HISTOCHEMICAL STUDIES ON THE PROCESS OF THE FRESH HIDES TO TANNED LEATHERS (REPORT II), ESPECIALLY ON THE RIPENING, SOAKING, LIMING, SHAVING & SPLITTING, BATING, PICKLING, CHROME-TANNING, AND STAINING & FAT-LIQUORING OF THE STEER-SKIN

HISTOCHEMICAL STUDIES ON THE PROCESS OF THE FRESH HIDES TO TANNED LEATHERS (REPORT II), ESPECIALLY ON THE RIPENING, SOAKING, LIMING, SHAVING & SPLITTING, BATING, PICKLING, CHROME-TANNING, AND STAINING & FAT-LIQUORING OF THE STEER-SKIN

By

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Histochemical studies on the process in which fresh hides become tanned leather has been done on hides ripening in cold storage. They have been compared for the degree of freshness, i.e. "not very fresh hides" and "decomposed hides". Also the changes of the hides during the processes of the ripening, soaking, liming, shaving, splitting, bating, pickling, chrome-tanning, staining, and the fat-liquoring of the steer-skins have been studied. Using these results, the morphology of the process was established from the standpoints of histochemistry and pathology.

In the previous report[1] the occurrence of the autolytic phenomena was described with necrobiosis, degeneration and nuclear changes. Also the bacterial invasion into the intercellular spaces and cell bodies in hides ripening in cold storage was reported. This is a report of the histochemical studies on the manufacturing process in which hides become tanned leathers. No one has investigated the alteration in which hides become leathers from a histochemical view.

Materials and Methods for Studies

Five fresh hides, ten ripening hides, five hides soaked in water, five hides limed in lime water, five hides in the shaving and splitting, five hides in the bating, five

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Table 1. History or the samples for studies on the process of soaking, staining and fat-liquoring from the hides of steer to the tanned

	Fresh hides	Ripend hides	Soaking
Name of hide-samples	3TF	No,382	18M12TF
Producing center	Tokyo	Zürich Swiss	Tokyo
Period of slaughtering	18 March, 1964	December 1963	October 1963
Period of ware hausing (cold storage)	18 March, 1964	20 Febr., 1964	22 Oct., 1963
Period of collecting of materials (fixation)	19 March 1964	19 March 1964	19 March 1964
Body weight of steer	about 260 kg	about 260 kg	about 260 kg
Weight of the steerhide	20 kg	20 kg	20 kg
Place of collection of materials	Senjyu-Midorimachi 1, Adachi-ku, Tokyo		

hides in the pickling, five chrome-tanning leathers, and five stained leathers in fat-liquoring, were employed in the investigation. These materials were given on the 18 and 19 of March in 1964 by the Nippon Hikaku Factory (Japanese Hide-Leather Co.). Fresh hides (No. 3TF) were stored for one day in cold storage just after slaughtering. Not very fresh hides consisted of two types, i.e., first group, "No. 382 of Swiss steerskin" stored for one month in cold storage (slaughtered on Dec. 1963 in Zurich, shipped from Marseille on Jan. 10 1964 and arrived at Yokohama Feb. 17 1964 and placed in stroage on Feb. 20, 1964 and second group, "No. 206 of Swiss steerskin stored five months in cold storage (slaughtered in Augst of 1963 in Zurich, shipped from Marseille on Sept. 10, 1963, and arrived at Yokohama on Oct. 12, 1963, and placed in storage on Oct. 18, 1963). These not very fresh hides were used as the materials of the ripend hides.

Materials used for studies on the process of the soaking, liming, shaving, splitting, bating, pickling, chrome-tanning, staining, and fat-liquoring, are shown in Table 1. The materials for the studies were fixed with buffered formol, and cut with freezing microtome into 15μ sections for DNA-, protein,-polysaccharides- and fat-staining. The stains employed were Hematoxylin-eosin stain, Azan stain for general histology, Feulgen reaction for DNA, PAS-hematoxylin stain with or without saliva-digestion for glycogen and for other polysaccharides, Duijn's acrolein Schiff reaction for protein, Sudan III staining for fat, Weigert's elastica staining for elastin, Van Gieson's staining for collagen, and Bielshowsky's staining for reticulin.

liming, shaving and splitting, bating, pickling, chrome-tanning, and leathers.

Liming	Shaving, splitting	Bating	Pickling	Chrome-tanning	Staining and fatting
13H11D	13H11D	12M11D	12M11D	10M11D	4M12TF
Kanazawa	Kanazawa	Kanazawa	Kanazawa	Kanazawa	Tokyo
October 1963	October 1963	October 1963	October 1963	October 1963	October 1963
22 Oct., 1963	22 Oct., 1963	22 Oct., 1963	22 Oct., 1963	22 Oct., 1963	22 Oct., 1963
19 March 1964	19 March 1964	19 March 1964	19 March 1964	19 March 1964	19 March 1964
about 260 kg	about 260 kg	about 260 kg	about 260 kg	about 260 kg	about 260 kg
20 kg	20 kg	20 kg	20 kg	20 kg	20 kg

Results

1. Histochemical changes in chrome-tanned steer-skins during water-soaking.

a. Changes of the hides keeping with the administration of the hide

Kawamura[2] said that the fresh hide suffered a qualitative change in cold storage like fresh food because of the semiproduct in the freshness. Okamura[3] divided nitrogen components of hide-protein nitrogen, into "lime-water soluble nitrogen", and "collagen nitrogen". According to Kawamura[2], the not very fresh hide with alopecia indicated an increase of non-protein nitrogen (thirteen times that of fresh hides), decrease of collagen nitrogen (nonuple), coagulated protein nitrogen (forty-five times) and lime-water soluble nitrogen (thirty times), in comparison with fresh hide.

In comparing the fresh hide stored for a day and those stored for 1 to 5 months, the former contained high polymer of DNA (methyl greenphilic) in the round nuclei of the epidermis and corium, and the latter indicated a decrease of DNA and depolymerization of DNA (pyroninophilic) in the pyknotic nuclei. In addition to the above-described changes there were a slight decrease of protein in the stratum corneum and a slight decrease of glycogen and protein in the hair follicle.

Old hides stored for 5 months indicated stronger degree of disappearance of DNA in the nuclei of the stratum basale, papillary layer, reticular layer,

arrector muscle and sebaceous gland than that of DNA in the old hides stored for 1 month.

Histochemical changes in fresh hides and ripening hides were shown in Table I.

b) Changes in the hides soaking in water

According to Kawamura[2] soaking in water played the role of dissolving the water-soluble substance and salt, absorption of water, and softening of the hides.

There were found the desquamation of the stratum corneum; decrease of glycogen in the stratum granulosum, spinosum et basale, and hair follicle; adhesion of fat on the epidermis; decrease of fat in the hair follicle and sebaceous gland; fat necrosis with fatty acids in the corneum. Generally there were shown pyknosis of nuclei.

2. Histochemical alteration in the chrome-tanned steer-skins during the process of liming and bating.

According to Kawamura[2] the liming in water played the role of dissolving the epidermal system formed in the hair root and the unhairing of the hair-shaft. In this step there were found a desquamation of the cells in the stratum corneum, granulosum et spinosum and unclear remain adhered as the glycoproteid masses in the only stratum basale and loss of DNA. Histochemical changes in the liming were shown in Table II.

In the hair follicles the glycoproteid cast by the desquamation liquidation and agglomeration of the stratum spinosum in the outer hair sheath continued to the surface of the epidermis. Glucoprotein in the stratum basale adherred to the transversal fibers as in other stratums in the epidermis. The space of the hair follicle enlarged by the unhairing. DNA of the cells in the outer hair sheath disappeared in this stage. Stratum basale was lost in this stage. There were indicated the increase of glycoprotein in the sebaceous glands. Toyota[4] found the acceleration of unhairing by sulphide compound as the decomposition of the hair in a lime water, and Burton[5] described that increase of glycoprotein in the sebaceous glands. Toyota[4] found the acceleration of unhairing by sulphide compound as the decomposition of the hair in a lime water, and Burton[5] described that mucoprotein in the hair root related to the unhairing. The present authors observed the disappearance of DNA and increase of glycoprotein in the unhairing.

a) Changes of the hides during shaving and splitting.

b) Changes of the hides during deliming or bating.

According to Kawamura[2] the limed hides in this process were delimed and elastin was digested by enzyme such as pancreatin and others. He observed that in the hides of mice decomposed by pancreatin there was a decrease of colagen-nitrogen (about 4%) in comparison to those with no treatment. Also he stated that the components decomposed by pancreatin or ammonium chloride were unclear besides that these was a some elastin. According to Kawamura it was

of interest that the deliming process might be related to the damage in the upper layer of the epidermis called silver plane, and to the mucoprotein just under the silver plane.

In the present investigation the authors found the desquamation of the stratum basale, the decrease of polysaccharides and fat in the sebaseous glands, and the isolation of the cells in the inner hair sheath. These changes might be based on the mechanisms of the hollow-pith formation.

3. Histochemical alterations in chrome-tanned calf-skins during the process of pickling, chrome-tanning to staining and oil-liquoring

a) change of the hides during pickling

According to Kawamura[2], the base of the hides or skin made by the preparatory process was modified to leather by the pickling and chrome-tanning.

In the present paper the authors observed pathologically those preparatory process of the hides to the modified one of the leathers as follows.

The hides in the process of pickling were influenced by the acidic effects of the treatment with a mixture of acid and salt at 2.5–4.0 of pH. There were found the decrease of fat, glycoprotein cast, formation of large fat droplets, melanin masses, bacterial multiplication, and the isolation of the cells in the hair follicles appeared in the snake-like vesicles or hollow pith. Also these was decrease of fat in the sebaceous glands. These results were shown in Table 3.

b) Change- of the chrome-tanned leathers

In the chrome-tanned leathers there were observed histochemically the increase of mucoprotein in the stratum basale, and in the collagen of the stratum papillare and reticularis. These increases of mucoprotein might be greater than they appeared. DNA in the papillary and reticular layers and fats in the hair follicles disappeared completely. No glycoprotein casts, large fat droplets, melanin masses, bacterial multiplication, and isolation of the cells occurred in the hair follicles.

According to Kawamura, a basic chrome solution was used in the chrome tanning, and it was prepared from reduction of dichromate by glucose, sulphurous acid gas and sodium thiosulphuric acid. Chrome-tanning was a kind of coagulation or precipitation, and it played the role of fixing protein and lipids morphologically like Zenker's and Bouin's fixation. Accordingly basic chrome might be absorbed in the collagenous fibers. In the chrome-tanned leathers mucoprotein of the papillary and reticular layers were strengthen more in that of the fresh and ripend hides.

c). Change of the stained and fat-liquored leathers. The dyes stained the stratum basale of the epidermis and fibers in the papillary and reticular layers of the corium, and the hair follicles, especially elastic fibers in the corium. The stained portion was negative in PAS for polysaccharides and in acrolein-Schiff reaction for protein. From the point of coloring mechanism of PAS which free aldehyde liberated from polysaccharide by periodic acid was able to combine the

Table 2. Histochemical alterations of fresh hides during the

Structure \ Process / Substances		Fresh hides				
		1	2	3	4	5
Epidermis	Stratum corneum					
	DNA	−	−	−	−	−
	Polysaccharide	−	−	−	−	−
	Protein	+	++	++	+	++
	Fat	+	+	++	++	++
	Stratum granulosum					
	DNA	+++	+++	+++	+++	+++
	Polysaccharide	+	+++	+++	+	+
	Protein	+++	+++	+++	+++	+++
	Fat	++	+	+	+	+
	Stratum spinosum					
	DNA	++++	++++	++++	+++	+++
	Polysaccharide	+	+++	++	+	+
	Protein	++	+++	+++	++	++
	Fat	+	++	+	+	+
	Stratum basale					
	DNA	+++	+++	+++	+++	+++
	Polysaccharide	+++	+++	+++	+++	+++
	Protein	+++	+++	+++	+++	+++
	Fat	−	−	−	−	−
Corium	Papillary layer					
	DNA	+++	+++	++	+++	++
	Polysaccharide	++	+++	++	++	++
	Protein	++	++	++	++	++
	Fat	−	−	−	−	−
	Reticular layer					
	DNA	++	++	++	++	++
	Polysaccharide	+++	++	++	+++	+++
	Protein	+++	+++	+++	+++	+++
	Fat	−	−	++	+++	+++
	Hair follicle					
	DNA	+++	+++	+++	+++	+++
	Polysaccharide	++++	++++	++++	++++	++++
	Protein	++++	++++	++++	+++	+++
	Fat	+++	+++	+++	++	+++
	Arrector muscle					
	DNA	++	+	+	+	+
	Polysaccharide	+++	++	+++	+++	+++
	Protein	+++	++	++	++	++
	Fat	+	+	−	−	−
	Sebaceous gland					
	DNA	+++	+++	+++	+++	+++
	Polysaccharide	+	+	+	+	+
	Protein	+	+	+	+	+
	Fat	++++	++++	++++	++++	++++
	Fat-necrosis with fatty crystals	−	−	−	−	−

water-soaking process on the chrome-tanning of the steerskins.

Ripening hides										Soaking in Water				
No. 206					No. 382									
A 1	A 2	A 3	A 4	A 5	B 1	B 2	B 3	B 4	B 5	1	2	3	4	5
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
+	+	+	+	+	+	+	+	+	++	++	++	++	++	++
+	+	+	+++	+++	+	+	−	+	++	++	++	++	++	++
+++	+	++	+	+++	++	+	+	++	++	+	+	−	−	−
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	++
+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
+	++	+	+++	+++	+	++	+	+	−	++	++	++	++	++
++	+	++	+	++	++	+	+	++	++	++	+	−	+	+
++	++	++	+++	+++	+++	+++	++	++	++	++	++	++	++	++
+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
+	++	+	+++	+++	+	++	+	+	−	+++	+++	+++	+++	+++
+++	+++	++	++	+++	+++	+++	+++	+++	+++	++	+	−	+	+
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	++
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	++	++	−	−	−	−	−	++	+	++	++	++
+++	++	++	++	+++	+++	++	++	++	++	+	+	+	+	+
++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	++	++	−	−	−	−	−	+	+−−	+−−	++	++
+++	+++	++	++	+++	+++	+++	+++	+++	+++	++	++	+	++	+
+++	+++	+++	+++	+++	+++	+++	+++	++	++	+++	+++	+++	+++	+++
+++	+++	−	−	−	−	+++	+++	+++	+c	+++c	+++c	+++	+++	+++
+++	++	++	+++	+++	++	+++	+	+	++	+++	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++
+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+	+	+	+	+
+	+	−	−	++	−	−	−	−	−	++	++	++	++	++
+++	+	++	++	+++	++	++	++	++	++	++	++	+	++	+
++	++	++	++	++	++	++	++	+	+	+	+	+	+	+
−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
+++	−	+	++	++	+	+	−	−	−	++	++	−	+	++
++	+	++	++	++	++	+	++	++	++	++	++	+	++	+
+	+	+	+	+	+	+	+	+	+	++	++	++	++	++
+++	+++	+++	+++	+++	+++	+++	+++	++	+++	−	−	−	−	−
−	−	−	−	−	−	−	−	−	+	+	+	−	−	−

Table 3. Histochemical alterations in chrome-tanned

Structure		Substances \ Process / Case No.	Liming 1	2	3	4	5
Epidermis	Stratum corneum, granulosum, et spinosum	DNA	\	\	\	\	\
		Polysaccharide					
		Protein					
		Fat					
	Stratum basale	DNA	−	−	−	\	\
		Polysaccharide	++	+	+		
		Protein	++	+	+		
		Fat	−	−	−		
Corneum	Papillary layer	DNA	−	−	−	−	−
		Polysaccharide	+	+	+	+	+
		Protein	++	++	++	++	++
		Fat	−	−	−	−	−
	Reticular Layer	DNA	−	−	−	−	−
		Polysaccharide	++	++	+	+	+
		Protein	+++	+++	+++	+++	+++
		Fat	−	−	−	−	−
	Hair Follicle	DNA	−	−	−	−	−
		Polysaccharide	+++	+++	+++	++	++∼−
		Protein	+++	++	++	++	+++∼−
		Fat	−	+	−	−	−
	Arrector Muscle	DNA	−	\	\	\	\
		Polysaccharide	++				
		Protein	−				
		Fat	−				
	Sebaceous Glands	DNA	−	−	−	−	−
		Polysaccharide	−	+++	+++	++∼−	++∼−
		Protein	++	++	++	++∼−	++∼−
		Fat	+++	++	++	+	−
	Alteration in hair follicles & sebaceous glands.	Desquamation	+	+	+	+	+
		Liquidation	+	+	+	+	+
		Agglomeration	−	+	+	+	+
		Decrease of fat	−	+	+	+	−
		Glucoproteln case	−	−	+	+	−
		Large fat droplet	−	−	−	−	−
		Melanin mass.	−	−	−	−	−
		Bacterial multiplication	−	−	−	−	−
		Snake-like vesiculation	−	−	−	−	+
		Hollow pith formatn.	−	−	−	−	−
		Isolation of inner hair sheath	−	−	−	−	−

Remarks: \ indicated disappearence or despuamation.

steerskin during the processes of liming to bating.

Shaving and splitting					Bating				
1	2	3	4	5	1	2	3	4	5
\	\	\	\	\	\	\	\	\	\
−	−	−	−	−					
S	S	S	S	S	\	\	\	\	\
S	S	S	S	S					
−	−	−	−	−					
−	−	−	−	−	−	−	−	−	−
S	+	+	+	+	+	+	+	+	+
ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
−	−	−	−	−	−	−	−	−	+
−	−	+	−	−	−	−	−	−	−
+	+	+	+	+	+	+	+	+	+
ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
−	−	−	−	−	−	−	−	−	−
−	+∼−	+∼−	+∼−	+∼−	+∼−	+∼−	+∼−	+∼−	+∼−
ǂ∼−	ǂ	ǂ∼−	ǂ∼−	ǂ∼−	ǂ∼−	ǂ∼−	ǂ∼−	ǂ∼−	ǂ∼−
ǂ∼−	ǂ	ǂ	ǂ	ǂ∼−	ǂ∼−	ǂ∼−	ǂ∼−	ǂ∼−	ǂ∼−
+	+	+	−	+	−	−	−	−	−
\	\	\	\	\	\	\	\	\	\
−	−	−	−	−	−	−	−	−	−
ǂ∼−	ǂ∼−	ǂ∼−	ǂ∼−	ǂ∼−	−	−	−	−	−
ǂ∼−	ǂ∼−	ǂ∼−	ǂ∼−	ǂ∼−	ǂ	ǂ	ǂ	ǂ	ǂ
+	+	+	+	+	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
−	−	−	−	−	+	+	+	+	+
−	−	−	−	−	+	+	+	+	+

Table 4. Histochemical alteration in chrome-tanned steerskins

Structure		Substances \ Process / Case No.	Pickling 1	2	3	4	5
Epidermis	Stratum corneum, granulosum, spinosum, et basale	DNA	\	\	\	\	\
		Polysaccharide					
		Protein					
		Fats					
Corium	Papillary layer	DNA	−	−	−	−	−
		Polysaccharide	+	+	+	+	+
		Protein.	−	−	−	−	−
		Fats	−	−	−	−	−
	Reticular layer	DNA	−	−	−	−	−
		Polysaccharide	+	+	+	+	+
		Protein	⫲	⫲	⫲	⫲	⫲
		Fats	−	−	−	−	−
	Hair follicle	DNA	⫲	⫲	⫲	⫲	⫲
		Polysaccharide	⫲∼−	⫲∼−	⫲∼−	⫲∼−	⫲∼−
		Protein.	⫲	⫲	⫲	⫲	⫲
		Fats	⫲∼−	⫲∼−	⫲∼−	⫲∼−	⫲∼−
	Arrector muscle	DNA	\	\	\	\	\
		Polysaccharide					
		Protein					
		Fats					
	Sebaceous glands	DNA	−	−	−	−	−
		Polysaccharide	−	−	−	−	−
		Protein	−	−	−	−	−
		Fats	⫲∼−	⫲∼−	⫲∼−	⫲∼−	⫲∼−
	Alterations in hair follicles & Sebaceous glands	Desquamation	−	−	−	−	−
		Liquidation	−	−	−	−	−
		Agglomeration	−	−	−	−	−
		Decreasee of fat	+	+	+	+	+
		Glycoprotein case	+	+	+	+	+
		Large fat droplet	+	+	+	+	+
		Melanin mass.	+	+	+	+	+
		Bacterial multipl.	+	+	+	+	+
		Snake-like vesicles	+	+	+	+	+
		Hollow pith formation	+	+	+	+	+
		Isolation of hair sheath	+	+	+	+	+
	Collagen	Fine fibration	+	+	+	+	+
		Loss of fuchsinophil	+	+	+	+	+
		Increase of picro-phile	+	+	+	+	+
		Stainability of Collagen dyes in fine fibrils	−	−	−	−	−

Remarks: \ indicated disappearance or desquamation

during the processes of pickling to staining and fat-liquoring.

Chrome tanning					Staining and fat-liquoring				
1	2	3	4	5	1	2	3	4	5
\	\	\	\	\	\	\	\	\	\
−	−	−	−	−	−	−	−	−	−
＃	＃	＃	＃	＃	＃	＃	＃	＃	＃
≡	≡	≡	≡	≡	＃	＃	＃	＃	＃
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
＃	＃	＃	＃	＃	＃	＃	＃	＃	＃
≡	≡	≡	≡	≡	≡	≡	≡	≡	≡
−	−	−	−	−	≡	≡	≡	≡	≡
＃	＃	＃	＃	＃	−	−	−	−	−
＃～−	−	−	−	−	−	−	−	−	−
≡	≡	≡	≡	≡	−	−	−	−	−
−	−	−	−	−	≡	≡	≡	≡	≡
\	\	\	\	\	\	\	\	\	\
−	\	\	\	\	\	\	\	\	\
−									
−									
＃～−									
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
+	+	+	+	+	+	+	+	+	+
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+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	−	−	−	−	−
+	+	−	−	−	−	−	−	−	−
−	−	−	−	−	+	+	+	+	+

Table 5. Stainability using Azan stain and changes of collagen,

Structure		Staining \ Process / Number of materials	Fresh-ness 5	Ripe-ning 10	Soaking 5
Epidermis	Stratum corneum	Azan staining	Orange, Yellow	Red Yellow	Light red Yellow
		Van Gieson's stain			
	Stratum granulosum	Azan staining	Blue, Yellow	Bule Yellow	Blue, red Yellow
		Van Giesons stain			
	Stratum spinosum	Azan staining	Blue, Orange Yellow	Blue, Orange Yellow	Orange Yellow
		Van Gieson's stain			
	Stratum basale	Azan staining	Blue, Yellow	Blue Yellow	Orange Yellow
		Van Gieson's stain			
Corneum	Papillary layer	Azan staining	Blue, Red	Blue Red	Light Bl. Red
		Van Gieson's stain			
	Reticular layer	Azan staining	Blue, Red	Blue Red	Light Bl. Red
		Van Gieson's stain			
	Hair follicles	Azan staining (Hair)	Red, Yellow	Red Yellow	Red Yellow
		Van Gieson's stain			
	Arrector muscles	Azan staining	Red, Yellow	Red Yellow	Orange Yellow
		Van Gieson's stain			
	Sebaceous glands	Azan staining	Light red, Yellow	Light red, Yellow	Light blue, Yellow
		Van Gieson's stain			
	Bielschowsky's Silver impregnation	Papillary layer	###	###	No practice
		Reticular layer	##	##	
		Hair follicle			
		Arrector muscles	###	###	
		Sebaceous Glands	##	##	
	Weigert's elastica staining	Papillary layer	No practice	No practice	###
		Reticular layer			
		Hair follicle			##
		Arrector muscles			
		Sebaceous glands			##

Remarks: × indicated no practica fo atain, ＼ showed no result.

reticulin and elastin in the process of chrome-tanning steerskins.

Liming	Shaving & Splitting	Bating	Pickling	Chrome tanning	Staining & Fat-liquoring
5	5	5	5	5	5
Blue, Light red	Blue, Light red	Blue, Yellow	Blue, Light red	Light Black Light red Yellow	Black, Black
Blue, Red	Blue, Red	Blue, Yellow, Red	Blue, Red Yellow, Red	Light Black. Yellow, Red	Black, Yellow, Red, Black
Orange Yellow	Red Yellow	Red, orange, Light yellow	Red, Yellow	Light-orange Red Light red, Yellow	Red, Black, Black
Light blue, Yellow	Yellow	– –	– –	– –	Black
+				+	Black
+ Segment	No practice	No practice	No practice	Fine +	Black
+					
+				Fine +	Black
⫫ Thick	⫫	⫩		⫩	Black
⫩	⫩	⫩ Fine	No practice	⫩ Fine	Black
⫫	⫫	Isolation +		Isolation ⫫	Black

Schiff reagent, it might be of interest that no decomposition was able to combine the Schiff reagent, and that these was no decomposition of the stained collagen.

Fat penetrated into the reticular layer and hair follicles of the corium which lost fat in the process of bating to chrom-tanning.

.4 Histochemical alterations of the chrome-tanned hides in the process of staining demonstrating the relationship between dye-diversion and tissue-condensity.

The hides were stained with Azan trichrome stains consisting of azocarmine, anilin blue and gold organe G, also, with van Gieson's stains consisting of picrinic acid and acid fuchsine. According to Seki[6] if the dyes are arranged from the micromolecule to macromolecule, there will be found the following orders: azocarmine (molecular weight 433, 921) (reddish coloring) organe G. (M.W. 452, 370) (orange coloring) aniline blue (M.W. 732, 718) (bluish coloring) in Azan staining, and picrinic acid (M.W.) (yellow coloring) acid fuschine (M. W.) (scarlet red) in van Gieson's staining. He assumed the size of the dye molecule from the point of the dye-dispersity following Stokes-Einstein's law and electron-microscopic measurement of the dye-molecule. Also he divided them into two groups as minute or higher dispersity, and rough or lower dispersity. Generally the macro-mollecular dyes with lower dispersity was easily absorbed by the tissue substances, on the contrary the micromolecular dyes with higher dispersity stained the same as were the macromolecular dyes. But when using these dyes in mixture, the loose structure contained wide holes stained with the macromolecular dyes such as aniline, on the contrary the fine structure contained narrow holes stained with the micromolecular dyes such as orange G in Azan staining. In van Giseon's staining, the erythrocytes, elastic fibers and muscle fibers contained condensed structure and narrow hole stained yellowish with picrinic acid with minute dispersity, and on the contrary the collagenous fibers contained loose structure and wide hole stained reddish with acid fuchsine with rough dispersity. The results were shown in Table 5.

a) Stainability of the epidermis

The epidermis stained with Azan stains showed variable stainability of blue, orange to red coloring, and also with van Gieson staining showed stainability of yellow coloring. Accordingly this might show a transformation of fine structure to rough structure owing to the degeneration of the cytoplasmic and nuclear substances. On the contrary, the stainability of the epidermis with van Gieson's staining indicate no changes but to yellow coloring.

b) Stainability of the corium

The corium consisted of collagenous fibers in the papillary and reticular layers, hair follicles, arrector muscle and sebaceous glands which were stained with Azan stains. They showed variable stainability of blue, orange to red coloring.

The collagenous fibers of the papillary and reticular layer indicated blue coloring in the fresh hides to bating hides, and light blue or red in the pickling hides to tanned and stained leathers by the Azan staining. On the contrary the collagenous fibers stained according to van Gieson's procedure, indicated red coloring in the hides from fresh to shaving, and light red or yellow coloring in hides from bating to tanned or stained leathers. Accordingly hides exposed to the process of bating, pickling to chrome tanning seemed to be decomposed or denatured from the above results.

Bielshowsky's silver impregnation for reticulin and Weigert's elastica staining for elastin were done to some sections to clarify the changes of reticulin and elastin. Reticular fibers on the process of liming seemed to be cut like segments, and that of chrome-tanning appeared to be like fine bundles. Elastic fibers on the process of liming seemed to be cut like segments, and that of bating appeared to be like fine bundles.

c) Stainability of the dyes on hides after staining and fat-liquoring

According to Dr. Niwa, the staining procedure of leather in the Nippon Hikaku Co. was done with a mixture of hematine oxide (ICI), iron sulfate ($FeSo_4$-$7H_2O$), direct Black B (Sumitomo Chemical Co.) and nigrosin Z (Orient Chemical KK) at 60°C. These dyes stained black collagen, reticulin and elastin in the papillary and reticular layers, hair follicles and sebaceous glands, especially indicated remarkable intensity in the superficial and converse sides of leather.

Discussion

For the investigation of the hide and leather, it is important to know the histochemical nature of the skin.

According to Ham[7] the skin is nearly waterproof; this enables a relatively fluid body to exist in dry air, and it likewise permits a body to be immersed in fresh water without becoming swollen and in salt water without becoming shrunken. The present result showed the swelling of the epidermis, dermis and subcutaneous tissue in the hide and leather by soaking in water, limed water and bating solution, and the shrinkage of those by the pickling, and the isolation or splitting of the dermis and subcutaneous tissue by the manufacturing process. These differences between skin, hide and leather might be based on the living, dead and treated states.

Bernard[8] found that there was an immerse quantity of glycogen in the soft cornified layers and that glycogen disappeared in the keratinization process. But the present investigation indicated no glycogen in the epidermis of the soaked hide.

According to Montagna[19] the epidermis contained traces of mucopolysaccharides that stained metachromatically and were stainable with PAS reaction. The stratum corneum contained abundant lipids released by the cells in the final

process of keratinization, and cholesterol could always be found in the stratum corneum. In the present study there was found decrease of mucopolysaccharide and fat in the epidermis on the process of the soaking in water, and the disappearance of the epidermis in the liming process.

According to Itikawa et al[10] there were principally polymerized DNA stained with methyl green in the nuclei of the normal epidermal cells, and depolymerized DNA stained with pyronine in the nuclei of the parakeratotic epidermis.

In the present paper not very fresh hides stored for 1 to 5 months showed decrease of DNA and depolymerization of DNA stained with pyronine in the pyknotic nuclei; also disappearance of DNA in the epidermal cells occurred in the hides soaked in limed water. DNA of the cells in the outer hair sheath disappeared in this liming stage.

Meyer[11] stated that connective tissue was ground substances which were soluble in slightly alkaline aqueous salt solutions, and that a portion of the ground substance is composed of acid mucopolysaccharides. According to Fullmer[12], there are nine known acid mucopolysaccharides of which two, hyaluronic acid and chondroitin, are nonsulfated, and of which five, chondroitin sulfates A, B, and C, keratosulfate, and heparin sulfate are sulfated. In the present paper polysaccharides in the corium decreased in liming to pickling, and increased apparently in chrome-tanning to staining. But it is very difficult to classify polysaccharides in the histochemical observations.

Recent evidence compels us to recognize that under usual histochemical conditions acid mucopolysaccharides in connective tissues are generally not stainable (Glegg et al[13] Leblond et al[14], Bradfield and Kodicek[15], and Dische[16]). Leblond et al isolated acid mucopolysaccharides and other carbohydrates from several connective tissue including tendon, skin, bone and cartilage. They found that, after the removal of glycogen, only hexose and methylpentose residues play a significant role in the periodic acid-Schiff reaction; and that acid mucopolysaccharides failed to stain. In the present paper the authors were not able to observe the detailed structure of the acid mucopolysaccharides around collagen.

Collagen is a white fibrous intercellular substance that swells in dilute acids and becomes gelatin when heated in water. Extracted collagens and gelatins contain small amounts of noncollagenous proteins (Kent and Whitehouse[17], Bowes et al[18], Jackson and Bentley[19]) and some carbohydrate which is firmly bound to collagen (Grassman and Schleich[21], Beck[21] Glegg et al[13'22], Leblond et al[14], Oneson and Zacharias[23]). According to Fullmer[12] these components must be considered in studies of staining reactions.

Collagen stains faintly to moderately with the periodic acid-Schiff method for carbohydrate. Glegg et al[22] and Leblond et al[24] secured extracts from several connective tissue sources and noted that fractions containing acid mucopolysac-

charides failed to stain with periodic acid-Schiff method, whereas fractions containing galactose, fucose, hexosamine, and sometimes glucose and sialic acid stained intensely. According to their opinions the evidence indicates that the periodic acid-Schiff reactions of collagenous tissues is due to carbohydrate and not to acid mucopolysaccharides or collagenous protein. Bangle and Alford[24] and Graumann[25] reached the same conclusion. Glegg et al[22] and Leblond et al[14] obtained a positive correlation between the degree of staining of several connective tissue structures with the periodic acid Schiff method and the amount of the above monosaccharides extracted; and moreover a lack of correlation between the degree of staining with the periodic acid-Schiff stain and the acid mucopolysaccharide content. In the present paper collagenous fibers were stained by the periodic acid-Schiff reaction for polysaccharides and by the acrolein-Schiff reaction for protein. As results, there were alterations of the collagenous fibers in the corium stained with the periodic acid Shciff reaction (PAS) and Duijn's acrolein Schiff reaction(AS) as follows: ⧺ (PAS) and ⧺ (AS) of the freshness, ⧺ (PAS) and ⧺ (AS) of the ripening, ⧺ (AS) and ⧺ (PAS) of the soaking, ⧺ (PAS) and ⧺ (AS) of the liming, + (PAS) and ⧺ (AS) of the shaving, + (PAS) and ⧺ (AS) of the bating, and pickling ⧺ (PAS) and ⧺ (AS) of the chrome-tanning, and ⧺ (PAS) and ⧺ (AS) of the staining.

Van Gieson[26] developed a staining method for connective tissue that persists today as one of the standards. Lillie[27] suggests that acid fuchsin in the Van Gieson method reacts with basic groups in collagen. Staining of collagen was slightly to moderately impaired in sections acetylated for 18 hours at 60°C, restored in sections deacetylated by an alcoholic saponification procedure. Some staining impairment with the Van Gieson method was observed in deaminated collagen. Lillie[27] also noted that collagen failed to stain with the Van Gieson method after sulfation. According to his speculation the latter effect may be due either to a change of pH or to a blockade of hydroxyls by sulfate. In the present paper, the collagenous changes of the staining impairment with the Van Gieson method are shown in every manufacturing process from hides to leathers as follows: red with acid fuchsin at the freshness, ripening and soaking, light red with acid fuchsin in liming, and shaving, and yellow with picrinic acid in bating, pickling, chrome-tanning and staining. Accordingly some staining impairment with the Van Gieson method was observed in deaminated collagen in bating to tanning.

Puchtler and Isler[28] and Bolduan et al[29] have provided evidence to indicate that either phosphotungstic or phosphomolybdic acid unites with basic groups on the collagen molecules, and its excessive anionic groups combine with basic groups of a strongly amphoteric dye such as aniline blue. The phosphotungustic or phosphomolybdic acid thereby serves to link the dye to collagen. In the present paper, some staining impairment with the Azan staining method was observed on the process of the chrome-tanning.

Summary and Conclusion

In the present study histochemical observations on the manufacturing process of making hides into tanned leathers have been done.

The results investigated are summarized as follows:

1) In comparing the fresh hides stored for one day and old hides stored for 1 to 5 months, the former contained high polymer of DNA in the nuclei of the epidermis and corium, and the latter showed a decrease of DNA and depolymerization of DNA. In addition to the above-described changes, there were a slight decrease of protein in the stratum corneum and a slight decrease of glycogen and protein in the hair follicles.

2) There were the following changes of the hides soaked in water: the desquamation of the stratum corneum, decrease of glycogen in the epidermis and hair follicles, adhesion of fat on the epidermis, decrease of fat in the hair follicles and sebaceous glands, and fat-necrosis with fatty acid crystalization in the corium. Generally there were nuclear pyknosis.

3) The changes in liming hides were desquamation of the epidermis, glucoproteid masses as a cast in only stratum basale, and loss of DNA in the nuclei of the epidermis and corium. The space of the hair follicle was enlarged by the unhairing.

4) The changes in bating hides were the desquamation of the stratum basale, decrease of polysaccharides and fats in the sebaceous glands, and isolation of the cells in the inner hair sheath. These changes might be based on the mechanism of the hollow formation.

5) The influence of pickling on hides was a decrease of fat, glycoprotein cast, formation of large fat droplets, melanin masses, bacterial multiplication, isolation of the cells in the hair follicle and a decrease of fat in the sebaceous glands.

6) There was an increase of mucroprotein in the stratum basale and in the collagen of the papillary and reticular layers of the corium. These increases of mucoprotein might be greater than they seemed. DNA in the corium and fat in the hair follicles disappeared completely. No glycoprotein casts, large fat droplets, melanin masses, bacterial multiplication, and isolation of the cells were occurred in the hair follicles.

7) The dyes stained the superficial corium, hair follicles and reverse corium. The stained parts were negative in PAS or acrolein Schiff reaction. It might be of interest that no decomposition of the stained collagen by periodic acid-oxidation were found. In the fat-liquoring fat penetrated into the corium which lost fat in the process of bating to chrome-tanning.

8) According to Seki, if the dyes are arranged from the micromolecule to macromolecule, there will be found the following orders: azocarmin (red coloring) orange G(orange) aniline blue (blue) in Azan-staining, and picric acid (yellow) acid fuchsine (scarlet red) in Van Gieson's staining. According to his opinion,

they were divided into two groups, i.e. "minute or higher dispersity, and "rough or lower dispersity of dyes". By using these dyes in mixture, the loose structure contained wide holes stained with macromolecular dyes, such as aniline blue or acid fuchsine, and on the contrary, the fine structure contained narrow holes stained with the micromolecular dyes, such as orange G or picric acid. Accordingly, on the process of fresh, ripend, soaking, liming, shaving, bating, and pickling hides becoming chrome-tanned and stained leathers, there were found the degeneration of the epidermis, corium, collagenous fibers, hair follicles and sebaceous glands, and denaturation of polysaccharides and protein in the cells and tissues from the point of coloring, such as aniline blue to orange, or acid fuchsine to picric acid.

9) With the mixture of hematine oxide, iron sulfate, direct Black B and nigrosin Z used for the staining of leathers, it was possible to stain collagen, reticulin and elastin in the corium.

Our present report showed histochemical observations of hides during the processes of manufacturing fresh hides to chrome-tanned leathers for the sake of clarification of the mechanism of swelling and splitting of collagen fibers and ventilation. Moreover it is very important to solve biometrically the problems of ventilation in the leathers, and this will become the subject for future investigation.

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Explanation of Figures

Plate 1

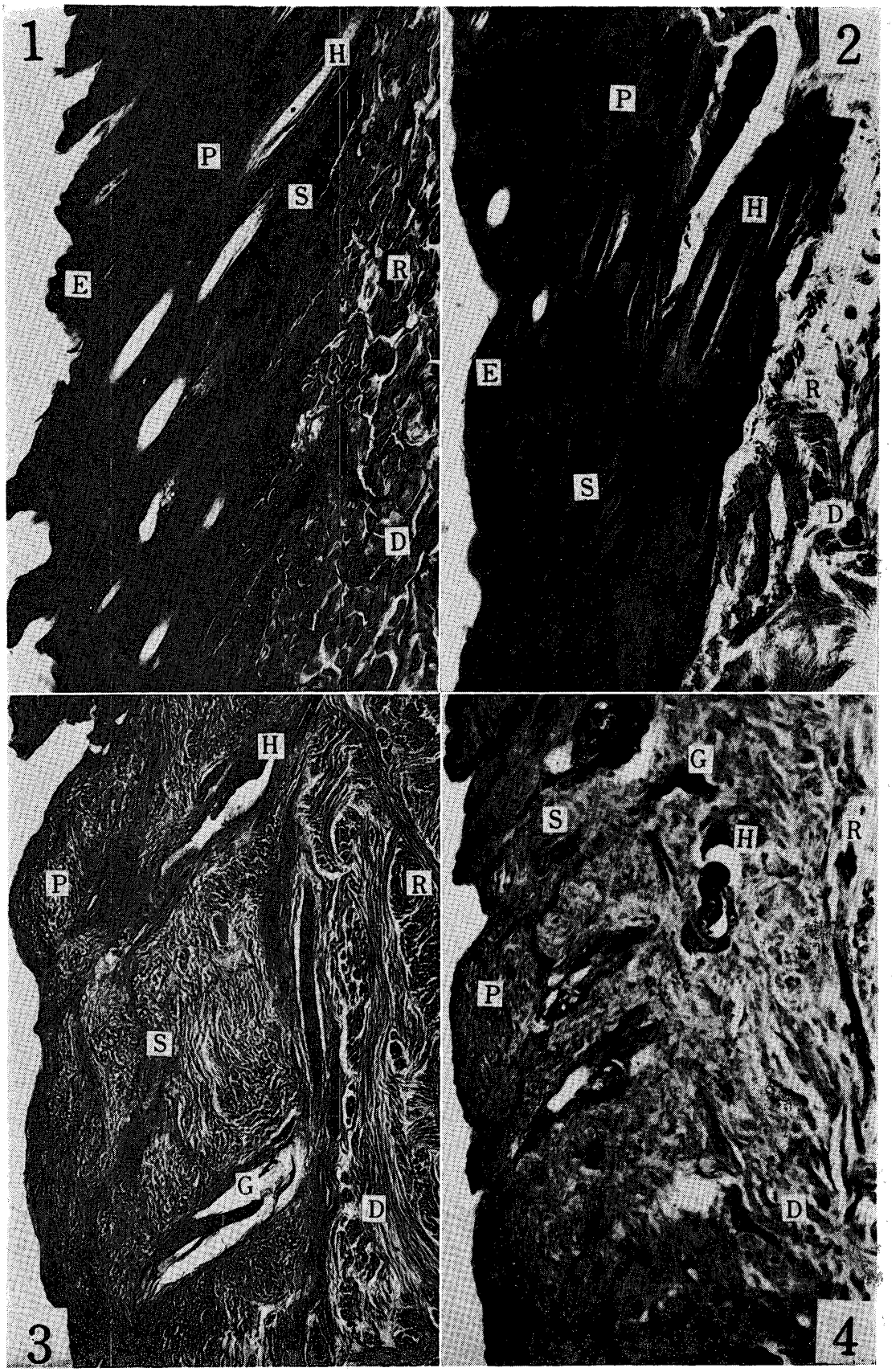
Explanation of Figures

Fig. 1. Section of the ripening hide stained with PAS reaction. The hide was taken from the steer slaughtered on August of 1963 in Zurich, shipped from Marseile on 10 September, 1963, arrived at Yokohama on 12 October, 1963, and stored on 18 October, 1963. This steer-hide was stored for 5 months in cold stroage, and then the materials for the studies were collected on 19 March, 1964 in Tokyo. The low-power photomicrograph indicated the structure of the epidermis (E), dermis(D), papillary (P) and reticular (R) layers, hair follicles (H) and sebaceous glands (S).

Fig. 2. Section of the soaking hides stained with PAS reaction. The hide was taken from the steer slaughtered on October of 1963 in Tokyo, and stored in cold storage on 20, February, 1964. After storage for 5 months the ripend hide was immersed in water, and the materials for study were collected on 19 March, 1964 in Tokyo. There were shown the desquamation shown of stratum corneum, swelling and splitting of collagenous fibers, and enlargement of the intercollagenous spaces. The abbreviations mean epidermis (E), dermis (D), papillary (P) and reticular (R) layers, hair follicles (H) and sebaceous glands (S).

Fig. 3. Section of the liming hide stained with PAS reaction. The hide was taken from the steer slaughtered on October, 1963 in Kanazawa, and stored in cold storage on 22 October, 1963 in Tokyo. After storage for 5 months and treatment by soaking, the soaked hide was immersed into liming water, and the materials for this study were collected on 19 March, 1964. There were shown the desquamation of the stratum corneum, granulosum et spinosum, swelling of the collagenous fibers, enlargement of intercollagenous spaces, isolation of the hair sheath, glycoproteid cast in the hair sheath, and disappearance of the arrector muscles. The abbreviation mean D, dermis; P and R, papillary and reticular layers; H, hair follicles and S, sebaceous glands. G. glycoproteid cast.

Fig. 4. Section of the shaving and splitting hide stained with PAS reaction. The hide was taken from the steer slaughtered on October, 1963 in Kanazawa, and stored in cold storage on 22 October, 1963 in Tokyo. After storage for 5 months and the soaking and liming treatment, the hide was treated with the shaving and splitting-process, and the materials for this study were collected on 19 March, 1964. There were shown the desquamation of the epidermis, isolation of the hair sheath, glycoprotein casts in the hair sheath, disappearance of the arrector muscle, and disappearance of fat in the sebaceous glands. The abbreviations mean D: dermis, P and R: papillary and reticular layers; H; hair follicles, and S: sebaceous glands. G, glycoproteid cast.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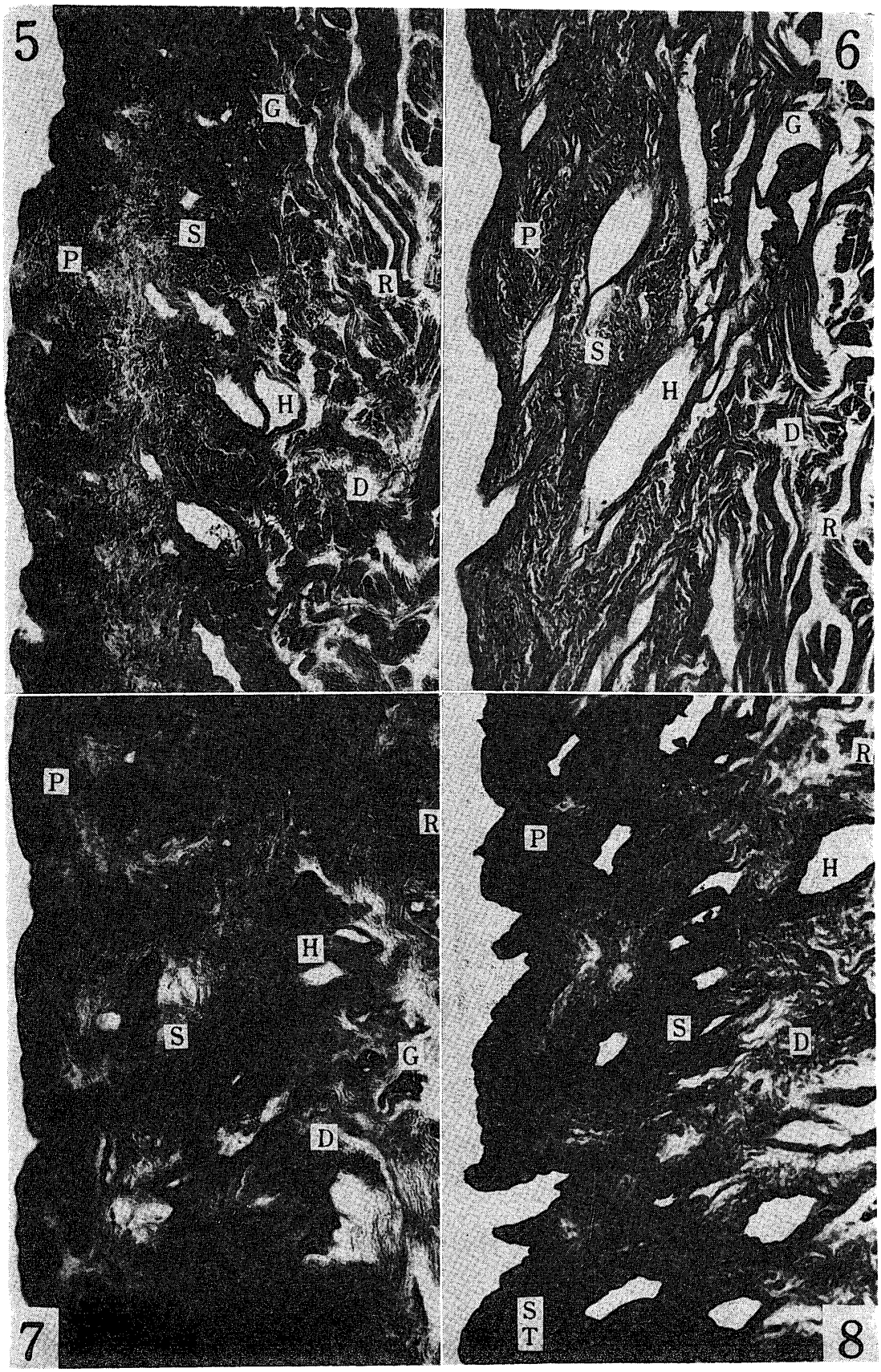
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Plate 2

Explanation of Figures

Fig. 5. Section of the bating hide stained with PAS reaction. The hide was taken from the steer slaughtered on October, 1963 in Kanazawa, and stored in cold storage on 22 October, 1963 in Tokyo. After storage for 5 months and the soaking, liming and shaving treatment the hide was immersed in a bating solution, and then the materials for this study were collected on 19 March, 1964. There were shown the desquamation of the epidermis, degeneration of collagen, swelling and splitting of the collagenous fibers, glycoprotein casts in the hair follicles with alopecia, hollow pit-formation, and disappearance of fat in the sebaceous glands. Abbrevations mean D: dermis, P and R: papillary and reticular layers, H: hair follicles, S: sebaceous glands, and G: glycoprotein cast.

Fig. 6. Section of the pickling hide stained with PAS reaction. The hide was taken from the steer slaughtered on October, 1963 in Kanazawa, and stored in cold stroage on 22 October, 1963 in Tokyo. After storage for 5 months and the soaking, liming, shaving, and bating treatment, the hide was immersed in the acidic solution, and then the materials for this study were collected on 19 March, 1964. The changes in the pickling hide were similar to the ones shown in the above-described Fig. 5. Abbreviations mean D: dermis, P and R: papillary and reticular layers, H: hair follicles, and S: sebaceous glands, and G: glycoprotein cast.

Fig. 7. Section of the chrome-tanning leather stained with PAS reaction. The hide was taken from the steer slaughtered on October, 1963 in Kanazawa, and stored in cold storage on 22 October, 1963 in Tokyo. After storage for 5 months and the soaking, liming, shaving, bating and pickling treatment, the hides were immersed into a chrome-tanned solution, and then were shown the materials for this study collected on 19 March, 1964. There were the desquamation of the epidermis, degeneration of the collagenous fibers, swelling and splitting of the fibers, enlargement of the intercollagenous spaces, hollow-pith formation in the hair follicles and sebaceous glands. Abbreviations mean: D: dermis, P and R: papillary and reticular layers, H: hair follicles, S: Sebaceous glands, and G: glycoprotein cast.

Fig. 8. Section of the staining and fat-liquoring leather stained with PAS reaction. The hide was taken from the steer slaughtered on October, 1963 in Tokyo, and stored in cold stroage on 22 October, 1963 in Tokyo. After storage for 5 months and the soaking, liming, shaving, bating, pickling and chrome-tanning treatment, the leather was immersed into a dye solution, and then the materials for this study were collected on 19 March of 1964. There were shown the several changes described in Fig. 7 above and fat infiltration in the corium, and stained with dyes. Abbreviation mean: D: dermis, P and R: papillary and reticular layers, H: hair follicles, S: sebaceous glands, and ST: stainable parts.