

STUDIES OF HYPERKERATOSIS OF CATTLEHIDES ATTRIBUTABLE TO PSOROPTIC SCABIES*

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Abstract

Results of a histological evaluation of hides from cattle infested with *Psoroptes ovis* (Hering) are presented. Five heifers were infested for periods of 10 days to 20 weeks before slaughter. The course of the progression of psoroptic mange was established microscopically. Two infested hides and one from a yearling which was infested and then treated with Ivermectin (Merck, Sharpe, and Dohm§), an anti-parasitic agent, were processed into leather and evaluated. The leather from the Ivermectin treated animal, though far superior to leather from the untreated infested animals, still showed some downgrading as compared to leather from hides from uninfested cattle.

Introduction

Because of the finding of hyperkeratosis in increasing numbers of cattlehides examined at the Tanners Council Laboratory, J.J. Tancous who is expert in this disorder (1a) conducted an informal survey and reported to the authors her estimate that 15 percent of all cattlehides arriving at U.S. tanneries are afflicted with hyperkeratosis (1b). Hyperkeratosis is the thickening of the epidermal layer of the hide, particularly the corneum, and is a symptom of a number of diseases. It has even resulted from ingestion of chlorinated solvents by cattle (2). In recent years the increase in the number of reports of hyperkeratosis in hides arriving at tanneries has arisen simultaneously with an epidemic of major proportions of *P. ovis* infestation in cattle, particularly in the west and southwest regions of the

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§Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

United States (3). Since hyperkeratosis is a major symptom of psoroptic mange the connection between the two is considered more than coincidental.

The newer methods of marketing, the current means of transportation of cattle, and the use of very large feed lots contribute greatly to the spread of the highly contagious psoroptic mange (3). Psoroptic mites do not burrow into the epidermis, but rather feed on the surface and were originally considered to do only superficial damage to the hides (2). The fact remains that *P. ovis* can cause listlessness, weakness, and in extreme cases death of the animal. The mites puncture the surface of the hide in search of sera, which may be mixed with blood, causing lesions and epidermal tissue reactions. The lesions start on the shoulders and withers, then spread down the back to the tail. In severe infestations the whole hide becomes involved. Hide damage can be much more extensive than originally supposed.

Extensive investigations have been conducted by Blachut *et al.* (4) and by other researchers on the life history, habits, and effects of the psoroptic mite on sheep; very little has been done on the histology of infested cattle hide (3). Indian researchers (5) have investigated and described psoroptic mange in buffalo calfskins. Grunder and Lange (6) described the damaging effects of *P. ovis* on the grain surface of finished leathers. A recently received publication by Von Rotz *et al.* (7) is a general study of ectoparasites in cattle. In their discussion, the authors report acanthosis, subcorneal pustules with eosinophile invasion, perivascular infiltration, and devaluation of leather as a result of psoroptic mange.

This paper presents the results of a cooperative program between the U.S. Livestock Insect Laboratory at Kerrville, Texas and the Eastern Regional Research Center, Philadelphia, PA, developed to study the effects of psoroptic scabies on cattlehide and tanned leathers. It covers the histological studies of the course of the disease, relationship to inflammation, and effects on leather made from both diseased animals and from a cured animal.

Experimental

The design of the experiment was that the Kerrville group provide, infest with *P. ovis* mites, kill, and sample the animals used. At Philadelphia, the Animal Biomaterials Laboratory carried out histological studies to determine the course of the response of the cattle to infestation and to suggest a method based on inflammatory response to promote early detection of the condition if possible. The Physical Chemistry and Instrumentation Laboratory isolated the mites and studied and characterized them by scanning electron microscopy.

Five Hereford heifers were selected for the experiment and exposed twice on the withers area of the shoulders to 500-1000 active *P. ovis* mites in September 1981. The animals were confined to anti-grooming stanchions. Table I shows the number of animals infested, length of time of infestation, and the number of live mites counted before slaughter.

TABLE I
DATA ON STANCHIONED* INFESTED ANIMALS USED IN TEST

Animal number	Date		Infestation period	Number of mites counted in scraping	Description of leather produced
	Infested	Killed			
1419	9/11/81	9/21/81	10 days	not counted	not tanned
1406	"	10/26/81	6 weeks	680	not tanned
1368	"	11/30/81	11 weeks	1332	not tanned
1409†	"	12/23/81	15 weeks	836	very poor
1186†	"	Died 1/23/82	20 weeks	not counted	very poor
144†§			9 weeks	938 5 days before injection with Ivermectin	fair to good

* Psoroptic scabies is considered to be seasonal; less active in spring and summer. This is not true if the animals are stanchioned (12).

† Sides were salted and sent to ERRC for processing into leather.

§ Animal recovered from sever *P. ovis* infestation after injection with the acaricide, Ivermectin. The animal was allowed to run free for 24 days to allow self-grooming prior to sacrifice.

ANIMAL TISSUE SAMPLING

Ten-inch square pieces of hide, taken from the manually infested withers area and the area in front of the hipbone of each animal after slaughter, were frozen quickly and sent to ERRC for histological evaluation and determination of the extent of visible damage. The pieces of hide taken from the hipbone area were used as controls. The control areas were not manually infested. Each square was thawed, photographed before and after clipping the hair, and sampled for analyses as shown in Figure 1. The subcutaneous tissue was removed from the square before sampling. Tissue samples were taken with a ½ in. round Osborne Arch punch from each of the five areas selected for determination of water content, and for examination by light and scanning electron microscopy.

DETERMINATION OF THE INDEX OF INFLAMMATION

One procedure examined to promote early detection of *P. ovis* infestation was the determination of the Index of Inflammation, a quantitative estimation of edema caused by inflammation and described by Weiss *et al.* (8). This requires the determination of the water content in the uninfested and infested tissues of the same animal. Different animals have different initial hydration states so that percent change in hydration of tissue is important rather than water content as such.

Samples from the tissue specimens were taken immediately after thawing and dried at 60°C and 5 mm pressure for 16-24 hr. From the results obtained, the

SAMPLED AREAS

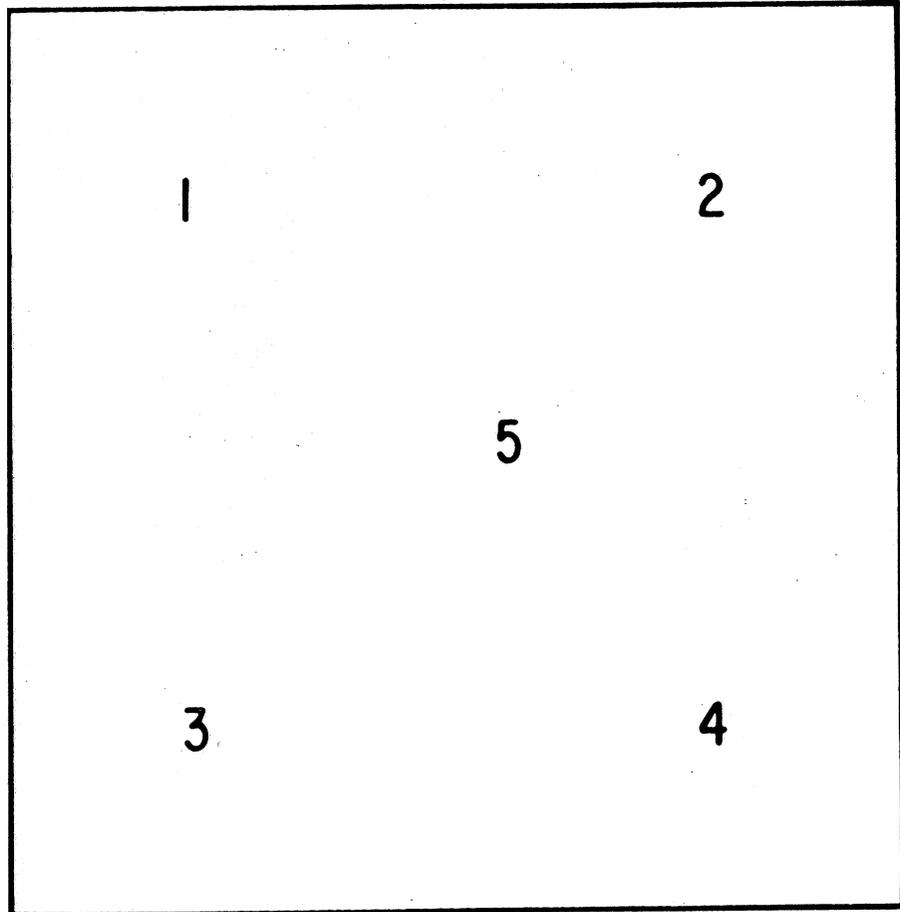


FIGURE 1. — Sampled areas on 10-in. square specimens.

Index of Inflammation was determined. This is dependent on the movement of body fluids into an inflamed area due to increased capillary permeability. The initial state of hydration of the animal must be known. A factor W was computed, calculating the water content of the tissue as percent of dry tissue weight. The Index of Inflammation is expressed as $\Delta W(\%)$ which is the percent change in water content as inflammation increases.

$$\text{Factor } W = \frac{\text{Weight of water (g)}}{\text{Weight of dry tissue (g)}} \times 100$$

$$\Delta W(\%) = \frac{W - W_c}{W_c} \times 100$$

Where W = water factor for the test area

W_c = average water factor for the control area (however, the lowest tissue water content found in the control area was used for W_c if the disease was sufficiently advanced so that invasion of the disease into the control area was beginning to occur).

HISTOLOGY

Half-inch plugs taken from areas, as shown in Figure 1, for histological evaluation were fixed in 10 percent neutral formalin*. Examinations were made from both frozen and paraffin embedded hide. Sections were cut at 50-60 microns and 10 microns, respectively, and stained. The following stains were used: Hematoxylin and Eosin to show nucleated elements and collagen, Orcien and Giemsa to show elastin and evidence of cellular infiltration, Weigert and Van Gieson to show the presence of elastin and collagen. However, only those stained with hematoxylin and eosin are shown in this report.

Photomicrographs were taken with a Zeiss Photomicroscope.

SCANNING ELECTRON MICROSCOPY

Small pieces of the infested skin were extracted in water for several hours to gently remove the mites. The supernatant was passed through a 0.2 μm Millipore filter to collect the isolated mites. While still on the filter, they were fixed in 3 percent glutaraldehyde in 0.07 M phosphate buffer for 4 hr, and washed four times with the buffer. Post-fixation in 1 percent OsO_4 in the same buffer occurred overnight. The mites were dehydrated through increasing ethanol-water series and critical point dried from carbon dioxide. The mites were mounted on stubs and sputter coated with gold. The specimens were observed in a JEOL JSM50-A Scanning Electron Microscope at an accelerating voltage of 15 kV. The structure of a female mite in fine detail is shown in Figure 2.

*1-10 dilution of 37 percent formaldehyde, saturated with calcium carbonate. Formaldehyde irritates mucous membranes and has been under study as a possible carcinogen for them, so contact with the chemical or its vapors should be avoided. For the latest information, Material Safety Data Sheets should be obtained from the manufacturer and the recommended precautions should be followed. In general, this is a wise procedure and we strongly recommend using Material Safety Data Sheets on all chemicals employed.

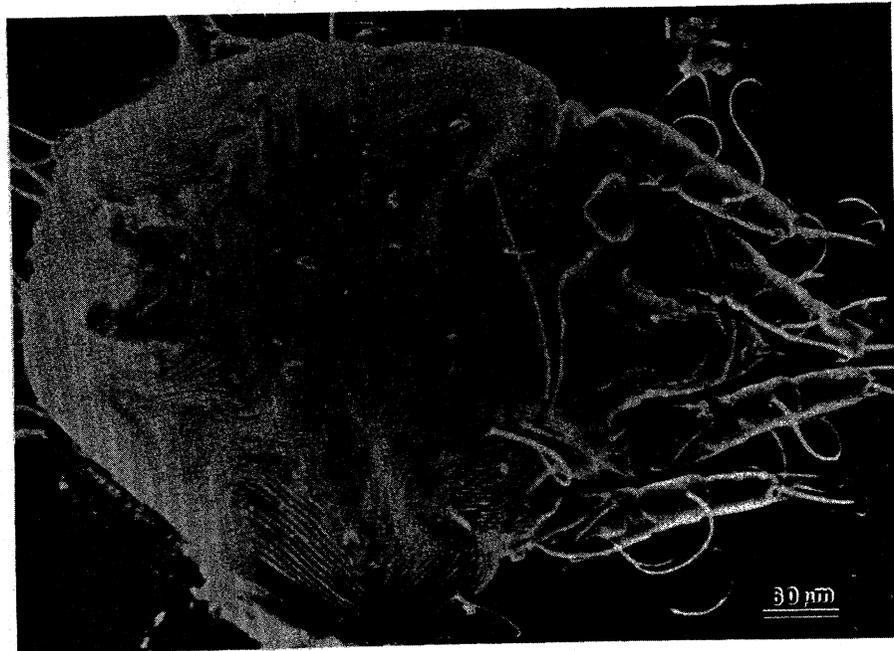


FIGURE 2. — *Psoroptes ovis* adult female mite (SEM photomicrograph).

USE OF IVERMECTIN TO EFFECT A CURE

Animal #144 was used specifically to test the curing effect of Ivermectin (9, 10), an antiparasitic agent, administered by injection to cattle infested with psoroptic scabies.

Immediately after purchase the animal was dipped in toxaphene to rid it of parasites. Two weeks later it was manually infested with *P. ovis* and placed in an anti-grooming stanchion. Nine weeks after infestation lesions had developed over 50 percent of the animal's body. At this time an injection of Ivermectin (200 micrograms/Kg) was administered in the prescapular area. Forty-one days after injection with Ivermectin the animal was cured but had residual scab material. The animal was released from the anti-grooming stanchion and was allowed freedom to permit self-grooming. Sixty-five days after injection (24 days of self-grooming) the animal was killed. The hide was salted and sent to ERRC for processing into leather and for evaluation of the grain surface for residual effects of psoroptic scabies.

Results and Discussion

INDEX OF INFLAMMATION

Results of moisture determinations or water losses from the five areas of the

TABLE II
INDEX OF INFLAMMATION ΔW (%)

Area sampled	Hide			
	1419		1406	
	W	ΔW (%)	W	ΔW (%)
Heavily infested	255	42.5	213	42.1
Heavily infested	247	38.0	204	36.0
Heavily infested	244	36.3	194	29.3
Moderately clear	232	29.6	186	24.0
Clear	210	17.3	184	22.7
Control area (noninfested)	179	-	150	

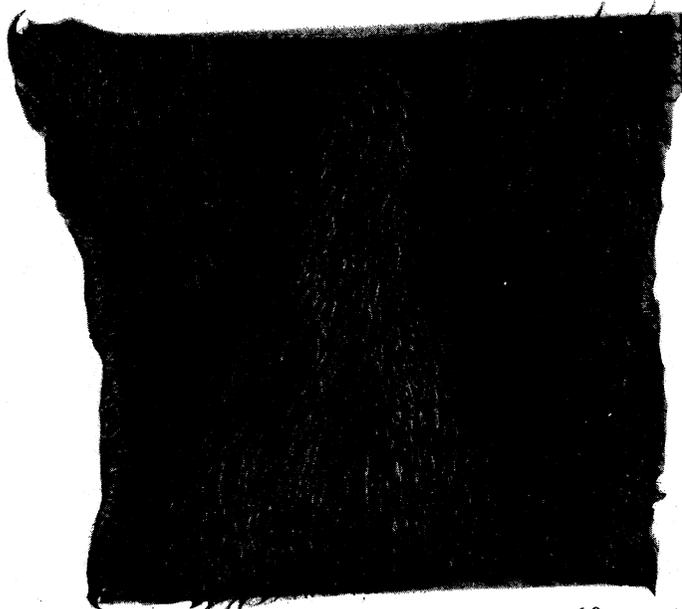
control and infested squares, indicated in Figure 1, were substituted in the formula for determination of W and ΔW (%), to obtain a quantitative estimation of edema in the infested area. The water content of the hide from an uninfested portion of the hip area from the same animal was used as a control.

The results of ΔW (%) for animals 1419 and 1406 are shown in Table II. Hide 1368 was edematous in both test and control areas and was not usable because there was no indication of the initial hydration state of the animal. The Indices of Inflammation are approximately the same for the two hides, ranging from 42 percent for the heavily infested areas to about 20 percent for the relatively clear but affected areas. The locations shown in Figure 1 were selected by appearance as to the number of lesions and turgidity of feel. This method has merit and it was originally considered that perhaps it might be used for biopsy sampling where the disease is suspected. However, the use of the biopsy technique for obtaining samples may be limited because of the danger of secondary infection in an already infected hide.

GENERAL OBSERVATIONS ON RECEIVED SPECIMENS

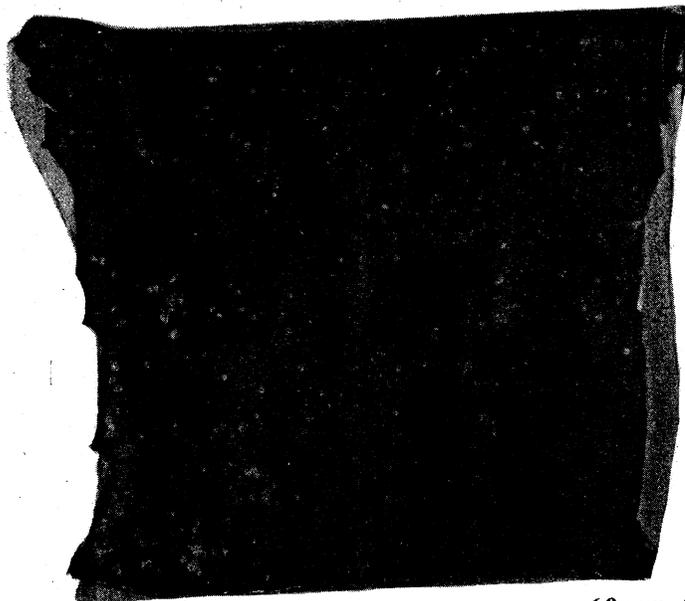
Hides from animals 1419, 1406, and 1368 shown in Table I were the most useful in following the progression of *P. ovis* infestation on the hide surfaces.

Figure 3 shows the control or uninfested sample taken from the hip area of animal 1419 as received. Figure 4 is the same area after clipping; notice the lesions present indicating incipient disease in what was to have been the control area. Figure 5 is the withers area from animal 1419, which was manually infested as stated in Table I. The center is noticeably raised under the hair. Figure 6 is the same area partially clipped, showing the hairy, scabby, encrusted center. Rather than clip the scab off, and create additional mechanical damage, it was pulled off by hand, allowing excellent observation of the many lesions present. Figure 7 shows a close-up photo of a cut through a 1/2-in. plug of hide taken from a scabby portion of the infested area of animal 1406 which was infested for 6 weeks before



60 mm

FIGURE 3. — Uninfested control from hip area of animal 1419 as received.



60 mm

FIGURE 4. — Control from animal 1419 clipped.

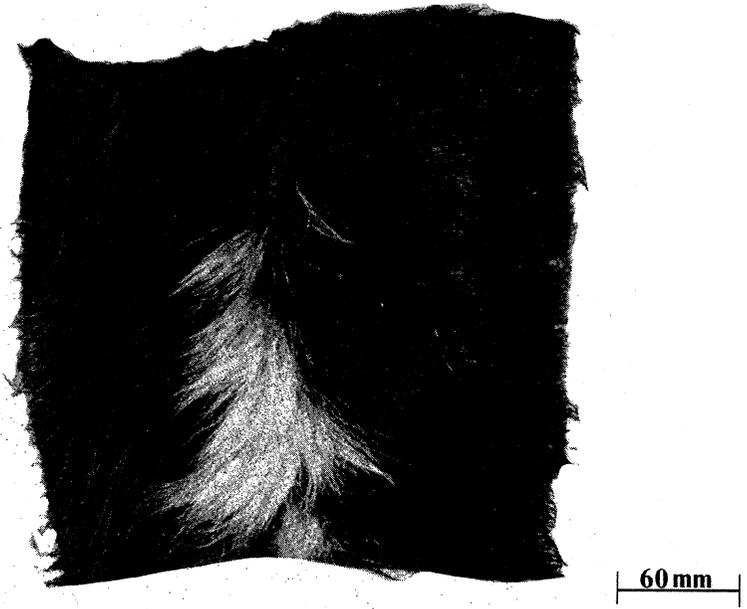


FIGURE 5. — Manually infested withers area of animal 1419 as received. Note the elevation due to scab formation under the hair.

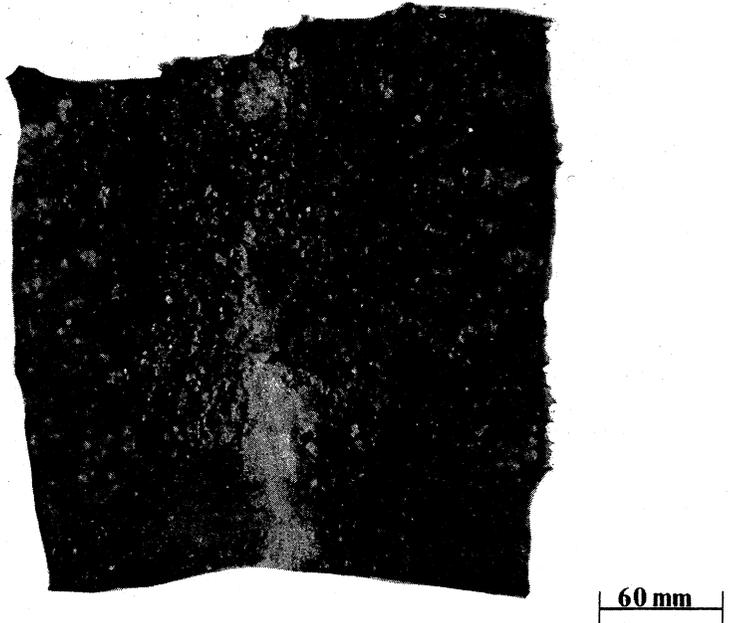


FIGURE 6. — Infested withers of animal 1419 after clipping off hair. Scab shows clearly.

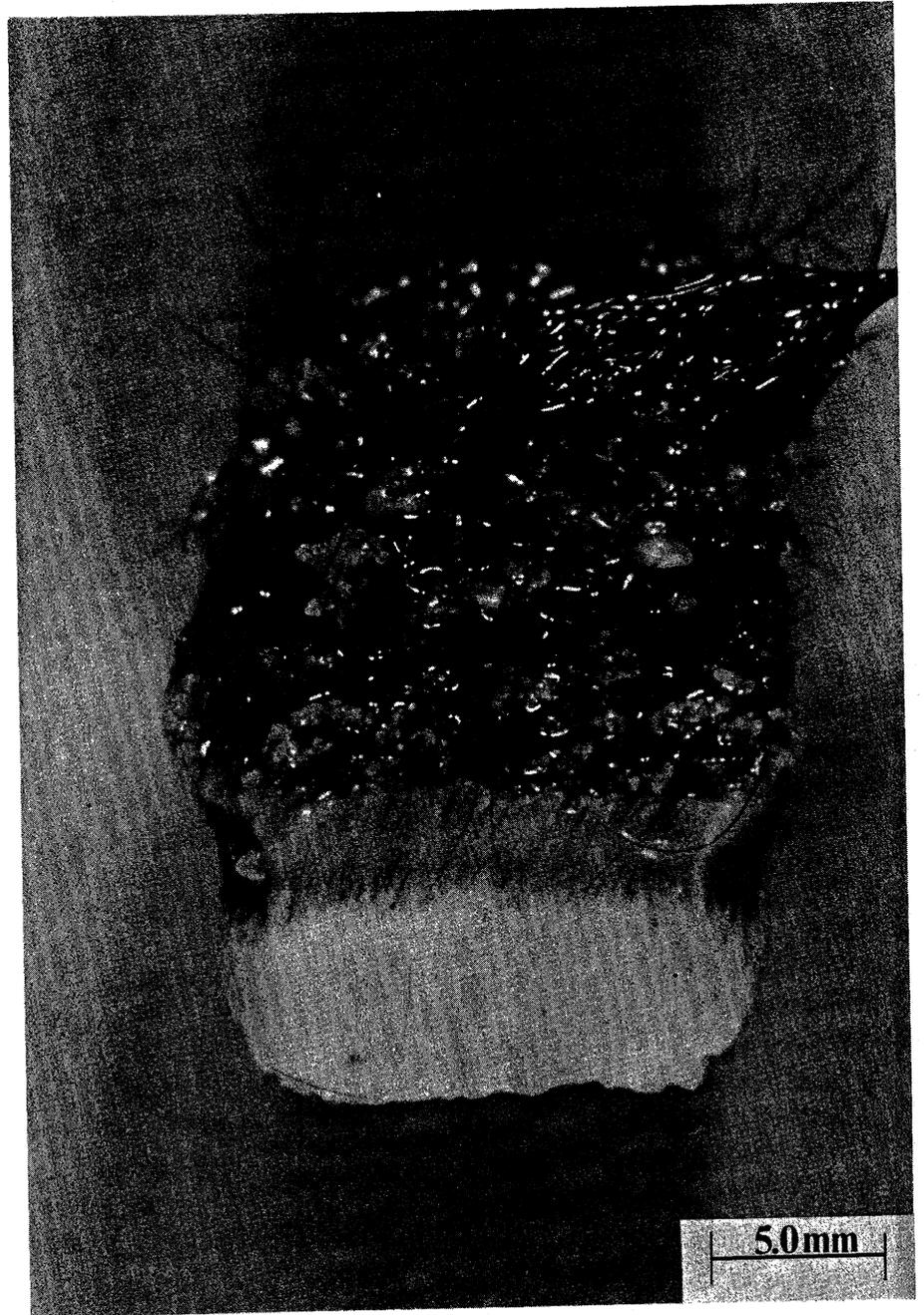


FIGURE 7. — Half plug from scabby section of hide from animal 1406 as received (unstained).

slaughter. It shows a proliferated epidermis, mixed with dried exudate from fluid produced when the hide surface is pierced by the mite. Many mites were found in the yellowish scabs.

HISTOLOGICAL STUDY OF THE PROGRESSION OF THE INFESTATION

Advantage was taken of the fact that there were areas in these animals of both incipient and advanced stages of the disease. Areas of intermediate progression were found between them. Where the disease was incipient the areas examined included clear areas immediately adjacent to the incipient areas to note histological changes not evident to the naked eye. Observations were compared with control areas farther removed from the disease process (e.g., control areas where no evidence of disease was found).

Figure 8 is a cross section of a normal control area showing the normal grain structure and thickness. Figure 9, from an area where the disease is incipient, shows slight increases in the stratum corneum and thickening of epidermis in spots, the beginning of breaks in the epidermis, and the sloughing off of epidermal tissue. Figure 10 shows a more pronounced epidermal thickening, acanthosis, and incipient papillation of the corium minor (grain area) brought about by inflammation. In Figure 11, an area showing medium involvement, the papillation is much more advanced, more corneal material is evident, and pronounced thickening of the epidermis (including the follicular lining) has occurred. Comparison of Figures 10 and 11 with Figure 8 brings out the increased depth of the corium minor (grain layer) accompanying this papillation. Figure 12 illustrates pronounced hyperplasia and the formation of cyst-like sacs which are beginning to detach under a generalized thickening of the corneal layer. Invasion of eosinophile cells, indicative of inflammation, was also found. Figures 13 and 14 show the final stages of the disease marked by pronounced cellular infiltration, the breaking off of the cyst-like sacs containing encapsulated cells, and the formation of scabs containing plasma exudate, corneal tissue, and the cyst-like sacs of epidermal tissue. Eosinophile cells were found in both the scabs and the residual grain area. As mentioned earlier, mites were also found in the scab tissue although none are illustrated here.

HIDE PROCESSING

Animals 1409 and 1186 either died suddenly or were killed because of physical collapse. Samples for histology and determination of water content were not available. When each animal died the hides were sprayed with toxaphene, salted, and shipped to ERRC where they were sided and processed into crust leather. When they were soaked, they showed loss of hair over the sides. After removal of the hair and epidermis, the grain clearly showed the roughened uneven appearance due to the papillation induced by the disease. It is this which is considered primarily responsible for the eventual downgrading of the leather. In the

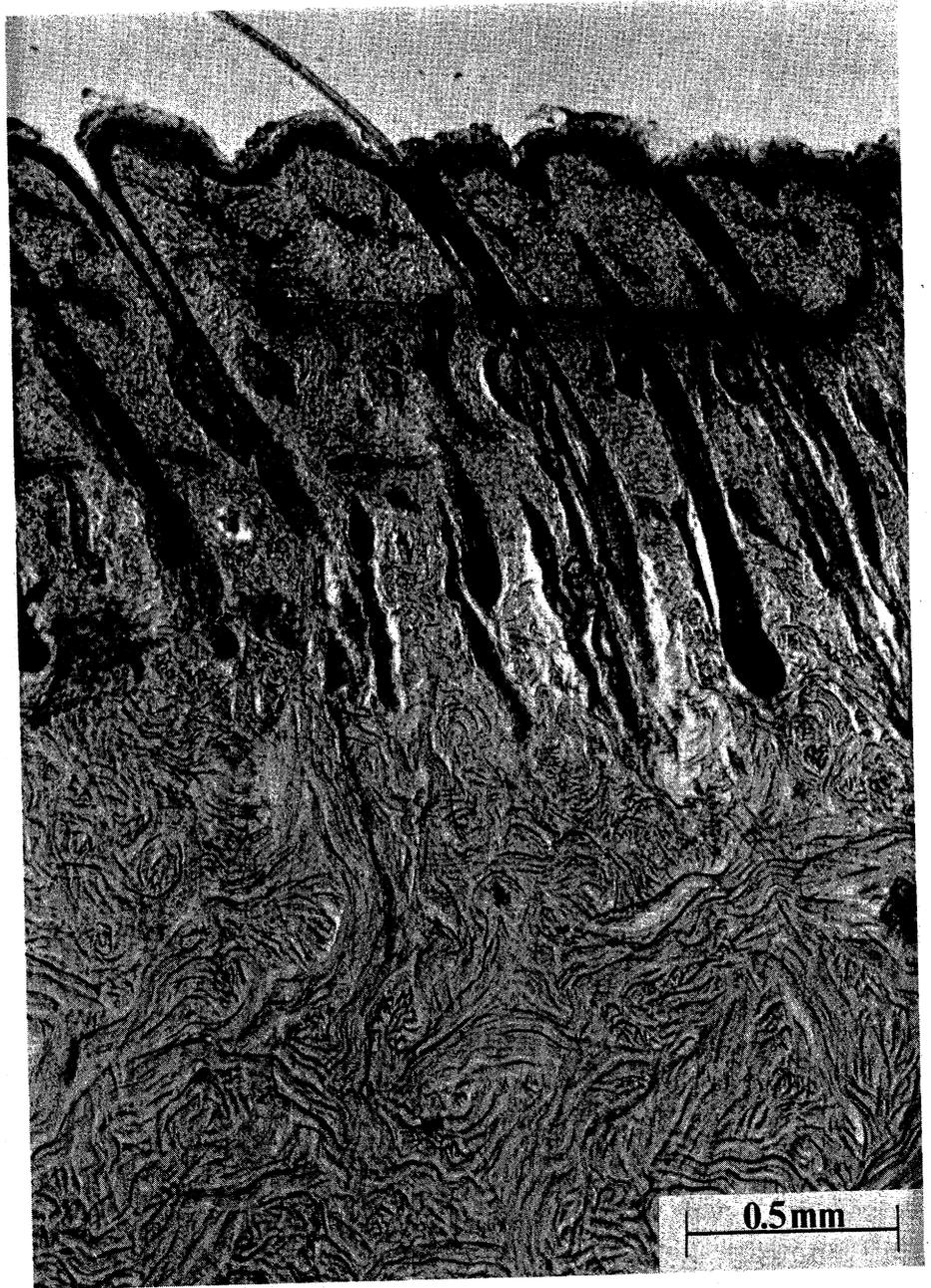


FIGURE 8. — Cross section of a normal grain area stained with hematoxylin and eosin (H + E stain).

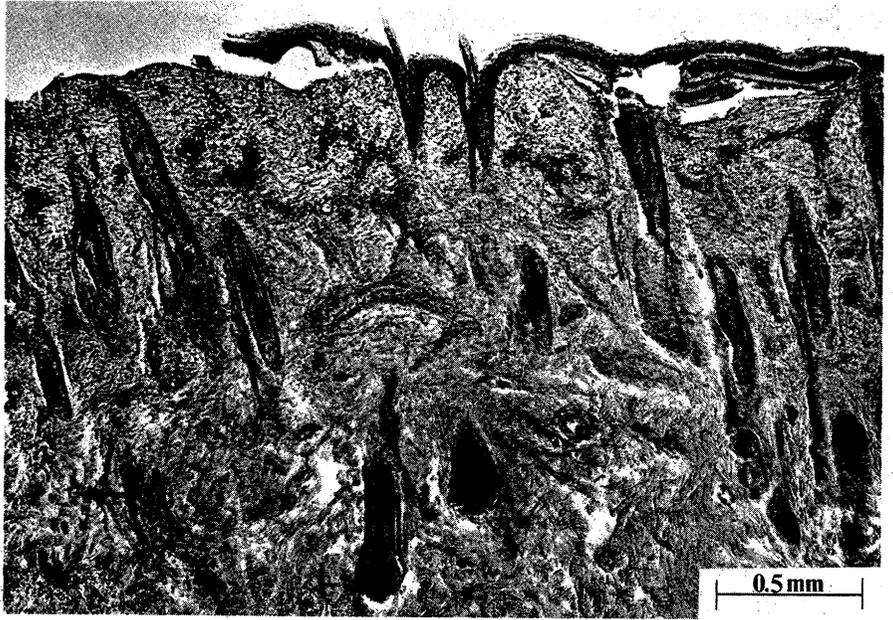


FIGURE 9. — Cross section showing an area where there is initial involvement. It shows slight increase in stratum corneum, epidermal thickening, and in this particular section, the beginning of a break in epidermis and the sloughing off of epidermal tissue (H + E stain).

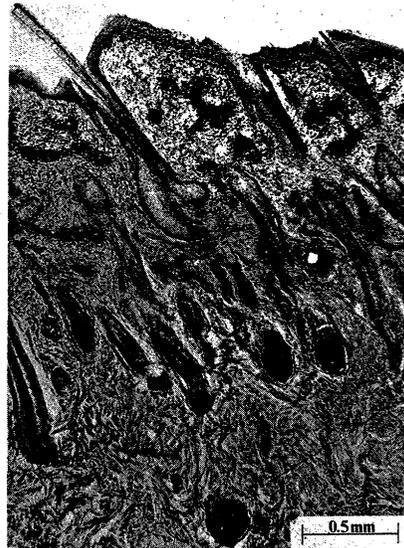


FIGURE 10. — Cross section showing acanthosis and the initiation of papillation (H + E stain).



FIGURE 11. — Shows pronounced papillation (H + E stain).

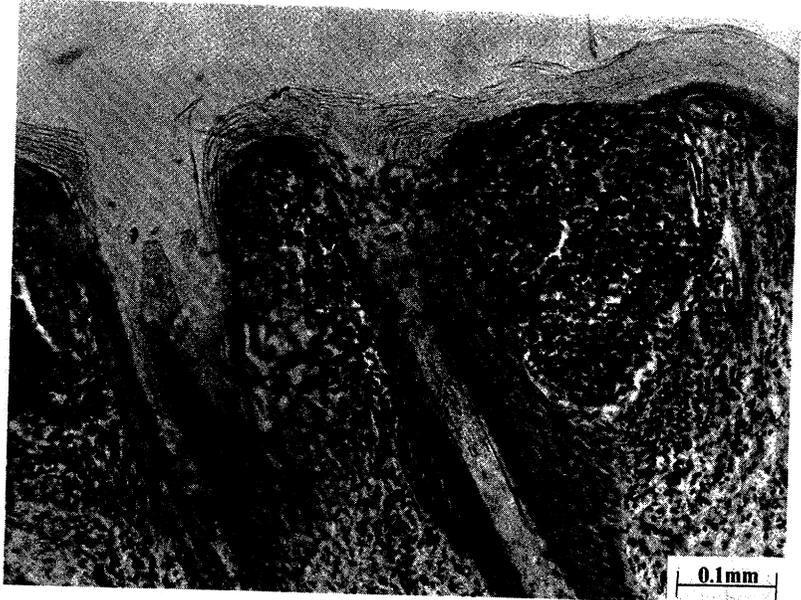


FIGURE 12. — Cross section shows hyperplasia, infiltration of cells into epidermal areas which begin to break away in cyst-like sacs, pronounced corneal thickening (H + E stain).

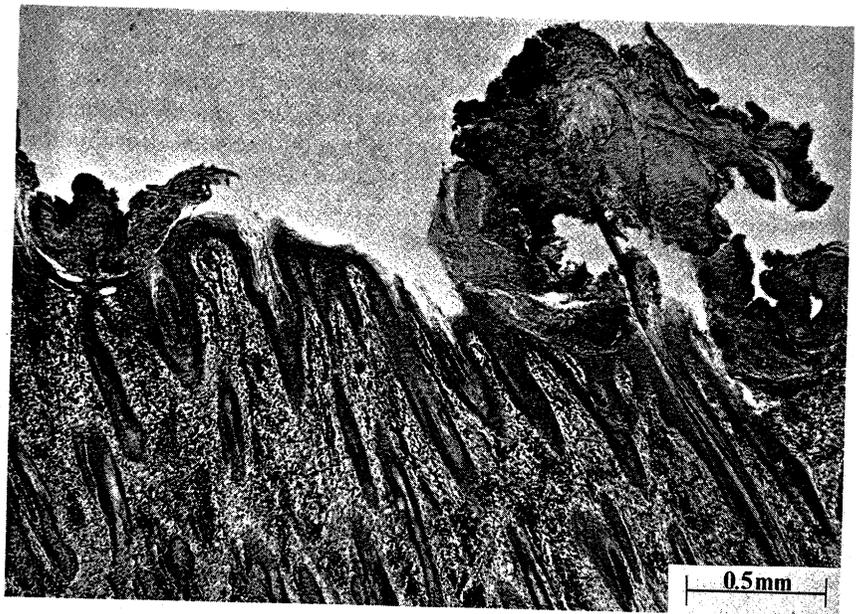


FIGURE 13. — Illustrates actual breaking away of cyst-like sacs (H + E stain).



FIGURE 14. — Cross section showing a scab containing proliferated stratum corneum, eosinophilic cells, dried plasma exudate (H + E stain).

blue (after chrome tannage), the wet sides were wrinkled overall and darkened in spots. Figure 15 is a close-up of one of these sides processed to the dried crust leather. Notice the rough and wrinkled appearance of the grain surface. Comparisons of these results were made with the processing and the leather made from the Ivermectin-treated animal, #144. Although histological evaluation of samples from animal #144 indicated that epidermal proliferation was still present in some areas on the side, behavior during processing was normal. The crust leather from this side (Figure 16) appeared to have only a few devaluating marks and some slight evidence of wrinkled grain when compared to the leather in Figure 15. This indicates that injection with Ivermectin offers much promise in efforts to combat the scabies problem. It lessens downgrading of the leather made from infested then treated animals.

At the present time there are other acaricides being tested by Wright *et al.* that also appear to be very promising (11) in treating infested animals. Effects on leather quality will need to be determined when selections of acaricides are made.

Conclusions

The suggestion is made that the recent increased incidence of hyperkeratosis in cattlehides reaching tanneries correlates with the finding of a major epidemic of *P. ovis* infestation of cattle. Findings of pronounced hyperkeratosis in cattle in-

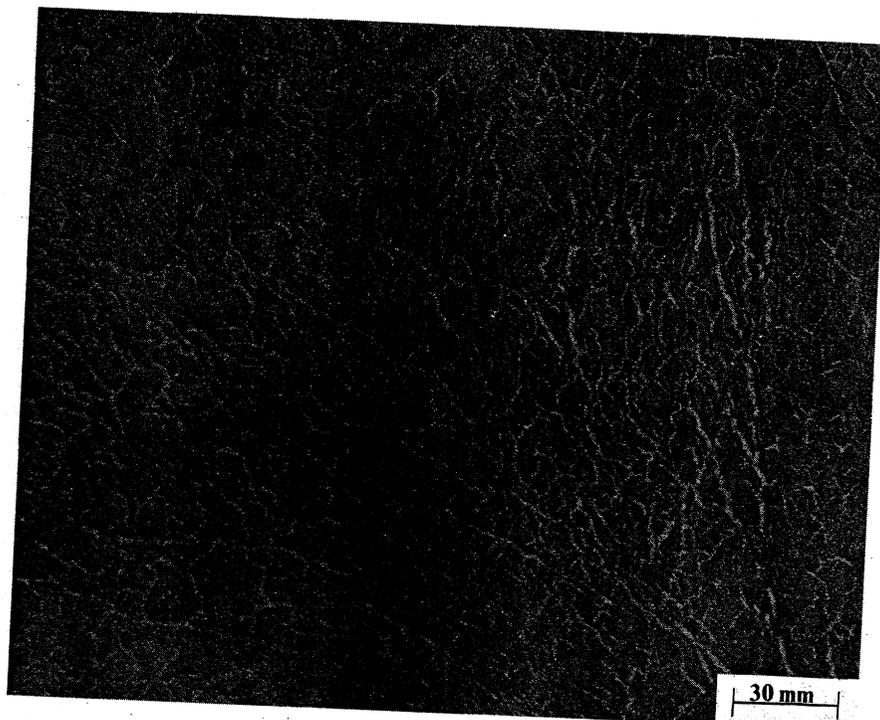


FIGURE 15. — A close-up photo of side 1409 processed to the crust leather. Note rough, wrinkled appearance.

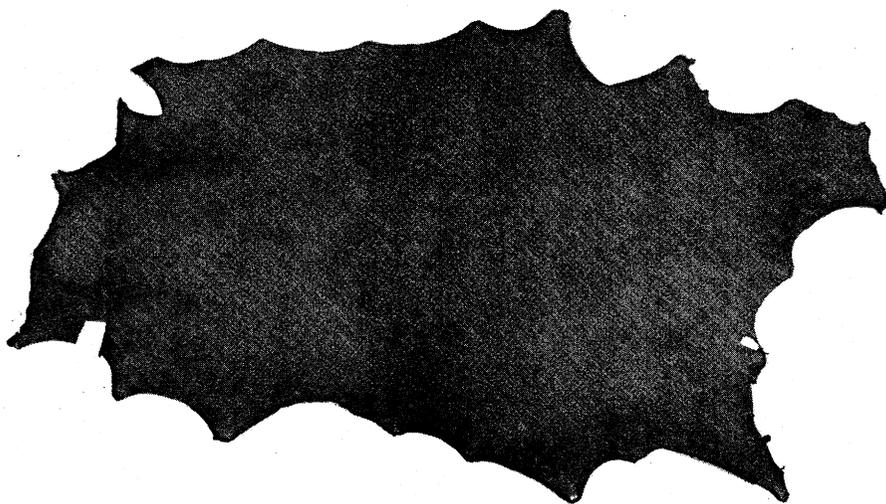


FIGURE 16. — A photo of crust leather made from a side of the Invermectin-treated animal (#144).

fested with *P. ovis* and devaluation of leathers produced from the test animals have been confirmed. Improved leather was obtained when an infected animal was cured by Ivermectin injection and permitted to groom in order to remove scabs. Index of Inflammation, a quantitative method of measuring skin irritation, correlated with the degree of disease. Due to the reported (3) paucity of data on the histological observations of the course of infestation of this disease in cattle, the detailed structure of the mite was studied by scanning electron microscopy. Also light microscopy studies on the probable course of the disease were carried out in considerable detail and found to be as follows:

Initial Stage - Slight increase in stratum corneum and epidermal thickening in minute spots. Sloughing off of epidermal tissue.

Middle Stage - Induced papillation of the surface of the corium minor and increased depth of the corium minor brought about by the inflammation. Acanthosis is pronounced. Breaking off of cyst-like sacs and encapsulation of cells in proliferated epidermal tissue mixing with exudate to form scabs. Eosinophile invasion into corium minor and scabs.

Advanced Stage - Extensive scab formation, with detachment of large pieces of epidermal tissue showing distinct hyperplasia. Immobilization of mites caught in the thick layers of scabs.

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