A COMPOSITIONAL BASED MODEL FOR THE TEAR FILM LIPID LAYER*

BY James P. McCulley, MD, AND Ward Shine, PhD (BY INVITATION)

ABSTRACT

Background: The tear film lipid layer is formed from lipids secreted by meibomian glands of the eyelid. After initial analyses of these lipids we concluded that an understanding of the function of the various classes of lipids in a normal lipid layer could only be understood after detailed investigations of both polar and nonpolar lipids of the meibomian gland.

Methods: Meibomian gland secretions were obtained from normals. Lipids were separated by thin layer chromatography and high pressure liquid chromatography, and analyzed by UV absorbance, gas chromatography and mass spectroscopy.

Results: Based on our analyses we concluded that the current understanding of lipid layer composition and function were inadequate or misleading. We therefore propose that the more polar lipids function as a structure (with surfactant characteristics) upon which the functional stability of the more nonpolar lipids are dependent. We further suggest that the interrelationships between lipid classes present, length of fatty acids and alcohols, their unsaturation, and hydroxylation are important for maintaining proper thixotropic characteristics of the lipid layer as well as optimal barrier properties.

Conclusion: The tear film lipid layer is composed of 2 phases: (1) a thin polar phase adjacent to the aqueous-mucin phase and (2) a thick nonpolar phase associated with both the polar phase and the air interface. The structural characteristics of the polar phase and the barrier functions of the nonpolar phase are a direct result of specific compositional parameters.

*From the Department of Ophthalmology, The University of Texas Southwestern Medical Center at Dallas. Supported in part by an unrestricted grant from Research to Prevent Blindness, Inc, New York, and grant EY03650 from the National Institutes of Health.

TR. AM. OPHTH. SOC. VOL. XCV, 1997
INTRODUCTION

Secretions of the eyelid meibomian glands form the outer lipid layer of the ocular tear film. Two conditions are necessary for this lipid material (meibum) to be effective: (1) it must be secreted in appropriate amounts and (2) it must form an effective lipid layer over the hydrophilic aqueous-mucin layer of the tear film. Our investigations have led to certain insights concerning necessary physical and biochemical properties of the tear film lipid layer.

METHODS

Meibomian gland secretions were collected from normal individuals. (Some analyses of meibum from chronic blepharitis patients are included to enhance an understanding of normality.) Meibum from each individual (not pooled) was analyzed by TLC, GC (cholesterol and wax ester fatty acids), GC-MS (wax alcohols and triglyceride fatty acids), HPLC with UV detection, and GC-MS (polar lipids and hydrocarbons). Cholesterol and wax esters were separated from each other by HPLC.

RESULTS

We propose that the tear film lipid layer is composed of 2 lipid phases: a thin polar phase and a thick nonpolar phase. The determining factor in positioning a lipid in either of these lipid-layer phases may not only be its lipophilicity but also the presence or absence of short-chain (carbon chain length C12-18) normal (not branched) fatty acids, especially saturated ones. On the basis of the presence or absence of cholesterol esters in meibum, we have identified 2 types of normals—those with cholesterol esters (NCP) or those without cholesterol esters (NCA). Thus, we have determined that in secretions from both types of normals, phospholipids and sphingolipids contain C12-18 saturated normal fatty acids, while C12-16 saturated normal hydroxy-fatty acids are present only in sphingolipids. Triglycerides also contain 15% to 25% short-chain saturated normal fatty acids. On the other hand, less than 6% (usually 5% to 7%) of these short-chain saturated normal fatty acids are found in wax esters. The corresponding wax ester short-chain fatty alcohols are less than 1%, except in NCA normals (40%). Cholesterol esters usually contain less than 1% of these short-chain saturated normal fatty acids. Hydrocarbons (all saturated) contain less than 1% of short chains (Table I).

Details concerning these results, including relative amounts (%) in meibum, will clarify certain points.

Wax esters (68%): fatty acids 20% oleic (monounsaturated 18-carbon
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<th>LIPID TYPE</th>
<th>ESTER COMPONENT</th>
<th>PERCENT CARBON CHAIN LENGTH AND TYPE</th>
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<th>N-HYD</th>
<th>N-SAT</th>
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AI, anteiso; HYD, hydroxlated fatty acid; I, iso; N, normal; SAT, paturated; UNSAT, unsaturated.
fatty acid, C18:1) and polyunsaturates present, 50% short-chain branched (iso and anteiso) chains; fatty alcohols few short chains, long chains 9% unsaturated and 85% branched. However, in NCA normals there is virtually no unsaturation in either the fatty acids or the alcohols but there are more short chain normal alcohols (over 40%). Cholesterol esters (16%): fatty acids few short chains, long chains very low unsaturation and 85% branched. These esters are absent in NCA normals (there was no corresponding increase in free cholesterol).

Triglycerides (6%): fatty acid short chains 45% oleic, polyunsaturates present, and 15% branched, long chain.

Free fatty acids (1.0%): 30% oleic and 30% short chain saturated normal.

Cerebrosides (1.5%): short-chain saturated normal fatty acids, 65% hydroxylated (NCP) but 95% in NCA normals. However this is 5-10% unsaturation in patients with blepharitis, except meibomian keratoconjunctivitis, 20% unsaturation; this blepharitis disease group is also very low in total cerebrosides.

Phospholipids (4%): short chain saturated normal fatty acids, except meibomian keratoconjunctivitis, 50% unsaturation.

Hydrocarbons (up to 1%): few short chains, long chains all saturated normal (up to C33 chain length).

Noteworthy are tightly controlled lipid parameters. The polar lipids (phospholipids and sphingolipids) contain predominantly short-chain saturated fatty acids with important hydroxylation in the sphingolipids; in NCA normals the amount of this hydroxylation is higher. Triglycerides from both types of normals contain 45% of the monounsaturated fatty acid, oleic acid (C18:1), and 55% total short-chain fatty acid unsaturation. Similarly, wax ester fatty acids in NCP normals are composed primarily of only two fatty acids; again oleic acid is quite important (30% to 50%), as is the branched-chain saturated fatty acid anteiso-C17:0 (8% to 33%). In fact, 15% or less of fatty acids are longer than C18 in both triglyceride and wax esters (Table I). However, in NCA normals, wax ester fatty acids are saturated and there is an increased amount of short-chain saturated normal alcohols.

**DISCUSSION**

An effective lipid layer, overlying the aqueous-mucin layer, must have certain characteristics. We propose the following as a model for an effective human tear film lipid layer. Adjacent to the aqueous-mucin layer is the polar phase. It is the sphingolipids and phospholipids which, in the presence of water molecules, initiated the segregation of the polar lipids from the more nonpolar lipids. We believe that highly hydroxylated sphin-
golipids, especially cerebrosides (which also contain sugar hydroxyls), are quite important in this initiation process.

POLAR-PHASE CHARACTERISTICS

It is, however, short-chain saturated normal fatty acids, with a narrow range in chain lengths, that promote structural stability in the polar lipid phase. In contrast, long-chain, branched-chain, or unsaturated fatty acids may promote instability or excess fluidity and are therefore preferentially excluded from the polar lipids. Specifically, this polar phase is composed of polar phospholipids such as PE (phosphatidylethanolamine), PC (phosphatidylcholine), SM (sphingomyelin), and others,\textsuperscript{10} and various other polar lipids such as ceramides and cerebrosides (sulfatides may only be present in NCA normals lacking cholesterol esters). It is important that a large fraction of the short-chain fatty acids are hydroxylated; however, these special fatty acids are only esterified to sphingolipids. Triglycerides (TG) are also incorporated into this polar monolayer\textsuperscript{11} to a concentration of 3\% to 5\%; here the presence of unsaturation (in addition to saturation) is quite important.\textsuperscript{11} On the basis of fatty acid composition, we suggest that wax esters can partially supplement a triglyceride deficit, but never replace their functionality entirely. This appears to be more likely in NCA normals where the wax alcohols contain large amounts of short-chain saturated normal alcohols. Some triglycerides and wax esters likely bridge between the polar and nonpolar lipid phases. When cholesterol esters are present, very small amounts may intercalate in a like manner,\textsuperscript{12} but this does not promote stability. We would expect a large fraction of free fatty acids,\textsuperscript{2} short-chain fatty alcohols, and monoglycerides and diglycerides with shorter-chain fatty acids also to be present in the polar phase. We believe that small amounts of free fatty acids\textsuperscript{13} may be quite important, since their presence can promote the formation of a gel phase (eg, SM and palmitic acid) and swelling of the lipid-water gel phase, especially in the presence of monoglycerides.\textsuperscript{14} Finally, we suggest that shorter-chain hydrocarbons may intercalate into the polar lipid layer in small amounts and further stabilize the structure of the polar lipid phase. It has been noted, however, that C12 hydrocarbons behave very differently from the longer C16 hydrocarbons\textsuperscript{15,16} and therefore only the longer hydrocarbons are effective; in fact, we have never detected meibum hydrocarbons shorter than C16. This observation is especially important when considering the important role of the polar phase in tear film stability.

The structural stability of the polar phase depends on an appropriate balance of polar lipid components, ions (such as calcium, potassium, sodium, chloride and bicarbonate), and pH. Thus, unusually great differences in polar lipid fatty acid chain length (eg, C12, lauric and C18, stearic)\textsuperscript{17} or the presence of fatty acid unsaturation (eg, C18:1, oleic)\textsuperscript{18} can lead to
demixing or segregation of a polar lipid type based solely on the fatty acid component. Alternately, an increase in pH (eg, above pH 8) in the presence of calcium ions can result in a decreased ability of PE to form hydrogen bonds\textsuperscript{19,20} necessary for structural stability.

The interaction of different lipid types is also affected by unsaturation. In the temperature range 35°C to 37°C, interactions between PC and TG depend on the degree of fatty acid unsaturation in the TG.\textsuperscript{11} Only when the esterified fatty acid in TG was the unsaturated oleic acid (C18:1), rather than the saturated palmitic acid (C16:0), was adequate fluidity and orientation maintained.\textsuperscript{11} On the other hand, cholesterol oleate forms a complex with triolein (TG containing only oleic acid), but when PC is also present the triolein and PC preferentially form a monolayer adjacent to the aqueous layer and the cholesterol oleate is preferentially relegated to a second layer.\textsuperscript{12} We believe that by this mechanism, TG unsaturation (eg, C18:1) plays a critical role not only in promoting the proper segregation of polar and nonpolar meibum lipids but also in promoting the stability of the polar lipid phase itself. We have determined that in meibum from normals without cholesterol esters (NCA),\textsuperscript{4} the only significant unsaturation retained is TG unsaturation.\textsuperscript{46}

NONPOLAR-PHASE CHARACTERISTICS
The total lipid layer, however, consists not only of the polar lipids but also much larger amounts of nonpolar lipids. The majority of these nonpolar lipids (wax esters, cholesterol esters, triglycerides, and hydrocarbons) form the bulk of the tear film lipid layer, the nonpolar phase. The longer-chain hydrocarbons (eg, C28) may not only increase cohesiveness but also decrease the water vapor transmission rate\textsuperscript{21} of the lipid layer. Thus, long-chain (C20-31) fatty acids, fatty alcohols and hydrocarbons, with similar chain lengths, are dominant and are important in determining the transmission rate for water vapor and other gases.\textsuperscript{22} Free fatty acids, if they are not ionized (ie, uncharged) may also be important in this nonpolar part of the tear film lipid layer. In this model, we propose that the polar phase not only has surfactant properties but, more important, acts as a structural matrix upon which the nonpolar phase depends. The nonpolar phase’s primary functions are to control the transmission rate of water vapor, carbon dioxide, oxygen, and ions; a secondary function is to act as a reservoir for triglycerides, wax esters, and other less polar lipids, including shorter-chain hydrocarbons important for maintaining optimal stability of the polar lipid phase.

ABNORMAL CHARACTERISTICS
Our investigations give some insight into the ramifications of abnormal meibum lipid compositions.\textsuperscript{23} For example, when compared with normals
(NCP), meibomian keratoconjunctivitis-type chronic blepharitis meibum wax esters are somewhat low in unsaturation, but the phospholipids have a significant amount of unsaturation; also, the amount of cerebrosides in this meibum is quite low. These differences may partially explain the low fluidity of the meibum and the instability of the tear film. This instability may be the result of demixing or segregation, which in turn results from the presence of unsaturated fatty acids in the phospholipids. Another example relates to KCS (keratoconjunctivitis sicca): It is now known that there are two types of KCS, the first due to aqueous tear insufficiency and the second due to excess evaporation. We have found a significant association between the latter type, evaporative KCS, and low meibum levels of the phospholipids PE and SM. Thus, the proposed structural role of the polar lipid phase appears quite important in maintaining proper functionality of the tear lipid phase; in this specific example, tear film water vapor transmission rate.

CONCLUSIONS

We believe that the importance of meibum composition, although generally known for many years, is often overlooked when considering function. For example, it is known that monolayers of cholesterol esters are much more stable if the esterified fatty acid is oleic acid (C18:1) rather than stearic acid (C18:0). However, in the tear film lipid layer, cholesterol esters contain only long-chain saturated fatty acids. We propose that it is the fatty acid moiety (primarily oleic acid) of wax esters and, to some extent, triglycerides that stabilize the nonpolar phase in which the cholesterol esters reside. Thus, the stability arises not from oleic acid esterified to cholesterol, but from esterified oleic acid in other lipid types (eg, wax esters), which are nevertheless associated with the cholesterol moiety which promotes stability. As previously discussed, the stability (and functionality) of this nonpolar phase may also be enhanced by the presence of long-chain hydrocarbons.

An understanding of the function of individual lipid classes and their composition can also be discerned by comparing the two types of normals. Normal individuals whose meibum does not contain cholesterol esters (NCA) nevertheless have polar lipids and triglycerides similar to those found in individuals whose meibum contains cholesterol esters (NCP). It is, however, the almost total absence of unsaturation in wax ester fatty acids and alcohols in NCA meibum that is most striking. We believe that in this meibum, where cholesterol esters are not present, any lipid properties necessary to maintain proper fluidity in the nonpolar phase are achieved by increasing the amount of branched-chain iso- and especially anteiso-fatty acids in wax esters. As previously discussed, we believe that
regardless of which type normal meibum is present, triglycerides and wax esters can act as transition lipids, bridging between the polar and the non-polar phases. Finally, the significance of uniformity in composition of polar lipids of both types of normals is noteworthy.

On the basis of our research, we propose a model for the tear film lipid layer that includes two lipid phases (Fig 1). This model more closely approaches that proposed with the mucin distributed throughout the aqueous layer and the polar lipids adjacent to this layer, rather than a model with separate mucin and aqueous layers. Recently reported photomicrographic investigations by others also support the former model. Finally, as has been suggested, we also believe that the tear film (lipid

![Proposed model of tear film.](image-url)
and aqueous-mucin layers including the glyocalyx) acts as a thixotropic system, which is essential for the proper fluidization and restructuring of the tear film during a blink. This system would only be optimally functional when the tear film lipid layer composition was appropriate and normal.

REFERENCES


**DISCUSSION**

**RONALD E. SMITH, MD.** I congratulate Dr McCulley and colleagues for their 20 years of contributions to our understanding of blepharitis and the lipids of the meibomian glands.

Drs McCulley and Shine propose a model of a two-layered structure of the lipid layer of the tear film, including a polar lipid layer providing surfactant characteristics to stabilize a nonpolar lipid layer, which seems to have a role in determining tear evaporation rates. These lipids need to be excreted in sufficient amounts with the proper biochemical composition to support the functions of the lipid layer of the tear film.

Rather than comment on the biochemistry aspects of this study, I would like to emphasize aspects of the lipid layer in the overall context of the ocular surface and the tear film.

In addition to advances in our understanding of the lipid layer of the tear film in the last decade, advances have been made in our understanding of blepharitis and its relationship with other common ocular surface disorders, such as dry eye.

A useful classification of dry eye resulted from a recent National Eye Institute workshop in 1995.1 This classification includes two major categories: (1) tear-deficient dry eye and (2) evaporative dry eye meibomian gland disorders. Blepharitis falls into the latter classification.

A two-layer theory of the tear film has replaced the traditional three-layer model.23 The two layers consist of a lipid layer on the surface of the tear film overlying a combined or mixed aqueous–mucin layer. Chen and
colleagues have stated that the tear film should be considered: "Dilute mucus with a lipid layer covering." McCulley and Shine's work reported today supports the two-layer theory for the tear film.

There is now an increasing consensus about the importance of osmolality of the tear film. Over a decade ago, Farris, Gilbard, and coworkers emphasized hypersmolarity as the final common pathway leading to ocular surface epithelial damage, including goblet-cell reduction, damaged epithelial cells, secondary inflammation, and ultimately clinical symptoms. Mathers and associates recently emphasized that hypersmolarity is the most important factor in assessing the environment of the surface epithelial cells. Farris feels that hypersmolarity is the "gold standard" measure of the tear film itself. Unfortunately, this is not an easy measurement to reliably determine, and there are still sources of error. Causes of hypersmolarity include the broad category of conditions that result in a decrease in tear secretion, and a second broad category of conditions that increase evaporation of tears. Meibomian gland disease and abnormalities in the tear lipid layer are in the second category.

Mathers reported on techniques to measure meibomian lipid volume and viscosity and emphasized the relationship of these changes to tear film dynamics and tear film turnover.

Robin and associates reported on the use of infrared photography techniques to visualize the structure of the meibomian glands and found abnormalities in patients with various disorders, such as acne rosacea.

Jester and coworkers studied a rabbit model of meibomian gland dysfunction that developed after prolonged topical application of epinephrine. These rabbits developed meibomian glands engorged with keratinized debris.

Bron and colleagues reviewed lipid production by meibomian glands and proposed a model of lipid production, including the effects of lids and blinking.

There have, therefore, been a number of advances in our understanding of the tear film in general and the role of blepharitis and meibomian gland lipids specifically in the context of ocular surface disorders, including dry eye syndromes. It is appropriate today, as emphasized by McCulley and Shine, that the lipid layer be viewed in the context of the entire tear film and in the even broader context of the lids, including blinking function. McCulley and Shine agree with Prydal and associates and Berman in considering the tear film in this broad context as a thixotropic system. Webster's dictionary defines thixotropy as the "property established by various gels of becoming fluid when shaken, stirred, or otherwise disturbed and setting again to a gel when allowed to stand." Viewing the tear film and its relationship to the ocular surface in this context makes sense; and I support the opinion of McCulley and Shine that
"the tear film acts as a thixotropic system, which is essential for the proper fluidization and restructuring of the tear film during a blink ... only functional when ... lipid layer composition was ... normal." This is a new way to consider the functional and physical properties of the tear film.

McCulley and his team continue to uncover the secrets of lipids of the meibomian gland and their role in various conditions, including dry eyes and blepharitis. It is likely that they will find specific lipid classes that correlate with specific clinical findings. They have made some preliminary observations in this regard as reported today.

Finally, O'Day,14 in his discussion of McCulley's original paper on classification of blepharitis in 1982, stated, "With the application of refined biochemical techniques ... uncertainties regarding the role of meibomian glands and lid disease will be resolved." O'Day's prediction 15 years ago was correct as McCulley and Shine and other investigation in this field are finding through ongoing biochemical and biophysical studies of the tear film lipids.

I have two questions for Dr McCulley:

1. How certain are you that specific lipid markers for keratoconjunctivitis sicca, blepharitis, and other conditions are primary events rather than secondary changes?

2. Have you considered the possibility of developing animal or other laboratory models to sort out the effects of various changes in lipid composition in the tear film?

REFERENCES

DON MINCKLER, MD. I would like to thank Dr McCulley for his talk and express appreciation on behalf of all of us who frequently see patients with tear deficiency. I would like to ask him to comment on the potential connection between some of these findings and what we observe in the Glaucoma clinic with the increased use of antifibrotic agents in filtering surgery. Antifibrotic agents, including 5-fluorouracil and mitomycin, aggravate tear deficiency, at least for the majority of my patients. We also have a problem with late breakdown of the surface of avascular blebs that we are creating in increased numbers. This is histologically a result of remarkable attenuation of the epithelium covering these filters and a virtual absence of goblet cells. We frequently see epithelial filaments over the blebs. In short, we are probably aggravating a pre-existing tear deficiency and we certainly need to know more about these inter-relationships.

JOHN T. FLYNN, MD. I would also like to thank Dr McCulley for his interesting paper. Let me ask a question. “What is a pediatric ophthalmologist doing up here talking about chronic blepharitis?” Well, the reason I am here is because I spend a day a week with our residents in Triage Clinic and never knew there was so much chronic blepharitis. The second reason I am here is I recently went, as I do every year, to our Cornell Alumni meeting and Dr James M. Sourani of Los Angeles, California, presented an enormous amount of his office data on the influence of dietary intake of fat on Meibomian gland secretion. He felt this played a very significant role in chronic blepharitis and its therapy. He took a dietary history on every one of his patients and the bottom line is that the people who ate junk food had thick, ugly-looking Meibomian secretions. The people who did not consume saturated fat in huge amounts had very fine, clear secretions. Dr Sourani put his patients, as part of his therapy for chronic blepharitis and Meibomianitis, on dietary restriction of the saturated fats. I would like to ask Dr McCulley, since the issue was not raised in his paper, whether what we take into our mouths finds its way into our Meibomian gland secretions and sets up culture conditions where all those organisms that play a role in this very common and very difficult condition can grow?
GEORGE L. SPAETH, MD. Congratulations on a beautiful paper with beautiful slides; your findings were very exciting. However, I wish to push the discussion a bit further along the lines of the last comments. Specifically, I have some thoughts regarding the basic cause of these lid abnormalities. There may be an appropriate model to look at, specifically, some of the systemic conditions in which there are abnormalities of lipid metabolism. I think specifically of the a-beta-lipoproteinemais and the sphingolipidoses. For example, Fabry's disease would be a good condition to examine. I wonder if you have looked at these abnormalities of systemic lipid metabolism to see about the lid manifestations.

JAY KRACHMER, MD. After John Flynn's comments, I am expecting to have a very greasy view of the world after the breakfast I had this morning. But, I also would like to congratulate Dr McCulley and ask you a question, Jim. It seems to me that there is a lot for us to learn by looking at babies. Infants do not blink. Their tear film looks like an oil slick and I have often said that if we could reproduce baby's tears, we would have a wonderful artificial tear. What can we learn from that? Do they have different lipids? Do lipids change throughout our lifetime? I would just like your comments.

JAMES MCCULLEY, MD. Great Comments. I especially appreciate that they were kind. While I am not sure whether lipid changes are primary or secondary or how the two might play together, we have shown that bacteria we recover in greater frequency from patients with various types of blepharitis, have lipolytic exo-enzymes. But, I think it might be a complex interaction. There are people that are most likely at more risk to develop blepharitis. Just as I pointed out, we found two groups of normals (those with and those without cholesterol esters), whereas, all blepharitis patients had cholesterol esters present, so I think there is a primary component. How many other primary components there might be, I am not sure. I think that there clearly are secondary pathways that can play a role.

Relative to the animal models, I am not sure of one that is better than the other. You have a very excellent animal model, Dr Smith, and others have animal models. We have not looked at animal lipids in the lab but their analysis might very well lead to important insights.

Dr Minckler, I do not think you can blame us cornea and external disease people for these nasty-looking blebs after mitomycin-C. I doubt that a tear abnormality is the culprit because the blebs are up under the lids. I think that it is probably something that is happening in the epithelial or vascular endothelial cell mitosis, so the blebs are very avascular. I do not think it is a tear abnormality that is primarily the problem. I have not thought about that and my initial knee-jerk response is that we are going
to kick that one back to the epithelium and not blame it on the tear film. Is there local goblet cell or total goblet cell dropout in these patients? I am not sure but I think it warrants further investigation.

Dr Flynn, your comments were great. We have talked about dietary influence and have thought about whether that is something we should pursue. We have not yet done that. We have been trying to churn through all the layers of lipids and it has been slow work because we had to develop techniques to be able to look at individual patient samples. I am sounding like I am making an excuse, and I am. It has taken a lot of time to figure out how to do from one patient, the lipids, and look at single lipid classes one at a time. It has been very slow, plodding, arduous work and it was disheartening for about 10 years because in the non-polars we would find abnormalities; well so what? Oh, there were abnormalities and it gave us some idea that it is appropriate to keep going but it did not allow us to have any insights into mechanisms. Then we got to the polar lipids and bingo. There we start to get some really good insights and everything starts to make sense, whereas, it had not made a lot of sense before. So, we have not done the dietary studies. We have thought about it. Now that we have all the techniques worked out, I think that is a very good route to consider pursuing.

We have not looked at patients with inborn errors of metabolism. In those rare patients that I see, I have not recognized any particular lid or lipid, or meibomian abnormality, but I certainly want to look at those patients and recover meibomian lipids from them. That is a very good point. Thank you.

And now to Dr Krachmer's comment. We always have wondered why babies were like rabbits and can just stare at us forever and never blink. We have not looked at the lipids in infants but that, along with the dietary issues and the inborn errors of metabolism, are things we certainly will do. I think that if one creates an effective polar lipid coating, we can probably "teflonize" the eye. Babies are maybe somewhat teflonized and it may be that we can re-create such a layer in patients that have a hyper-evaporate dry eye state. It may very well be that we can create a super normal tear film by creating a very effective polar layer. I think we can create a polar layer that really will hold together, that will then support the non-polars, that will in turn prevent evaporation and allow us to go long periods without blinking. Thanks for your comments as well. Thank you all.