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OPTOMETRY AND VISION SCIENCE

FEATURE ARTICLE ON LINE

About Vital Staining of the Eye and Eyelids. I. The Anatomy, Physiology, and Pathology of the Eyelid Margins and the Lacrimal Puncta by E. Marx

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ABSTRACT
This article is a translation of the original article authored by Eugen Marx and published in 1924.[1] Amazingly, many of the issues addressed in the 1924 publication are now, >80 years later, of prime interest for both understanding the lid margin and ocular surface and thus for dry eye diagnosis and treatment. To assist the reader and possibly to provoke further contemplation on a particular section of the translation, we have inserted comments, identified throughout the text. All references, in their original format, have been included in this translation, except those referred to in a few paragraphs that were not readily understood in today's technical language and which were omitted. The first figure of the original article is not included in this translation because it was referred to in one of the few omitted paragraphs.
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Key Words: Marx's Line, mucocutaneous junction, vital staining of the eyelids

Investigators who evaluate the anatomy of the eye in vivo by slit lamp microscope have shown only minimal interest in the margins of the eyelids and the lacrimal puncta. This is understandable, because these ancillary organs are not exciting from the anatomical in vivo view, although they have important functions. (Authors' comment: Marx's comment regarding the relative neglect of the eyelid margins is still relevant, because we remain without adequate answers for a plethora of questions concerning the function and mechanism of actions of the lid margins.) Interesting features of these organs are only noticed when a special stain is used. It is important to commence with a brief description of the eyelid margin and the lacrimal punctum, observed with a strong magnifying slit lamp. To facilitate the analysis process, we will only be talking about the lower lid. First, it is a matter of patient comfort to examine the lower lid, whereas it is more difficult to keep the upper lid everted for a prolonged period as for the drawing of a sketch; and second, there are no noticeable differences between the two. (Authors' comment: Marx did not appear to investigate in detail whether there were differences between the upper and lower lid. Our research suggests that there are morphological differences between the two, and we predict that this area will be the subject of further investigation.)

The epithelium of the epidermis of the eyelid is somewhat less translucent than the epithelium of the conjunctiva of the eyelids; it is also a bit more irregular, striated at the surface, and abuts against the epithelium of the conjunctiva (Authors' comment: forming the mucocutaneous junction), which is often situated on a higher level relative to the epithelium of the epidermis of the eyelid. (Authors' comment: This observation, validated by us, describes a possible important anatomical feature of the mucocutaneous junction, which is apparently overlooked in contemporary literature.) The meibomian gland orifices also open onto the epithelium of the eyelid; they look like small, round, yellow discs; and their margins are just as translucent as the margins of the lacrimal puncta. In addition, hairs can be observed running through the epithelium in a totally different direction; some of them have small bubbles attached to their roots; these are retention cysts of the Moll glands. It is not possible to distinguish the individual cells of the tissue or of the palpebral conjunctiva as Koeppe[2] had previously observed. (Authors' comment: Note that what is being described is an examination of the tissue prior to the instillation of vital stain, and hence, the inability to visualize individual cells.) Numerous vessels of various thicknesses are beneath the epithelium. Their direction is usually perpendicular to the longitude of the eyelid. Many of

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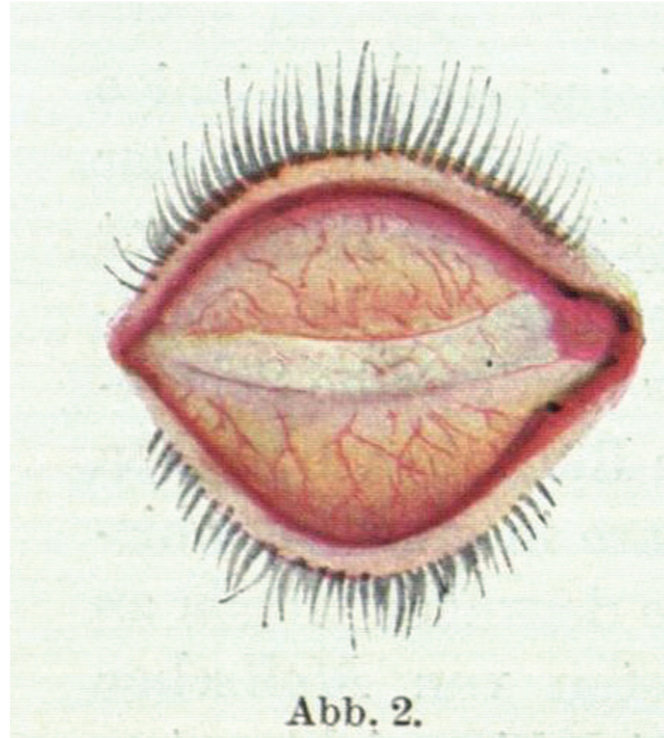

FIGURE 1.

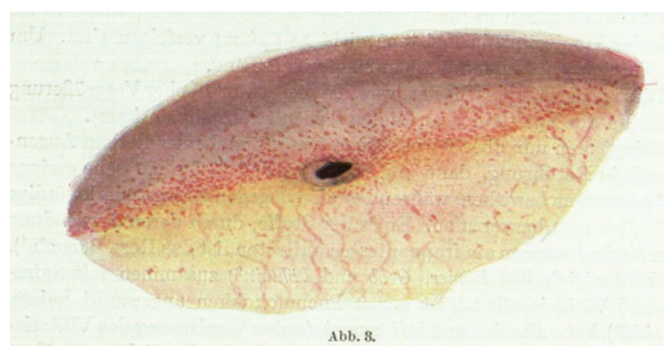

FIGURE 2.

these vessels lead into the subcutis (Authors' comment: referring to the deeper parts of the dermis) of the eyelids; they are fairly hard to follow from the surface of the skin. Sometimes they join into a ring that surrounds the lacrimal punctum. These observations have been made with a slit lamp, with 30× magnification.

By using vital stains on the conjunctiva and the eye, impressive results can be obtained in some of the cases. The effect depends on the type of stain. One of the substances that causes some of the most impressive changes is rose bengal,[a] a stain that was combined with safranin and victoria-yellow by Römer et al.[3] and used for treating pneumococcus. Kleefeld[4] was the first to use rose bengal, without combining it with victoria-yellow, for identifying defects on the cornea and did it in a very distinct and persuasive way. Furthermore, this technique was much more applicable in detecting desquamation of the epithelium compared with the use of fluorescein. Rose bengal in 5% water solution appears to show the most curious effect on the posterior eyelid edge. (Authors' comments: We believe that the term eyelid edge was used by Marx to convey the termination of the epidermal epithelium of the lid margin where the epithelium of the palpebral conjunctiva begins—now known as the mucocutaneous junction. The term mucocutaneous junction has been used throughout to represent this term. In addition, Marx advocating the use of 5% rose bengal is 5× the concentration recommended by contemporary authors[5–7] and should have caused severe stinging; however, neither the use of an anesthetic or stinging was reported by Marx.)

Even macroscopically, after only a drop has been placed in the conjunctival sac, it can be clearly seen that the sharp edge (Authors' comment: of the mucocutaneous junction), which is formed by the palpebral conjunctiva and keratinized epidermal epithelium of the lid margin joining together, is colored with a fine red line. There is more staining on the nasal side than the temporal side, where the line is thinner. Both upper and lower eyelids are colored. The lines of the upper and lower lid connect nasally and temporally, and there is more staining nasally than temporally; this way, the entire conjunctival sac is surrounded by the posterior side of mucocutaneous junction observed by the stained line (Fig. 1). The line is built of numerous small dots that are more coalesced posterior to the mucocutaneous junction, observed by slit lamp microscope.

That is why the sharp line is formed (Fig. 2) (Authors' comment: referring to the line of staining "the line of Marx"), which, as said before, is elevated from the surface of the epidermis of the eyelid margin. The reason for this is the abrupt ending of the stratum granulosum and corneum, which reappear in a different shape and thicker at the beginning of the conjunctiva.[8] The anterior side of the red line (Authors' comment: the part closest to the eyelashes) is situated precisely on the border between conjunctiva and lid margin. By staining just one part of the mucocutaneous junction, precisely where the stained tissue meets the unstained tissue, we will notice that the red line and the mentioned border lie in their respective planes. (Authors' comment: This is best observed in cross section with the slit lamp.)

The thickness of the named line varies between individuals; in some cases, clear dots can be distinguished one from another, whereas in other cases, it is difficult to locate singular dots because of their being so crowded. The dots coalesce into thin lines but, invariably, these dots are less dense on the palpebral conjunctival side of the eyelid margin, just a short distance from the mucocutaneous junction. After that, no more compacted bunches can be found and they become less frequent and smaller until only a few scattered dots remain. (Authors' comment: Observing moving posteriorly.) These independent dots are to be found everywhere in the entire conjunctiva of the lids and ocular surface. A half or 1 mm away from the posterior side of the mucocutaneous junction, the staining is gone. (Authors' comment: The staining of the palpebral conjunctiva stops 0.5 to 1.0 mm posterior to the red line toward the ocular surface side of the line.)

Many more peculiar features occur around the lacrimal punctum, because the line becomes thicker and takes the shape of an outside curved semicircle or ellipse surrounding the punctum. The punctum is located inside this semicircle, but the distance between this semicircle and the punctum varies. However, the punctum always tends to be positioned more to the temporal side of the semicircle rather than the nasal side. The stained dots partly follow the curved rim of the epithelium encircling the punctum where they disappear into the lacrimal punctum. Usually, no

[a]To be purchased from "Dr. Grübler" company, in Leipzig.

dots appear within the duct, but if they are present, the quantity will be minimal.[2]

This is the general and most frequently occurring finding that is characteristic for young people. Despite the fact that numerous variations can be observed, the most important of them have to be addressed (Fig. 3).

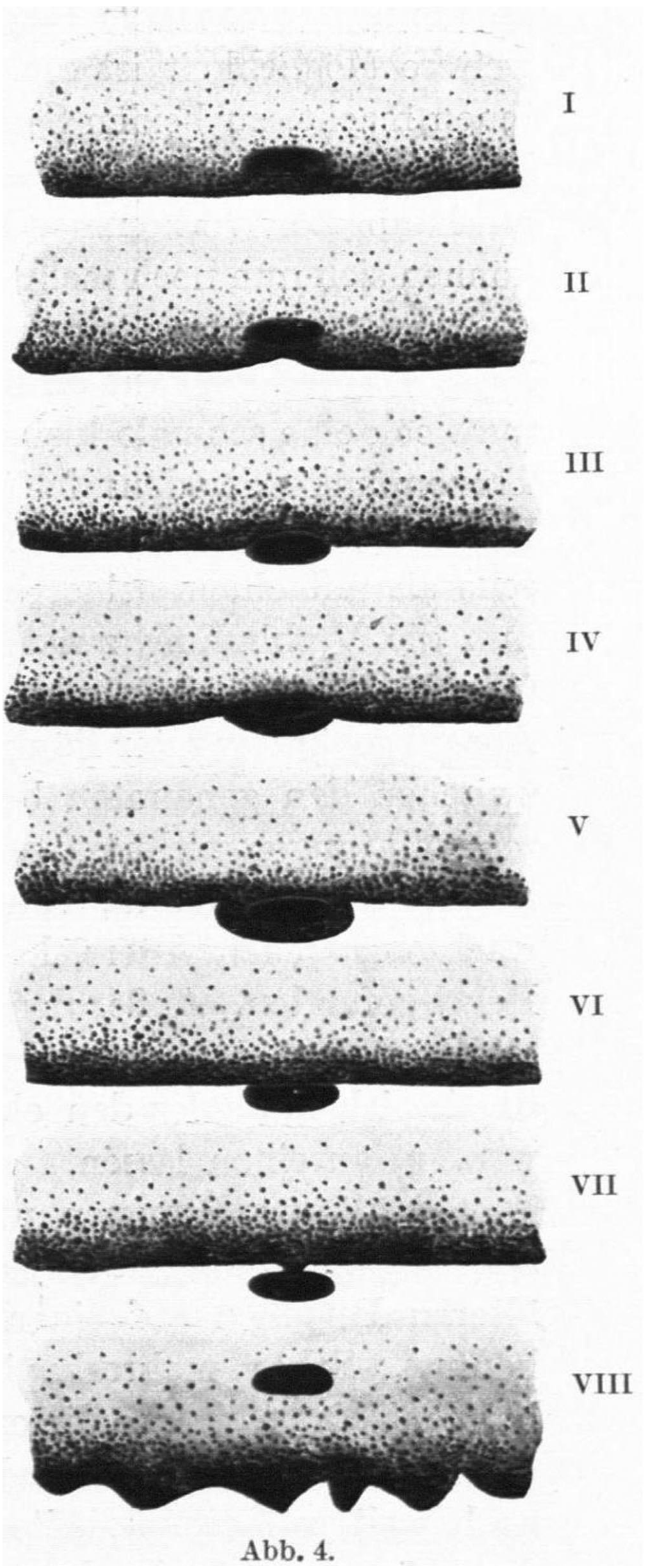

FIGURE 3.

In the first case, the red line can pursue its undeviated path almost undisturbed, around the lacrimal punctum, which is now more or less posterior to the mucocutaneous junction but totally surrounded by stained tissue (I). Second, the line could continue its regular path but create a kink of red stain toward the middle of the opening of the lacrimal punctum (II). There is also the possibility of the lacrimal punctum not being encircled by the red line but having the red line pass through it (III). The line could also create two kinks posteriorly at both sides of the lacrimal punctum (IV). The most common occurrence is these two kinks being more distinct (V). The line can continue its way away from the lacrimal punctum, resulting in the line just barely touching the lacrimal punctum posteriorly (VI). Finally, the connection from the line to the opening can get as small as a narrow bridge (VII). In all of the cases, the lacrimal puncta can be in contact with the stained line centrally or at its edges. It does not matter whether the connection is broad or narrow—there will always be a certain number of red dots that bridge the punctum and the stained line, at least when the patients are young and the tear flow is not exaggerated. This conclusion resulted from analyzing hundreds of eyelids from newborn babies to middle-aged grown-ups. There are many minor variations of the possibilities shown above, but because of being so similar to the ones mentioned, it would not be productive to analyze them here separately. (Authors' comment: We have observed all seven of Marx's descriptions, verifying his observations; however, the implications of these observations require further research.)

Older people do not appear to fit into the scheme. Schirmer had already discovered that the lacrimal puncta of older persons (age >50 years) tend to be raised and prominent from the surface of the lid margin; sometimes, this can be even seen without magnification. Using the slit lamp microscope and rose bengal, we find other interesting phenomena. With increasing age, the lacrimal punctum moves away from the red line situating itself posteriorly. (Authors' comment: Indicates movement toward the ocular surface.) Thus, with older people, the lacrimal punctum moves further posteriorly, away from the red line, in comparison with younger people. (Authors' comment: Because the median age is greater today than in 1924, Marx's comment to >50 years might be even more pronounced in the contemporary geriatric population.) The borderline is less sharp and contains a lot more undulations (VIII). Even in these cases, (Authors' comment: where the punctum is relatively further away from the line) the line shows clear signs that the lacrimal punctum is close to it.

In conditions like these, in cases where the lacrimal puncta are perfectly normal and functional, we can still find traces of the red dots that make the connection between them both. It has to be mentioned that the reported variations only vaguely correspond to the most common situations encountered. There are many other possibilities, e.g., such as the influence of the age of patient, nasolacrimal ducts that are very different between right and left eyes of the same patient, and further curious findings.

The following is the resulting question: what are the small stained dots made of? The answer is fairly readily obtained, if a gentle sweeping of the eyelids is done with a small knife—a process that is absolutely painless. The small amount of red tissue that was collected is added to isotonic saline solution and analyzed under a regular microscope with the same magnification as previously used with the slit lamp microscope on the eye. The same small red dots are seen by raising the magnification. The dots are observed as red-stained epithelial cells, many of them having a dark red nucleus, whereas others have only an evenly pink cytoplasm or just small red dots around the nucleus. Most of the cells, irregular quadrangular or pentagonal and some of them are also round or elliptical, are sometimes bound together by a transparent substance, but many of them are independent. Therefore, we conclude that the "small dots" visible on the slit lamp microscope were epithelial cells, rendered visible by staining.

Evaluating the spot at the mucocutaneous junction where the cells were removed, all the red dots had disappeared. But adding some more rose bengal to the same area, the dots reappear, now being even more dense with diffuse coloring in between. Thus, epithelial cells of deeper layers were colored. On that spot the epithelium is ~10 to 12 cells layers thick and that process can be repeated several times.

It is now important to consider the pathologic variations on man to fully understand the meaning of the staining of the mucocutaneous junction and its parts. Every eye that secretes tears in an unusual way is pathologic. The only pathological states that will be mentioned are the ones that cause an overflow of tears, but the reason for this is unknown. We will also refrain from analyzing the mechanical obstructions that might get in the way of the out-flowing tears. This way, the only remaining possibilities are the ones where an unusual position of the lacrimal punctum causes the overflow of tears and the ones when the "wet" feeling of the patient cannot be the result of abnormal positions of the lacrimal puncta or other apparent reasons.

If rose bengal is instilled into the eyes of people with abnormal and also apparently normal punctal positions, significant differences in regard to normal variations can be observed. First, the lacrimal puncta appear to be situated clearly anterior to the red line but are occasionally completely set apart from the red line. (Authors' comment: In so defined abnormal situations.) Despite the fact that the tissue surrounding the lacrimal punctum is marked by the same red dots, they do not establish any connection to the red line (Fig. 4). However these cells, although they can be stained too, are different from the cells of the red line. This phenomenon sometimes occurs after an enucleation of the eyes of youngsters or after a long period of eversion of the lacrimal puncta.

If the artificial eye that was implanted does not fit well, the lacrimal punctum will also have an incorrect position so that a certain overflow of tears will soon be established, just as described previously. The same situation can occur on youngsters with shrunken eyeballs. If the artificial eye is well fitted, the tears drain off regularly; then, the same results can be seen when staining the mucocutaneous junctions. Eyes where no reason can be found for the overflow of tears are particularly peculiar.

Using rose bengal, it can frequently be observed that the lacrimal punctum is situated anterior to the stained line. (Authors' comment: On the eyelash side of the line. This observation reflects the abnormal situations described above.) I have observed this several times; on the same patient, the eye with tear overflow corresponded to the description above, whereas the normal eye showed regular staining.

A second category of eyes with tear overflow shows the lacrimal puncta lying posterior to the stained line, in some cases even fairly far away. (Authors' comment: On the ocular surface side of the stained line.) Even in such conditions, the surrounding of the lacrimal puncta can be stained but again not making any (or just a minor) connection to the main line. Something similar can be found on the elderly who complain about abundant tears and who have a minor ectropion (Fig. 5).

The third category is defined by eyes with tear overflow, which occurs in acute or chronic conjunctivitis. In this situation, it can be noticed that the line is not as straight as before: it shows bizarre inlays anteriorly and the border of the line is not sharp anymore at many areas. In the case of a diplobacillus conjunctivitis, angular and even acute undulations can be found nasally to the lacrimal punctum and in the – corner of the eye, exactly on the spot where the alterations caused by the disease occur. Not only the cells will absorb the stain but also the entire surrounding tissue, which can lead to extensive staining on the surface of the conjunctiva. The tears can easily flow out at the broken mucocutaneous junction and then lead to the known complaints (Fig. 6). (Authors' comment: The tears flow out through a break in the line which may function as a drainage/overflow channel.)

The relation between the tear-flow and the deviant ratios in the staining of the eyelids and the lacrimal puncta, existing because of the pathological cases, leads one to question whether there is any physiological connection between tear flow and these staining phenomena. The existence of such a connection has not yet been demonstrated; however, several arguments for the likelihood of such a connection will be presented.

The red line is primarily situated along the entire length of the eyelids, both, upper lid and lower lid, increasing in thickness na-

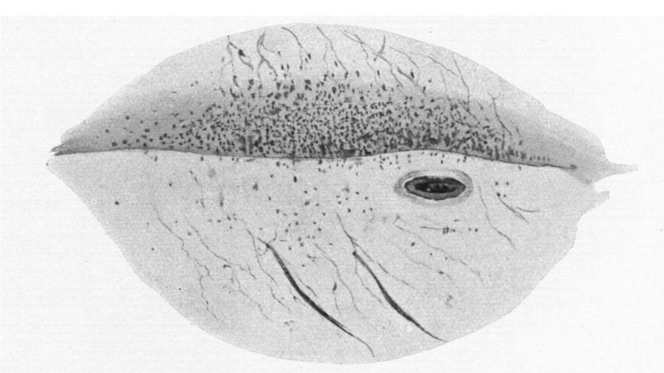

FIGURE 4.

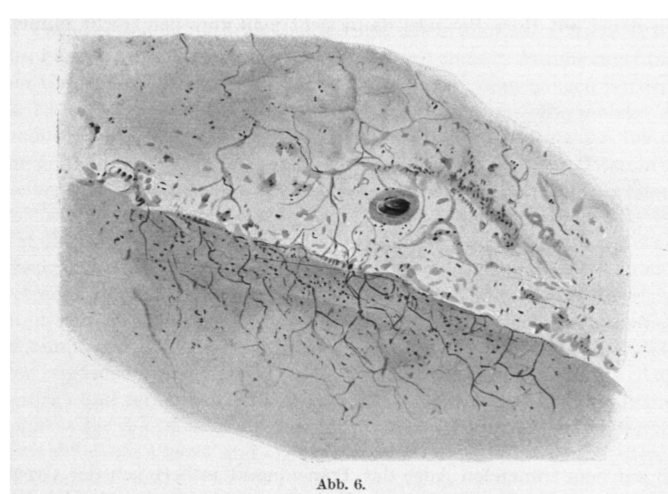

FIGURE 5.

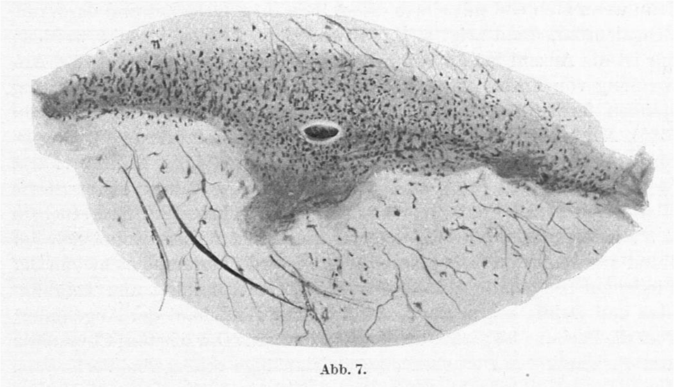

FIGURE 6.

sally, reaching its greatest width at the lacrimal punctum. This line creates a closed ring; representing a barrier to the flow of tears onto the lid margin, thus the supposing existence of a relationship between form and function has to be correct. It is this reason that leads us to believe that there is a connection between the flow of the tears and the interesting staining of the eyelids. There are other facts that would lead us to believe that we are wrong in our approach: in pathological situations, where the connection between the stained epithelial cells of the mucocutaneous junction and the ones of the lacrimal punctum is absent, epithelial cells, stained red, spread more anteriorly than usual. Both are associated with tear overflow. The following can be concluded: different forms of staining of the epithelial cells of the mucocutaneous junction imply changes in the draining of the tears, and vice versa. (Authors' comment: An interesting observation and area for further investigation.)

In addition to this partially answered question, some others will appear: how is it that the epithelial cells of the outer edge of the conjunctiva of the eyelid are so susceptible to staining with a certain dye, whereas other cells of the conjunctiva are not? (Authors' comment: There are many complexities to this 1924 question, which remain currently relevant and unanswered.)

Is the utilized substance or the special properties of the cells the reason, or are they both the reason? First, it has to be determined how other substances affect the same tissue. Because the effects of these substances on the eye were unknown, the first experiments were conducted on animals and on conjunctival sacs with no eyeball.[b] It was fairly complicated to obtain certain substances. All the tests were conducted with the test ingredients dissolved in water. The result was that three of the substances have almost the same effect as rose bengal: eosin (10%), water blue, and nigrosin (both 5%). The named substances have affinity for the same cells as does rose bengal. If one of the three were used before rose bengal, the same cells will be stained; but the number of stained cells is larger by use of rose bengal. I have used fuchsin (5%), scarlet red (2%), malachite green (5%), naphtol green (5%), methyl violet (5%), safranin (4%), orange dye (5%), methylene blue (1% and 4%), chrysoidin (1/2%), gentian violet (2%), toluidin blue (4%), and dahöia dye (5%).

In experiments, these substances, malachite green, scarlet red, and fuchsin, only stained the conjunctival sac slightly and for a short period of time, the mucocutaneous junction almost not being stained at all; naphthol green has no affinity for the conjunctiva; methylene blue, gentian violet, toluidine blue, and dahöia are strongly attracted by the tissue; still, the mucocutaneous junction remains almost unstained. Chrysoidin and bismark brown create an even, light yellow coating over the palpebral conjunctiva; orange dye results in a diffuse orange staining, with no differences regarding the distance from the mucocutaneous junction. Methylene blue has the same characteristics as methyl violet and toluidin or toluidine blue; but the stain is less persistent than the latter ones. Knüsel and Vonwiller[9] had already obtained excellent results by using methylene blue on the conjunctiva.

The selectivity of these substances, some tending to influence only the mucocutaneous junction and the others, wider areas, demonstrates that staining with two stains can be achieved. First, when staining the conjunctiva blue or violet by using methylene blue or gentian violet, the mucocutaneous junction is just slightly stained. Then, by adding rose bengal in the sac, the well-known red line will form on the mucocutaneous junction, verging on the blue or red of the rest of the conjunctiva.[c]

Knowing what the effect of some staining on the conjunctiva is, or at least the spot where they are applied, we can return to the question whether the cells or the stains or both, are responsible for the strong selective character. In my opinion, it does not make any sense to consider the tears themselves as being the reason for the alteration of the mucocutaneous junction cells. (Authors' comment: Referring to cellular changes resulting in staining). First, the bottom of the tear meniscus is usually not as stained as the rest of the mucocutaneous junction cells. Second, the epithelium of the canaliculi does not take any stain at all, and third, the stain can be clearly detected on newborns. By analyzing the stains that stained the mucocutaneous junction much more intensely than the other areas, it can be observed that they are all acid stains. (Authors' comment: Most histologic stains are classified either as acid or as basic. An acid stain exists as an anion (negatively charged) in solution, whereas a basic stain exists as a cation (positive charge).) Despite this fact, not all of them have this effect. However, no basic stain induced the staining of the mucocutaneous junctions, which might provide an explanation for the selectivity of the staining.

This leads into the domain of vital staining, where reactions and selectivity have a major role, and such effects might have to be considered. (Authors' comment: This refers to different effects of basic and acid stains.) First, it has to be answered what vital staining is, because there is no current agreement. Vital staining—first introduced and used by Ehrlich[10]—generally defines the staining of a cell with a dissolved substance, such that the cell can be distinguished from the others, which are not stainable. But this definition is not precise, because substances exist that stain live cells and terminal ones; furthermore, it is difficult to decide whether a stained cell is alive, dying, or already dead.[11] (Authors' comment: It is remarkable that almost a century later, vital staining and its definition remain obscure.)

Not only is the word "vital" connected with vague definitions, "stained" means, while describing the substance in a living cell, the stain is either diffuse or granulated. But in the opinion of several authors, these stained granulae are not living material. It has to be assumed that because of the penetration of the stain into the cell, new

[b]Römer, Gebb, and Löhlein (l.c.) have studied a couple of tinctures to evaluate the possibilities of them damaging the cornea or the conjunctiva.

[c]Some very interesting double staining of other types have been achieved by Knüsel and Vonwiller (l.c., Page 171).

elements will be created, or that these are passive cell structures, when already existing particles adopt the stain. These stained granulae are formed under physical conditions, but we cannot say exactly what they consist of in either acidic or basic stains. The fine spreading of the staining particles is assumed to be an indicator only, if a stain can depose in a cell as granulae or not. The deposition of stain in cells takes place through adsorption, according to Bethe,[13] but the reaction between stain and cell still plays a major role in the process. He hypothesized that the reason for the different capacity of staining between base and acid is due the electric charge of the particles inside the cell. Bethe discovered that all the cells, which he had analyzed, possessed a strong affinity for basic stains but did not absorb acid ones, either having neutral or alkaline pH. Without exception, the cells with acid reaction mainly absorb acid stains; the same opinion was shared by Rohde.[14] This might be helpful in looking at things: because of the epithelial cells of the mucocutaneous junction absorbing acid stains, we could conclude that these cells are different from all the others, by possessing an acid reaction.

Several reasons strengthen that hypothesis. The cells of the conjunctiva (Authors' comment: presumably both bulbar and palpebral conjunctiva) can be induced to absorb the already mentioned stains by instilling a lotion consisting of 0.2% silver nitrate, argentum nitricum, 0.25% zinc sulfate, 1% acetic acid, and 1% holocain into the conjunctival sac. According to the electrolytical dissociation theory, these substances are all acid lotions.

This means that the number of free acid ions is greater than the number of free basic ions, so that the hydrogen ions might intrude into the epithelial cells, making the cytoplasm pH acidic and this way empower the cells to absorb acid stains. After a short period following the instillation, the normal state returns; the hydrogen ions were neutralized and the surface of the conjunctiva remains unstained by the rose bengal.

The following can be observed when using basic substances: by instilling a biborax natricus lotion of 1% onto the margin stained with rose bengal, the leaching of stain occurred more quickly than by using physiologic salt solution. The lotion containing caustic sodium solution also prevented the treated area from absorbing the same amount of stain afterward. However, complete lack of stain could never be achieved by these basic drops.

Care must be taken not to cause any superficial lesions when dripping, as this would induce the coloring of the conjunctiva. Research on this subject, and with other basic substances, still has to be continued, because the results do not allow for a definitive conclusion.

Another interesting observation is that the epithelial cells of deeper layers are also positive on staining; when gently removing the upper layer of the palpebral conjunctiva, the effect too soon fades away. I have to add that the mucous membrane of the oral cavity also accepts rose bengal only after wounding.

To sum up my observations, I would temporarily conclude that the upper layer of epithelial cells of the conjunctiva have an alkaline pH, apart from the ones of the mucocutaneous junction which is of "acid-reactive" cells, just as all the other ones that are situated underneath the surface layer. (Authors' comment: This refers to the exposed cells once the surface cells of the conjunctiva are removed.)

The given explanation should be sufficient for these facts, although other plausible explanations exist.[15] New facts will lead to new viewpoints; for now, nothing is entirely sure. (Authors' comment: It is remarkable that many of these questions raised in 1924 remain unanswered to this day.)

The actual extent of acidity of these cells is very hard to evaluate, because the Sörensen or Clark indicators—which are normally used to determine pH consist of alcohol based stain of certain color (e.g., methyl orange, methyl red, and neutral red)—show different colors when used in acid mediums than in alkaline ones. This way, they are of no use, as they are not vital stains. The given explanation appears to be adequate, although further research on this field is welcome.

If temporarily marginal epithelial cells appear to be "acid-reactive," the following aspect arises: the lacrimal fluid is an alkaline fluid, which has to be adequately excreted to protect the cornea against drying out, being antibacterial, and obtaining possibly other purposes. But, normally, the lacrimal fluid is not allowed to pass the mucocutaneous junction. The fluid must be conducted inside the line to reach the lacrimal punctum with maximum ease. The acid pH of the cells of the mucocutaneous junction might attract the alkaline tear flow, thus contributing to leading the tears to their destination, the lacrimal punctum, promoted by blinks. How these attractions take place is unknown at the moment. Also, the existence and impact of the capillary attraction are without confirmation.

These facts prove that we are dealing with a vital staining, at least a staining related to "life," because the effects on a corpse are totally different. Rose bengal, which was instilled into the conjunctival sac of cadaver eyes, stains the entire conjunctiva red, not even allowing the sharp line to take shape. But this does not necessarily make it clear that we are dealing with a vital staining or that the cells are simply keratinized. If waterblue and nigrosin are used to identify eleidin,[16] a substance present in the keratinized cells, and if it is correct that these cells are responsible for absorbing the entire red stain, then we could infer that the cells are protected against the penetration of the stain by the sebum of the meibomian glands.

However, this demonstration is misleading; degreasing the margins with ether and then applying alcohol-based lotion of rose bengal, only a slight staining of isolated cells can be noticed, just like it happens if the upper epithelium layer is damaged. Another argument could eventually be introduced to consider the possibility of the keratinized cells getting colored as a result of them being imbued with the tincture. Some cases of ectropion show an irregular borderline that extends further on over the palpebral conjunctiva and the eyelids, more than usual.

The changes in keratinization could also be due to prolonged exposure to air. This possibility, analyzed microscopically, reveals not only keratinized cells but also very often surface abrasions, which facilitate the stain penetrating the deeper layers and causing a diffuse staining, eclipsing the inhibition of the cells. Additional arguments against keratinization can be added. Virchow[17] supports the idea of the existence of an "admarginal" zone of the conjunctiva, which is placed along the mucocutaneous junction and just before the skin. Here, pikrin and eosin were absorbed better than in other parts of the epithelium. This more intense staining sometimes is visible in superficial layers, sometimes at all epithelial layers. I could not make consistent observations in my specimen, but this should not surprise us, because the eyelids I have worked on were either pathologic or on corpses. Virchow was able to experiment with new and normal human tissues. The area where he discovered these facts is exactly that area which allows acid reactions and where no keratinized cells can be found. Apart from that, it is also interesting that this admarginal zone allows intense staining with acid tincture using sections (Authors' comment: presumably biopsy sections); the same zone, in the living organism, absorbs mainly the same substances.

Another aspect that supports the vital character of the staining is that some cells from the surface of the conjunctiva—as said before—stain themselves with acid solutes. Observing the scraped specimen by microscope, most of the cells appear without stain, but some did absorb red stain; these look similar to the ones of the margins, they do have a slightly darker nucleus and do not have anything in common with a keratinized cell. Does the biogenesis of these parts explain the special position of the epithelial cells of the lid margin, investigated by Nussbaum[18] and Asks[19] on man and by Klees[20] on cats and mice?

To be concise, the process of an embryonic phase is as following: approximately in the third month, the eyelids grow together by migration of the epithelial cells. The cells situated in the front of the connective-tissue merge into the epitrichium of the eyelid, (the outer layer of the fetal epidermis) and the posterior ones unite with the epithelium of the conjunctiva without having a clear border (Authors' comment: forming the fornix). Initially, the layer connecting the eyelids is only two to three cells thick, thickens later on, while the structure of the tissues also changes. Insufficiently nourished cells are unable to absorb stains, receive vacuoles, and become keratinized. This process of keratinization commences on both sides of the lids connecting cell layer: anteriorly, the process is connected to the stratum corneum of the skin, whereas, posteriorly, the process runs fully independent in the intermediate cell layer. This is the reason, among others as well, why the eyelids separate.

Although this knowledge is not totally sufficient for understanding the unusual cells of the mucocutaneous junction, we are learning that keratinized cells arise only from the intermediate layer and not in the cells of the epithelium of the conjunctiva.

The entire study can be summarized as follows:

1. There is special staining at the mucocutaneous junction and at the lacrimal puncta, which can be induced by several different acid stains.
2. One of the practical uses of this staining technique is to determine the location of the lacrimal punctum, in regard to the mucocutaneous junction, as well as the form and existence or absence of the punctum.
3. The several types of stain provide clues for determining the normal course of tear drainage along the lacrimal rivus.
4. The normal stained line maybe missing for some types of tear chemistry.
5. For several reasons, these stains have to be considered as vital, although there is no certainty.
6. Further investigation is required to explain these phenomena.

(Authors' comment: Writing this summary in 1924, Marx could not have anticipated the breadth and magnitude of the relevance of his observations to contemporary dry eye and anterior segment practice.)

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[d]The references from the original article are presented in the exact format of the original article. References added by the translators are presented in standard journal style.