

MICROSCOPIC STUDY OF LEATHER DEFECTS

I. VEININESS IN GLAZED CALFSKIN*

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ABSTRACT

Prominent blood vessel pattern on the grain surface of leather is an esthetic defect which has long been troublesome and costly to the industry. In spite of numerous attempts to solve the problem, as summarized from the literature, the basic cause of the defect remains unknown. Inadequate curing and drastic processing were most frequently suspected, but so far there has been no conclusive proof. Therefore a continuing search for preventive measures would seem less practical than systematic testing of corrective treatments.

Microscopic examination of cross-sections, improved by a suitable aqueous embedding technique, is shown to be helpful in guiding such a program. Studies of blood vessel distribution have established that they are typically arranged in three layers. The deepest and largest vessels, especially the arteries, are the principal offenders in this problem, but it is shown that their depth can be so variable that shaving is not a dependable solution. More significantly, there is almost always a large void space around the deep vessels in veiny leather but not in non-veiny samples, even from skins processed together in the same tannery pack. Thin skins are an especially difficult problem. The suggested approach for correction would be to fill these voids, either by plumping the fibers with a suitable retannage or by the use of impregnating materials. Cross-sections illustrating an experimental zirconium retannage indicate a definite reduction in void space and in consequent veininess. Likewise a resin treatment, being studied elsewhere, seems quite promising after preliminary trials.



INTRODUCTION

Veiny leather is objectionable because of its unsightly appearance. Since calfskin leathers are prized for their beauty of grain, veininess is understandably a costly problem to the tanner. The term "veiny" is used because it is well under-

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stood in the trade and not because it pertains only to veins. Actually both arteries and veins are involved since they are always closely associated, as indicated in the diagram of Figure 1a. In fact, the arteries are likely to be more troublesome because of their larger diameters and heavier walls. Figure 1b shows a horizontal (parallel to the surface) section of calfskin through layer 1 of diagram 1a while

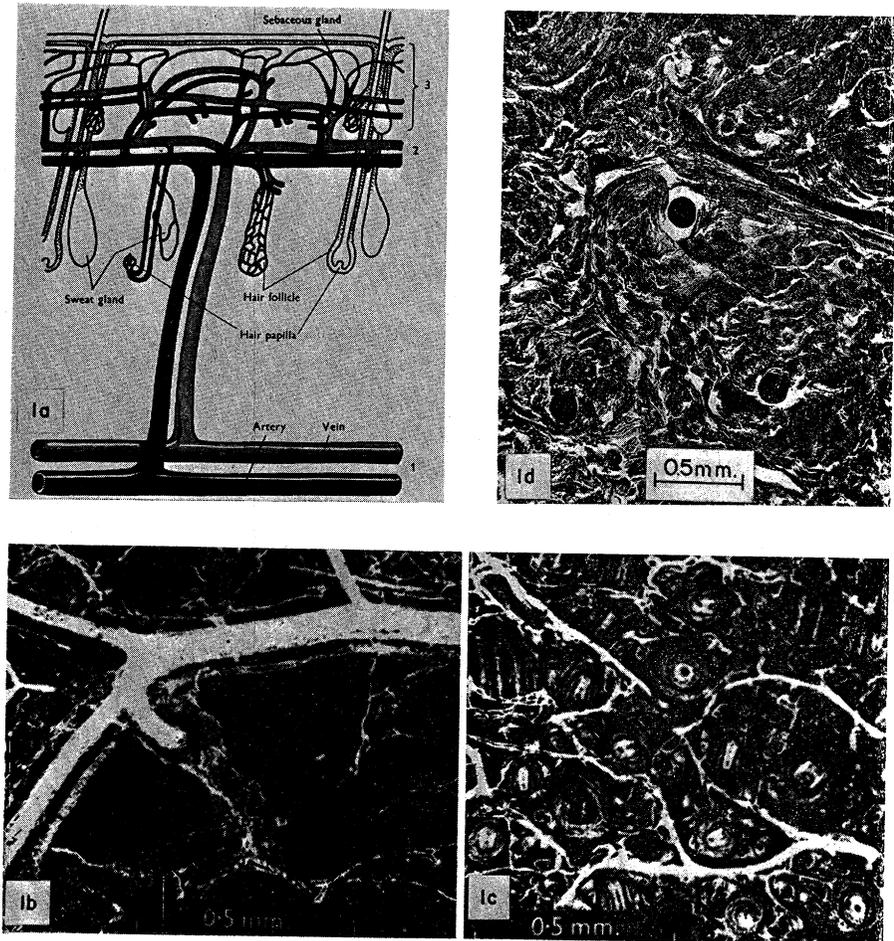


FIGURE 1.—Distribution of blood vessels in animal skin: (a) diagram showing general scheme of arrangement in three layers; (b) horizontal section of ink-injected calfskin through layer 1; (c) same through layer 2 (b and c are reversed contrast prints); (d) horizontal section of leather through layer 2 (positive print) showing vessel in same plane as hair papillae. See acknowledgments section.

Figure 1c shows a similar section through layer 2, indicating the comparative size of the vessels. Layer 3 can safely be ignored in this problem. These figures are from a study of blood vessel distribution in calfskin by Goodall and Yang

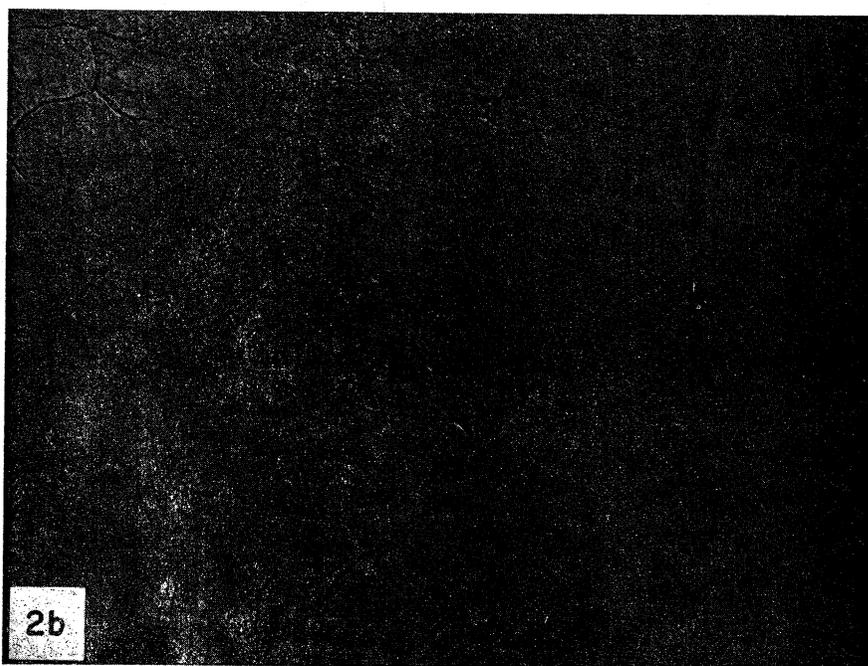
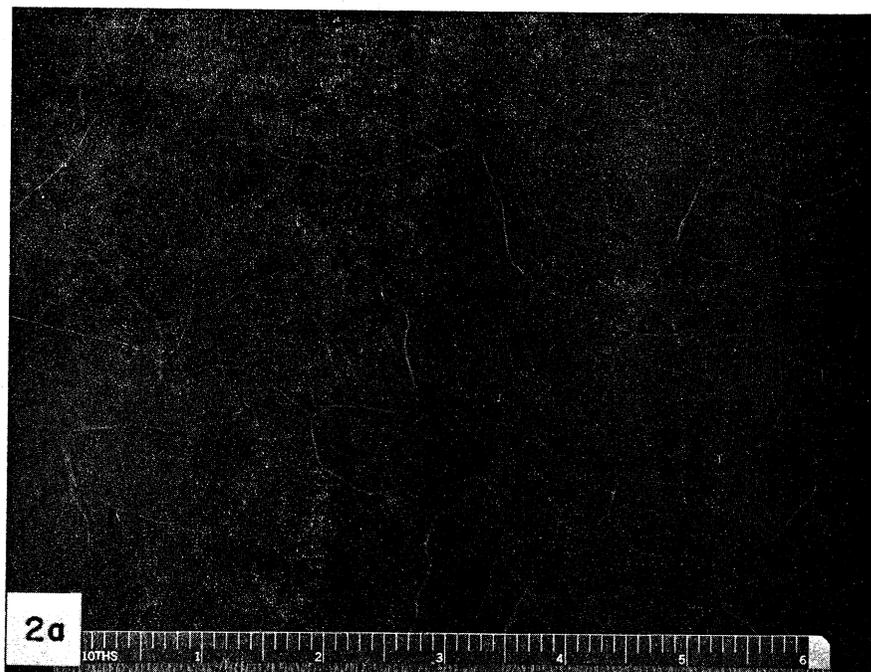


FIGURE 2.—Surface views of veiny glazed calfskin leather: (a) grain surface, and (b) flesh surface. Scale in inches.

(1) utilizing the technique of India ink injection. The authors found that the vessels consistently occurred in three layers or plexuses, increasing in size from the upper to the lower levels, with vertical feeders between them and to larger vessels beneath the skin. This general pattern, with minor variations, holds true for all mammalian skin (2, p. 212). From our observations the second layer is more often found at the base of the hair roots rather than higher up as shown in the diagram. This is illustrated in the horizontal section of Figure 1d showing hair roots at a much lower level than in Figure 1c.

Wilson (3) has defined veiny leather as "leather in which the pattern of the larger blood vessels appears on the grain surface in the form of indentations." This is the usual form of the defect seen in calfskin leathers. The grain surface of an extremely veiny sample is illustrated in Figure 2a, and the corresponding flesh side is seen in Figure 2b. The indentations are usually so shallow as to be difficult to feel, but they can be easily seen by their difference in darkness and gloss compared to the background. In 1925, Wilson and Daub (4), noting the open channels around the vessels, reasoned that these channels would offer less resistance to the pressure of glazing and thus project their indented pattern to the surface. Likewise the surface would receive less gloss within the pattern, making it more visible. This explanation is still valid today.

As to the basic cause of veininess, there is so little agreement in the literature that most likely there are a number of causes. Some believe that season of slaughter and mode of feeding are important (3, 5). Rivière (6) has commented on the relative absence of veininess in leathers from thicker skins (related to red-haired breeds) and from skins of a certain geographic origin. Strong evidence has been offered to implicate improper curing, with its resulting putrefactive destruction of either the vessel walls in skins with hair-slip (7, 8) or of the tissue surrounding the vessels (4; 9, p. 282; and 10). But some recent comparative tests (6) suggested that poor cure had no effect. Orthmann and Higby (11) investigated an uncommon variety of calfskin veininess consisting of a raised blood vessel pattern. They were able to demonstrate the defect in skins from animals that had died in transit to the slaughterhouse. Some excellent photographs showed the responsible vessels filled with coagulated blood and located in the intermediate (second) layer at the base of the hair roots.

Many tanners feel that beamhouse procedures have a strong influence on the extent of veininess encountered. Merrill (12) warned especially against under-soaking and underliming; he advocated prolonged or accelerated soaking and drastic liming to minimize veininess. However, Rivière (6) and others (13) feel that drastic liming should be avoided, including warm liming (14). Becchio (15) also recommended thorough soaking but warned against using fresh water before liming to avoid excessive swelling. Jones (7) advised milder liming and bating of hair-slip skins, and claimed that overbating of good skins would cause veininess, yet Merrill (12) insisted that the extent of bating had no effect whatever. Tancous, Roddy and O'Flaherty have summarized many of these varying

viewpoints in their manual (16, p. 16) on leather defects and in a recent review (13). Everett and Carroll (17) have also dealt with the problem briefly in a recent article on microscopy.

Another variety of veininess commonly called "wild grain" is believed to be caused by the vessels of layer 2 in diagram 1a. It is more prevalent in skins from winter take-off, presumably because the vessels are more distended. To combat this difficulty, Wilson (3, 18) advised carefully controlled bating and prevention of excessive swelling. The role of bating in this connection is still unresolved. Hollander's early work on the histology of bated skins (19, 20) indicated that bating enzymes might be influential because of their possible effect on elastic tissue. As mentioned previously, Jones (7) claimed that overbating could cause veininess while Merrill (12) found no effect.

The importance of elastic tissue has probably been overemphasized. Even though Ornes and Roddy (21, 22) reported a higher content of elastic tissue in veiny skins (in the blue) as compared with a non-veiny one, their method was not specific for blood vessel tissue, nor were the analytical differences large enough to be reliable for characterizing the defect. The comparative microscopic appearance of the arteries in autoclaved residues (21, p. 134) would probably be too variable and subjective to distinguish a veiny skin, but it is interesting that different sides were found to be similar in distribution and size of arteries.

It has been suggested that the elastic coat of arteries becomes hard and brittle after chrome-tanning (23) and that the resulting rigid, tubular structure might cause the surface pattern (16). If so, it would be more likely to produce a raised pattern than the usual indentations, and veininess would be the normal condition rather than the abnormal.

Modified pickling and tanning processes have also been suggested (15), including the use of certain masking agents (polymeric metaphosphates) to improve the fiber structure (24). Considerable attention has of course been given to the art of shaving. Repeated removal of thin slices is preferred over thicker cuts (15), with spot shaving in veiny areas (6). Proper shaving should cut well into the main arteries to reduce their surface effect (3). Due to the variability in depth of these vessels, as will be shown later, and the limitations on how much thickness can be sacrificed, it is apparent that shaving is not the complete answer to the problem. Also, it is of interest that attempts to redye the surface or to vary the drying methods were of no help (6). Experimental proof for all these varied claims is far from adequate.

A pilot matched side experiment has recently been described by Reihsmann and Fugikawa (25) in which a two-resin treatment was applied to chrome-tanned veiny kipskins. It was intended to improve uniformity by filling flanky areas, but it also resulted in reduced veininess. Cross-sections indicated that the resins partially filled the void spaces and yet produced a more mellow leather than the

controls, with equivalent break. This seems a promising approach to minimizing veininess.

EXPERIMENTAL METHODS

Sectioning.—Cross-sections of the leather samples were prepared in the usual manner by cutting at 50 microns (about 0.002 in.) on a freezing microtome, staining if desired and then mounting the sections with glycerol-jelly on slides. With compact tissues this is usually satisfactory, but with fragile, fragmented samples there is too much loss of material and distortion of the loose components. For these reasons gelatin embedding was usually employed to maintain the tissue integrity in an aqueous medium. This method (26) has the primary advantage of avoiding solvent dehydration, as required for paraffin or resin embedding, which would remove the fats.

Gelatin embedding.—Prepare a stock solution of 20 percent gelatin (ordinary grade) and 1 percent phenol in water:

Gelatin (powdered)	100 gm.
Phenol (melted)	5 ml.
Water (cool)	400 ml.

Soak the gelatin by sprinkling it on the surface of the water. When wetted, gradually warm the mixture on a steam or water bath to dissolve, avoiding high temperatures. Stir in the melted phenol and store the stock in tightly capped bottles. For use, heat a bottle in boiling water to melt the gelatin and hold it in a water bath at 37–40°C.

Infiltration of the leather samples (about 1/4 inch square) is first necessary to assure penetration of the gelatin into the internal spaces. Soak the samples in water containing a wetting agent until thoroughly wetted. If formalin-preserved or chrome-blue samples are used, wash them very thoroughly before proceeding to embed in gelatin. Infiltrate the samples in three stages: first soak them in five percent gelatin (1:4 dilution of stock) for six to eight hours; then transfer them to ten percent gelatin (1:2 dilution) and hold overnight. To improve the efficiency of gelatin penetration during the first stage, use intermittent application and release of vacuum (in a vacuum desiccator) until no more bubbles appear. Finally place the samples in 20 percent (stock) gelatin for several hours to complete the process. All of this should be done in an incubator or oven at 37–40°C.

The infiltrated samples may be embedded in a shallow dish of appropriate size. At room temperature pour a thin layer of melted gelatin stock into the dish and allow it to become slightly firm. Orient the samples on this layer as desired, then cover them with more of the stock. When firm enough to handle, harden the material in the refrigerator and cut out the individual blocks, leaving a thin margin of gel on all sides of the sample. Harden the gel blocks further by soak-

ing them for one or two days in ten percent formalin (1:10 dilution of commercial 40 percent formaldehyde). The hardened blocks are of excellent consistency for frozen sectioning and can readily be cut at 20 microns or less. The main disadvantage of using gel sections is that they shrink severely when dehydrated in organic solvents, and the gel cannot be removed after formalin fixation. Therefore they are not suitable for stains that require non-aqueous mounting, although they can be handled fairly well in 50 to 70 percent alcohol. If necessary, they might be attached to slides first with albumin-glycerol to keep them flat before staining. As an alternative the gel blocks may be sectioned without hardening in formalin, the sections attached to slides and the gelatin removed with warm water; but this is a difficult procedure and requires special equipment.

Fat staining.—Because of the variety and intensity of colors imparted by tanning materials, dyes and pigments, staining of leather sections is necessarily limited in scope. Also there is less need for staining for the same reasons. For present purposes, fats added during the fat-liquoring operation were the only important constituents not visible in unstained sections, so this was the only type of staining attempted. Furthermore the fat stains require aqueous mounting methods, permitting their use with gelatin-embedded sections.

A wide variety of fat stains is available, but color and contrast are important considerations for photographic reproduction. Oil Red O and Sudan Black B are the most generally useful ones. With dark-colored leathers the red stain serves well to distinguish fats from dyes; likewise, with light-colored leathers the black stain is very effective. Directions for their use are found in standard texts (26, 27).

Oil Red O.—Prepare a saturated stock solution of the stain in 99 percent isopropanol. For use mix 12 ml. stock solution with 8 ml. water, let stand for ten minutes and filter. This is an unstable suspension and must be prepared fresh each day. Sections are conditioned first in 60 percent isopropanol, stained for ten minutes in the fresh dilution and then washed in water before mounting. Lipids are stained a bright red. Aqueous counter-stains may be used as appropriate; such as, light green or hematoxylin.

Sudan Black B.—This may be used in the same manner as the other Sudans. A saturated stock solution in 70 percent ethanol is also the staining reagent. Sections conditioned in 70 percent alcohol are stained for 30 minutes, rinsed in 70 percent alcohol, washed in water and mounted; or the stain may be dissolved in propylene glycol. This is claimed (26, 27) to be less likely to remove alcohol-sensitive lipids.

Microscopy.—A Zeiss‡ Photomicroscope with built-in 35 mm. camera was used for preparing photomicrographs. Adox‡ KB 14 (135–20) black and white film and Kodachrome‡ II Professional (KRA 135–36) color film were used

‡Mention of brand or firm names does not constitute an endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

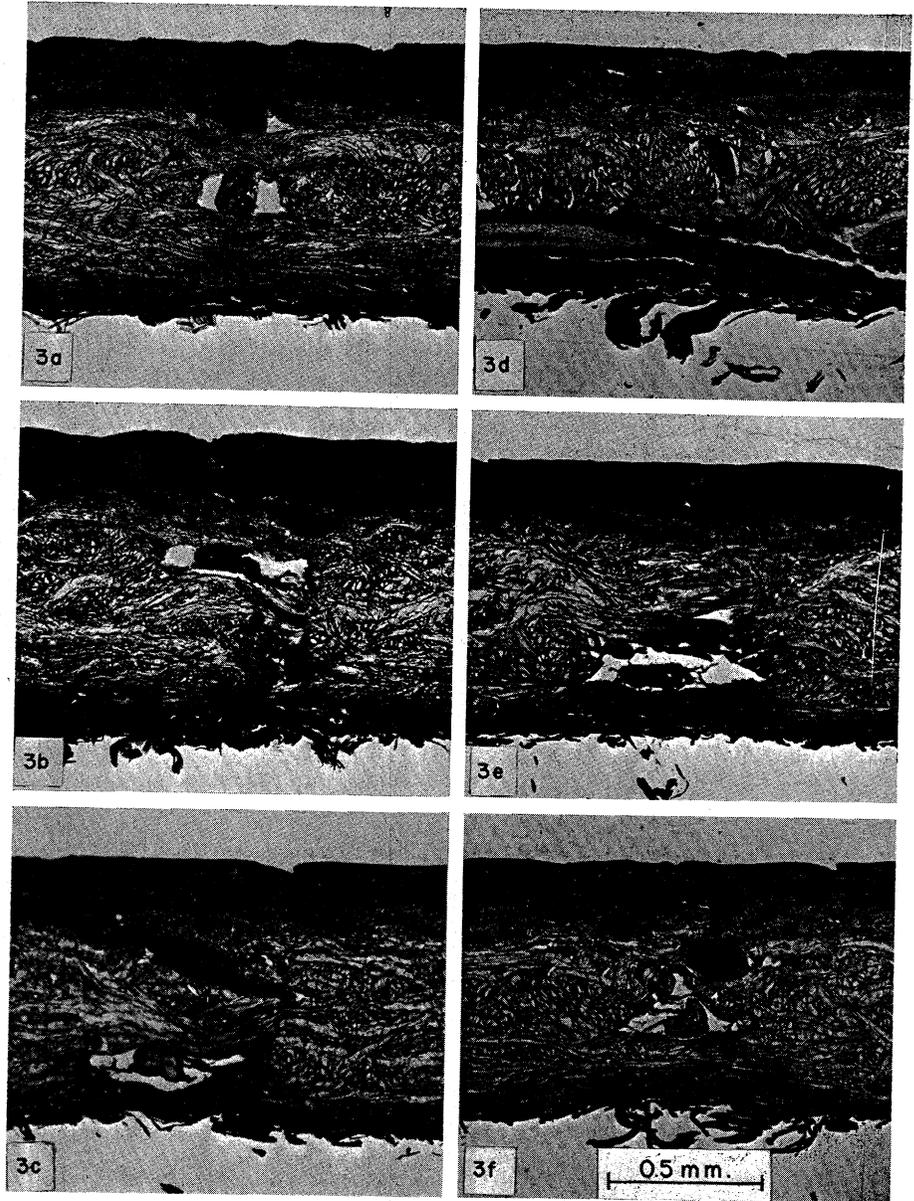


FIGURE 3.—Cross-sections of veiny glazed calf, showing typical void spaces around the blood vessels, from same tannery pack as sample of Fig. 4. Sections 3c, 3d, 3e and 3f were embedded in gelatin; the rest were not. Sections 3b and 3e were stained with Oil Red O; the rest were unstained. Scale applies to all sections.

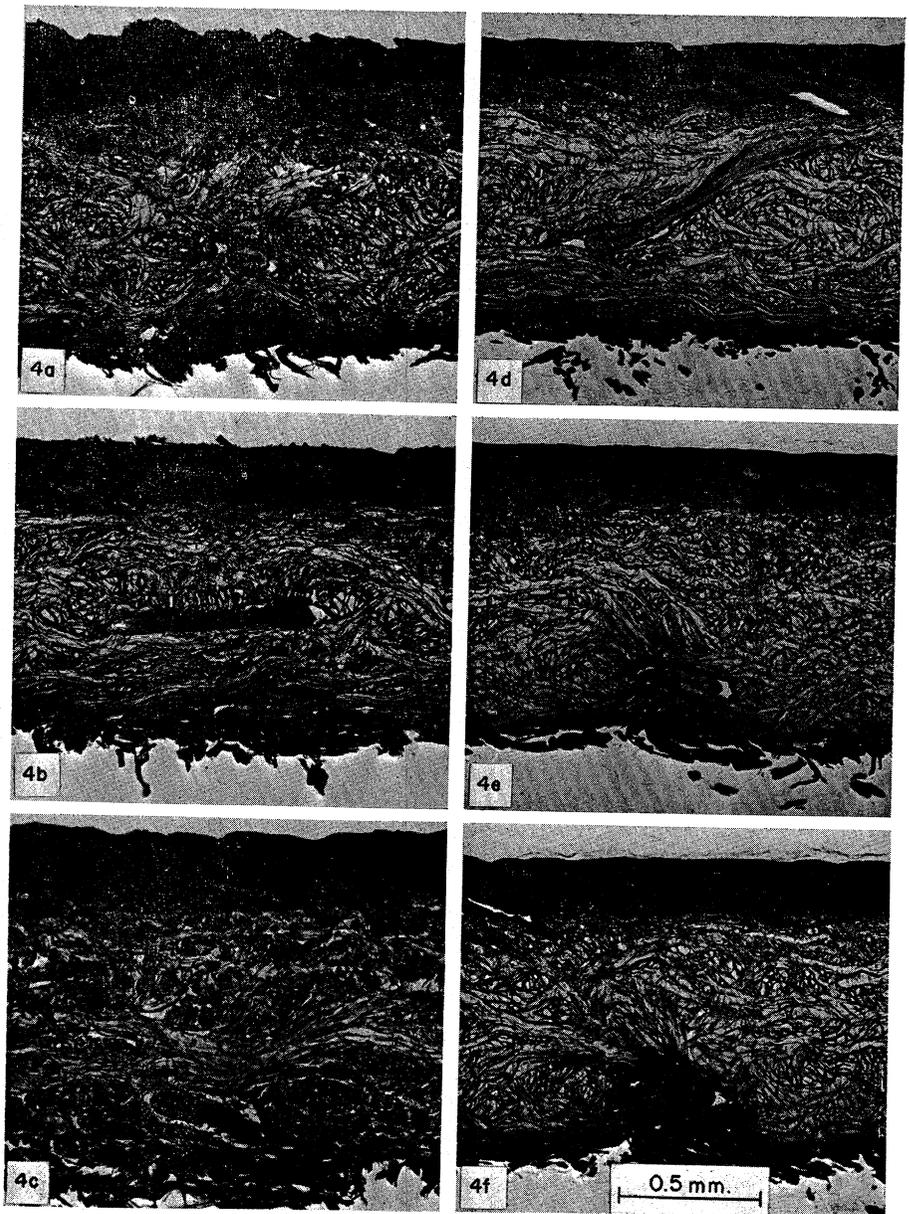


FIGURE 4.—Cross-sections of nonveiny glazed calf, practically free of void spaces, from same tannery pack as sample of Fig. 3. Sections 4d, 4e and 4f were embedded in gelatin; the rest were not. Sections 4a, 4c and 4f were stained with Oil Red O; the rest were unstained. Scale applies to all sections.

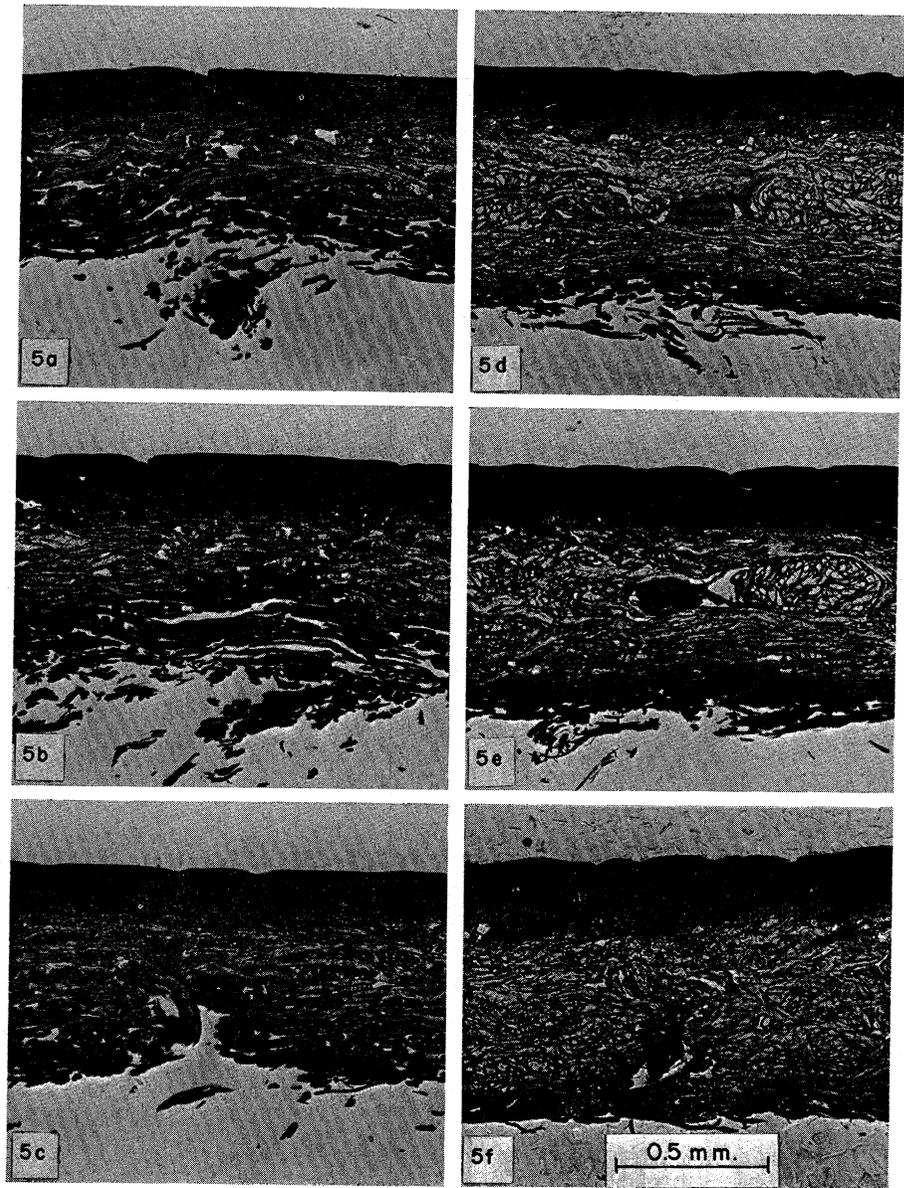


FIGURE 5.—Left side: cross-sections of thin-skinned veiny glazed calf with vessel fragments and fiber damage. Right side: cross-sections of veiny calf with unusually small voids. All specimens were embedded in gelatin. Sections 5b, 5e and 5f were stained with Oil Red O. Scale applies to all sections.

interchangeably. A green filter in the microscope was employed to enhance red sample colors on black and white film. Photographic enlargements were prepared as needed.

RESULTS AND DISCUSSION

Four varieties of veiny leathers were examined microscopically, using multiple specimens of each in a search for additional information on the nature of the defect.

Veiny vs. non-veiny skins.—The seriousness of the problem came to our attention when a tanner submitted two contrasting pieces of black glazed calf, both from the same tannery pack and the same purchase lot. One had several large veiny areas while the other had none. Figure 3 shows a selection of cross-sections representing four different specimens of the veiny sample, cut perpendicular to the surface lines (except for section 3d, which is cut obliquely). It is apparent that the responsible vessels are: 1. usually collapsed; 2. surrounded by a relatively large void space or channel; and 3. occur at varying depths, mostly in the lower half of the skin (layer 1). The over-all width and emptiness of the channel seem to determine the severity of the surface defect.

Figure 4 illustrates the non-veiny sample in a similar manner, also including four different specimens. Section 4d shows a vertical feeder vessel between layers 1 and 2. The significant difference to be noted here is the relatively small amount of void space around the vessels, which correlates with the relative absence of surface indentations. Very faint lines were visible enough to guide cutting of specimens but not severe enough to be objectionable.

Veiny thin skins.—It would be expected that thinner skins might be more troublesome because the deep vessels are closer to the grain surface, and less thickness can be sacrificed for proper shaving. In addition, there is more mechanical damage from the stretching and pounding of the glazing operation. An extremely veiny thin skin was examined which exhibited a raised vessel pattern instead of the usual indented one. The left half of Figure 5 illustrates three specimens from this sample. All had to be embedded in gelatin because of their fragility. It was difficult to distinguish the vessels in these sections due to mechanical damage, although it was usually evident where they had been. Fragmented portions often clung to the flesh side, as in 5a, from incomplete shaving or were torn out completely to leave an empty channel, as in 5c. The vessels were often obscured by deep penetration of dye and fat-liquor, as in 5b. It is believed that excessive stretching of these thin skins is responsible for the raised pattern. Failure of the grain above the vessel channels to relax when tension is released produces an upward pucker or embossed design.

Veininess without void space.—The veiny leather pictured in Figure 2 was unusual in not showing the typically large void spaces around the vessels. The right half of Figure 5 illustrates two specimens from this sample. It is ob-

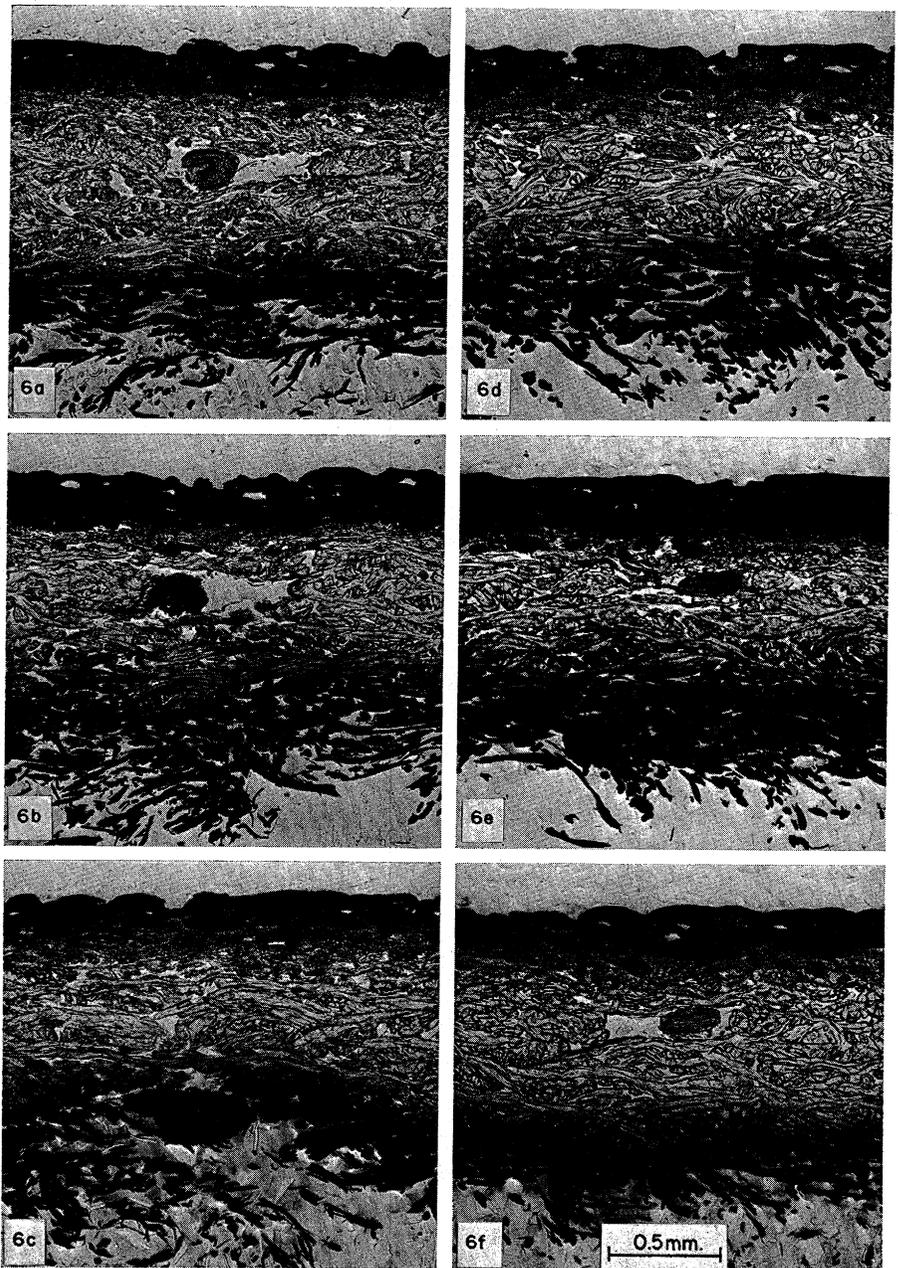


FIGURE 6.—Cross-sections of veiny calf from matched side experiment. Left: control side with normal chrome tannage. Right: experimental side retained with zirconium. All specimens were embedded in gelatin. Sections 6b and 6e were stained with Oil Red O. Scale applies to all sections.

vious that the spaces are much smaller than those seen in Figure 3. However, there is a distinct impression that the fibers below and above the vessels have been compacted and stuck together without returning fully to their original positions, thus making the original channels less apparent in sections but still producing their usual surface effect.

Veininess reduced by retannage.—A cooperating tanner suggested plumping the fibers with a suitable retannage in order to achieve some filling of the void spaces around the vessels. He performed a comparative matched side test on a veiny skin after chrome tanning. One half was experimentally retanned with zirconium, then both halves were finished together by their regular process for black glazed calf. The left half of Figure 6 shows three cross-sections representing two specimens of the control side tanned only with chrome. The right side of Figure 6 shows corresponding views from the retanned portion. Most of the deeper vessels had apparently been shaved off, except for some areas, such as, section 6c. The majority of vessels seen were located close to the grain layer and might possibly belong to layer 2 rather than layer 1. At any rate there was a pronounced reduction in the size of void spaces which correlated with reduced veininess. The retanned half was appreciably improved in appearance although the veininess was not completely eliminated. This would also seem to be a promising approach to minimizing the defect and upgrading the leather.

SUMMARY AND CONCLUSIONS

Although veininess does not involve the essential properties of leather, it is of serious economic importance for its strong effect on the selling price. Preventive measures would be the ideal solution but would require full knowledge of the basic cause of the defect. No progress has been reported in this direction. It is known that the blood vessels in the skin are always arranged in three layers, with the largest ones at the bottom. Anatomically there is no real difference between skins in this respect except for thickness. Also it is agreed that the surface indentations are produced by pressure treatments, such as glazing. This is explained in terms of findings from microscopic study of cross-sections, where typical void spaces were found around the responsible vessels. Shaving was previously considered to be the best way to eliminate the offending vessels, but again the microscope revealed that the vessel depth is too variable for this to be practical in all cases. Corrective treatments designed to fill these voids and equalize resistance to pressure would thus seem to offer the most promise at the moment. Zirconium retannage for indirect filling and resin impregnation for direct filling are steps in the right direction which should be further evaluated.

ACKNOWLEDGMENTS

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DISCUSSION

PRESIDENT MEO: The discussion leader on this paper is Mrs. Jean Tancous.
MRS. TANCOUS: These excellent slides presented to us by Mr. Everett have demonstrated that histological techniques are a necessity in leather research.

Without an inside look, as Mr. Everett calls it, into the structure, we should be handicapped in our interpretation of chemical and physical analyses and would be unable to diagnose the causes for many defects encountered by tanners.

Mr. Everett is to be commended for his application of polarized light and the special filter at 45° to the plane of polarization to leather microscopy. Both tools have effectively demonstrated the difference that exists in the fiber bundle orientation between hides.

Mr. Everett's contribution to the problem of veininess is worthwhile, especially his observation that retannage improves the condition. We have tried this retannage on calfskins and can verify his findings. The results of the two-resin treatment, which Mr. Reihsmann will present tomorrow, will be of a more specific approach to this problem.

As a question to Mr. Everett, I should like him to explain the embedding technique he used for preparing the cross-sections of calf leathers having veininess. It is a useful histological technique and one that all of us working in histology should be applying to our work.

MR. EVERETT: This will appear in publication. It consists briefly of soaking the sample, first in a 5 percent gelatin solution and then progressing to 10 percent, 20 percent gelatin, until it is soaked all the way through. Then we harden the gelatin in the refrigerator, cut out a small block and further harden it in formalin overnight. Then this hardened gelatin block can be placed on the freezing stage of the microtome and cuts even easier than ordinary leather samples. This can also be applied to all sorts of skins as well as leathers. The main advantage is that it gets around the use of organic solvents which are usually required for a paraffin base. In this way, we don't disturb any of the fat content and keep to an entirely aqueous system.

MRS. TANCOS: Thank you.

In the demonstration you have with the three layers of blood vessels, you mentioned that the flesh-side layer contributed most to the veininess, but your cross-section showed the inside second layer. Would you explain this to us? Do you think the center layer contributes more to veininess or the flesh layer?

MR. EVERETT: I think it is mostly the flesh layer. In other words, the diagram indicates only the ideal average. They don't follow as straight a pattern. It can fluctuate up and down. Of course, you do find some cases that suggest it is the middle-layer vessels rather than the bottom layer. But I think in calfskin at least, it is almost always the bottom layer. It varies considerably in depth.

DR. R. M. LOLLAR (Armour and Co.): Another point on the question of the layers, you mentioned veins and arteries both in the diagram. We call it veininess. But would you comment on whether you think the defect is truly veininess or whether both arteries and veins contribute?

MR. EVERETT: I can't comment because I'm not quite sure. It seems more logical that the arteries would be mostly at fault because they are heavier walled and tend to be slightly larger. You can usually differentiate between them in a section of skin, but once you get into leather, you can't always be sure. I don't know the final answer, but I suggest both could be playing a part. Probably the arteries are the principal offenders.