tears, and mixtures of various tear proteins, Nagyova and Tiffany implied that tear lipocalin was interacting with the surface lipids to lower the surface tension (2). This idea that proteins are interacting with the lipid layer has been taken a step further by our group. We now believe that proteins are an integral part of the air liquid interface of the tear film and not simply interacting with the lipids from below. The evidence in part has come from comparing dynamic surface tension changes of Meibomian oils, phospholipids, ocular mucin, whole tears, and tear proteins in a Langmuir trough. From this, it is clear that proteins, and not Meibomian lipids alone, are needed for lowering the surface tension to that found in whole tears. Moreover, these proteins include the major tear proteins such as lysozyme and mucins (3,4). Enigmatically lipocalin is giving confounding results (unpublished) which are indicating that apolipocalin is able to penetrate a lipid film, but hololipocalin (lipocalin loaded with lipids) appears to be trapped in the aqueous. By contrast, other tear proteins unfold at the surface and become trapped in the lipid layer. By labelling the proteins and lipids with fluorescent tags, we have been able to show that the presence of proteins causes the surface layer to become gel like rather than mobile which is the case when lipids alone form the surface film. This is comparable to clinical findings, where the surface pattern of the tear film is stable through multiple blinks.

In terms of tear viscosity, a recent exciting, but confounding paper by Gouveia and Tiffany (5), indicates that pure solutions of lysozyme, lactoferrin, IgA, or tears without lipids behave as Newtonian fluids i.e. as an object is moved faster through a fluid (shear rate) the resistance it incurs (viscosity) does not change. Whole tears (with lipids) behave quite differently: their viscosity drops rapidly with increased shear rate. This is known as shear thinning or non-Newtonian behaviour, and is thought to be important for reducing drag on the ocular surface when blinking. In contrast with the pure protein solutions, mixtures of pairs of the proteins above or apolipocalin alone showed shear thinning similar to normal tears. Hololipocalin (with lipids) showed Newtonian behaviour. At this stage, there are no explanations for these confusing
phenomena other than it being the mixture of proteins with proteins, and proteins other than lipocalin with lipids which give tears their non-Newtonian properties. Important here is the concept of protein interaction in the tears rather than the role of individual proteins. This interaction of proteins has not gone unnoticed by Ben Glasgow’s team who have described that lipocalin interacts with both lactoferrin and lysozyme, but not albumin and consequently has suggested that there is a «functional interdependence of these proteins in the formation of the tear film» (6). Importantly this interaction is ionic and is disrupted at high salt concentrations. Speculatively, this could help explain the common thread of increased osmolarity in dry-eye: increased salt concentrations disrupts the protein-protein interactions leading to altered viscoelasticity and surfactant properties of the proteins in tears which in turn leads to rapid tear breakdown.

These studies on the contribution that proteins have to the physical nature of tears hold many exciting possibilities. Do the inflammatory proteins disrupt the physical performance of the common tear proteins? What are the contributions of some of the quantitatively small proteins to the physical properties of the tears, or similarly proteins that are relatively abundant, such as lacritin, but have as yet no clearly defined role? I look forward to seeing the results from intrepid young workers who have the courage to take up the challenge of examining the tear film as a dynamic interaction of molecules.

REFERENCES

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