SALT STAINS ON SOUTH INDIAN HIDES AND SKINS.

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The term salt stain has been applied to defects in hides or skins which arise during the curing period. Stather (*Collegium*, 1928, 567) groups these defects under three heads: (1) those in which curing salt does not play any part, *e.g.*, red heat and blue and purple colorations, which are due to the pigments produced by bacteria growing on the hide substance (Lloyd, *J. Int. Soc. L. T. C.*, 1929, **13**, 538; Bergmann, *ibid.*, 1929, **13**, 599; Stather and Leibscher, *Collegium*, 1930, 151, 170), (2) defects produced mechanically by the action of salt crystals within the hide, or on its surface and (3) true salt stains. The last are spots caused by impurities in the curing salt, or the denaturant added to it.

According to Stather, there are two kinds of salt stains, (*a*) pale yellow to orange scale-like specks on the flesh side, measuring 3–5 mm. in diameter (Becker, *Collegium*, 1912, 408) and (*b*) lemon-yellow to orange-brown stains of diameter 4–8 mm. (Becker, *loc. cit.*; Abt, *Collegium*, 1912, 388; 1913, 204). Becker succeeded in producing yellow or orange stains by using pure cultures of bacteria isolated from the corresponding stains. He was not able to produce blue stains by mere bacterial action, however, and concluded that these might be due to chemical changes not involving bacterial activity. Abt maintained that micro-organisms are only secondary in stain-formation; he found the stained regions always gave more intense qualitative tests for iron than the unstained. According to him, calcium sulphate in the curing salt is precipitated as calcium phosphate by reaction with ammonium phosphate derived from the nucleic acid of the skin. The resulting ammonium sulphate reacts with insoluble ferrous carbonate forming soluble ferrous sulphate which forms a stain by combining with skin protein. He recommended adding small quantities of sodium carbonate to the curing salts, since the former precipitates calcium sulphate and thus prevents further changes.

The peculiar type of stains with which the present investigation deals belongs to the third type described by Stather. These stains occur chiefly on the wet salted hides coming from the Malayalam districts, a tract parallel to the western sea-coast of the South Indian Peninsula. The colour of the stains ranges from blue-green to violet, and is not seen either in the cure, or in the soaks, developing only when the stained hides and skins are soaked in the lime liquor. Thus it will be seen that the tanners purchasing hides and skins from these areas suffer a loss owing to the absence of any criterion by which they could distinguish the stained from the unstained goods at the time of buying. There is no hair-slipping accompanying these stains as is generally the case with

stains described by previous workers. The colour of the stain is greenish blue, deep blue, or bluish purple in the lime liquors, but changes to black as the vegetable tannage proceeds, remaining as black spots on the finished and dressed hides. The defective hides are useless for light colouring and, in consequence depreciate in value.

The various problems arising out of this investigation have been taken in the following order:—(1) A study of the curing conditions in Malabar, (2) the nature of the micro-organisms connected with stain production, (3) chemical examination of stained and unstained hides and of curing salts, (4) experimental production of stains, (5) treatment for the removal of stains and (6) prevention of stain-formation.

Curing Conditions in Malabar.

Large curing centres in Malabar are situated in towns which are far apart. The hides and skins are collected in small numbers at a time by villagers who cure them with a very low grade of salt, generally mixed with earth to increase their weight. The goods are bought by small collectors who sell them to big merchants in towns, where they are salted and stored in godowns till the accumulated thousands are despatched wet-salted to various tanning centres in South India.

A study of the conditions prevailing in the godowns throws considerable light on the problem in hand. The godowns are generally very dirty, badly ventilated and unprovided with adequate drainage. The hides are not washed, but are directly rubbed with a low grade of sea-salt which is often mixed with salt previously used for curing; they are then folded and piled on the floor or over a cement platform a foot from the floor. Generally two to three days elapse between the purchase of the hides and their salting and storage in godowns, during which period they are saved from partial decomposition by the crude cure originally given by the villagers. After the first salting in the godown, the hides are left in piles for about a fortnight, while the brine formed by abstraction of water from the hide percolates through the pile and collects on the floor. In many cases the volume of brine thus formed is so great that it actually rises above the platform and covers the lower layers of the pile. After fifteen days a second salting is given, the hides being refolded and repiled; in this condition they remain in the godowns for more than six weeks before despatch to the tanning centres.

The climatic conditions in Malabar are peculiar to those prevailing on the south-western coast of India with a high average rainfall, for eight months the atmosphere being practically saturated with moisture. Reports from the tanners point out that the highest percentage of stained hides, which sometimes reaches as much as 30 per cent., occurs in the lots despatched from Malabar during the second half of October and the whole of November. After that period the percentage of stained hides decreases till it reaches a minimum at the beginning of the monsoon in June. Although the tanners do not maintain detailed statistics, the information is based on years of experience in the Malabar hide trade, so that the figures cited above can be safely assumed to be very nearly correct. A comparison with data for the rainfall of Malabar

will reveal the fact that hides accumulated during the south-west monsoon, which extends from June to September or October, show a great tendency to become stained ; thus during the wet season there is increased incidence of stains.

In this connection it should be noted that the goat or sheep skins coming from the Malayalam districts contain a much lower stained percentage than hides from the same area. The reason for this difference may lie in the fact that the skins are carefully salted with greater cleanliness, and are not generally allowed to accumulate in the godowns over so long a period as the hides. The skins fetch comparatively higher prices than the hides and hence the extra care bestowed on them.

The brine accumulated on the floor is very offensive in odour, and contains much decomposing and putrefying organic matter ; it is rich in hydrogen sulphide and saprophytic organisms, and is drained away only when the collected hides are despatched and the next collection begins. The atmosphere of the godown is saturated with moisture.

In some places the hides are kept immersed in saturated brine in large cement-lined pits, such brine being generally very dirty. The hides are allowed to accumulate in the pits till favourable market conditions warrant their being sold. When fresh hides are introduced the old brine is removed, but more dirty salt is thrown in to make up for the dilution.

Micro-organisms in curing salts and on hides and skins.

Stained portions of the mechanically delimed hides were introduced into sterile nutrient broth and the more prominent organisms then developing isolated by the usual methods. A number of such micro-organisms have been systematically studied and will be described in detail elsewhere. Generally speaking they are of two types, those occurring in the soil and those associated with the skins of animals.

Attempts were made to induce stain formation by smearing thoroughly washed pieces of skins of freshly slaughtered sheep with pure or mixed cultures of isolated bacteria, followed by rubbing with good quality common salt for preservation. In all the cases the results were negative, indicating that none of these bacteria was specific in producing the characteristic stains, although they may be an important factor, as will be explained later. Halophilic bacteria of the pigment-producing type were also isolated ; they produced red or orange pigments, and may resemble those described by Lloyd (*loc. cit.*), but their study was not pursued since they could not be responsible for producing blue, purple or violet stains on hides.

Analyses of stained and unstained hide portions and of curing salts.

(*a*) *Curing salts.*—Table I shows the percentage of the more important constituents of three samples of salt. Khari salt consists mainly of sodium sulphate and is exclusively used in the provinces of Bengal and Behar for salting hides. Ernakulam and Trivandrum salts are locally manufactured along the sea-coast ; these with others prepared at individual centres are used in Malabar for curing.

TABLE I.

Constituent	Khari		Ernakulam		Trivandrum	
	Water soluble	Soluble in conc. HCl	Water soluble	Soluble in conc. HCl	Water soluble	Soluble in conc. HCl
Na	19.21	..	35.47	..	32.41	..
Mg	2.358	0.772	1.074	0.127	0.440	0.163
Ca	0.207	2.251	0.229	0.236	0.136	0.300
Fe	0.003	0.08	..	0.032	..	..
Cl	5.697	..	54.71	..	49.98	..
SO_4	36.42	..	1.44	..	1.608	..
Insoluble	..	..	..	1.33	..	2.66

(b) Stained and unstained leather portions.—The material was taken from vegetable tanned and dressed hides in local tanneries. The hides were examined, stained portions removed, and then cut or chopped as fine as possible and mixed thoroughly. Unstained portions from the stained hides were also treated in the same manner. The materials were then ignited and the resulting ash analysed, typical results being given in table II.

TABLE II.

Sample	Fe	Ca	Mg
Stained	0.024	0.0752	0.0088
Unstained ..	0.017	0.0859	0.0054
Stained	0.0579	0.0545	0.0134
Unstained ..	0.0358	0.0550	0.0104
Stained	0.0542	0.0487	0.0182
Unstained ..	0.0394	0.0787	0.0132

From the above it will be clear that iron is invariably higher in stained than unstained portions; magnesium shows a similar variation, but the differences in calcium are inconsistent. The quantity of calcium in any part of the skin will depend upon the thoroughness of deliming, which in South Indian tanneries is effected mechanically by repeated scudding and washing with water. The higher percentage of iron in the stained portion has a significance which will be seen from later observations.

Experimental production of stains.

In Malabar, the salt generally used for curing is a mixture of used and unused salts, the used salt commonly containing a large quantity of earth, and some organic matter derived from the hide. The quantity of sodium chloride

in the used salt is very much less than in the fresh, and, consequently, when a mixture is applied there is always danger of there being less salt than is required for effective curing. Moreover, the used salts may contain several types of micro-organisms forming a heavy inoculum and thus leading to rapid stain-formation. To determine whether such was really the case, experiments were conducted with different mixtures of fresh and used salts, and of these with soil. Skin pieces were cured with (1) best grade of bazaar common salt, (2) a mixture containing equal weights of used and fresh salts, (3) a mixture of soil with the best grade bazaar common salt, and (4) a mixture of soil and used salt. The pieces thus treated were kept moist for over two months before examination, when it was observed that none had developed any stain, either in the soaks, or after liming and de-hairing.

In another series of experiments the skin pieces were preserved by immersion in brine instead of rubbing with salt, the cures being made up from best grade bazaar common salt, used salt and soil. The brine was saturated initially, and the skins were kept in the cure for six weeks before examination. Results are given in table III.

TABLE III.

Cure	P_H	Stain
Fresh salt	7.2	Nil.
Fresh salt+soil	7.2	,,
Used salt	6.6	,,
Used salt+soil	6.6	Positive.
Used salt, sterilised	6.4	Nil.

The stains on pieces cured with used salt mixed with soil resembled the natural stains in colour. This observation together with the previous one that a greater amount of iron is present in stained portions than in the corresponding unstained ones led to the inference that iron was one of the constituents of the stains, and that it may have been derived from either the curing salt or the soil.

To determine whether iron only was responsible for the stain-formation, the following experiment was made. The brine (30 and 25 per cent.) was prepared from pure sodium chloride, and 500 c.c. of brine was used for each bottle, to which ferrous sulphate (0.5 per cent.), calcium chloride (0.5 per cent.) and magnesium chloride (0.5 per cent.) were added in different combinations. Skins from freshly slaughtered goats were washed thoroughly, cut in pieces of 2″×3″ or 2″×4″ and kept immersed in these solutions for about four months, when they were examined. Not one showed stains in the soaks, but after liming for three days, the stains came up in all cases where iron was present. De-hairing showed the patterns to be strikingly identical with those obtained in nature.

TABLE IV.

Cure	Stain
NaCl alone	Nil.
$NaCl+FeSO_4$	Positive.
$NaCl+FeSO_4+CaCl_2$	,,
$NaCl+FeSO_4+CaCl_2+MgCl_2$ ..	,,
$NaCl+CaCl_2$	Nil.
$NaCl+CaCl_2+MgCl_2$	,,
$NaCl+MgCl_2$	,,
$NaCl+MgCl_2+FeSO_4$	Positive.

From the above table it will be obvious that calcium and magnesium salts have no influence on stain-formation, and that when iron salts are present in the cure the stains develop even in absence of added calcium or magnesium salts. It may further be adduced that iron which might be present on the skin pieces as hæmoglobin from unwashed blood is not sufficient for stain-production.

The accompanying photographs show the appearance of the artificial as well as the natural stains.

In a fourth series of experiments the pieces were soaked in different cures containing either iron salts or specimens of laterite soil together with sodium chloride (30 per cent.) and examined for stains in the manner described already.

TABLE V.

Brine with	Stains
Laterite soil	+
Laterite+NaF	+
$FeSO_4$	+++
Fe_2O_3	+
$FeSO_4$+water extract of laterite soil ..	++
Fe_2O_3+ ,, ,, ..	+

+, visible ; ++, fairly heavy ; +++, very intense.

Discussion.

That iron salts are responsible for the stain-production has been amply proved by the curing experiments described in the last section. An attempt will now be made to explain the action of iron which is present in the curing salts as an impurity, unknowingly added when the salted skin is weighted with earth. Malabar soil is essentially laterite and is naturally rich in iron oxides, but insoluble iron is not by itself capable of producing a stain. Soluble iron salts alone are also ineffective, so there must be an agency which fixes the soluble iron salts on the hide and transforms them so as to impede their removal by water. The part played by bacteria though not to be overstressed is by no means an unimportant one. Iron present in an insoluble form in the soil and accompanying the curing salt is solubilised by the activity of micro-organisms. Starkey and Halvorson (*Soil Sci.*, 1927, **24**, 381 ; *ibid.*, 1931,

32, 141) in an extensive study on the transformations of iron in nature have found that insoluble iron can be brought into solution and reprecipitated in the form of ferric hydroxide by the activities of micro-organisms other than iron bacteria. They emphasise particularly a very clear distinction, first made by Winogradsky (*Centralbl. Bakt.*, 1922, II, 57, 1), between iron bacteria and organisms which may be associated in the precipitation of iron. They have shown that under ærobic conditions micro-organisms may effect solution of iron by the acid produced, while under anærobic conditions in dextrose or peptone media, micro-organisms may dissolve and reduce iron present as ferric hydrate by decreasing the oxygen pressure and by the formation of acid, even at reactions approaching neutrality. Such conditions are likely to occur in the curing pits or godowns, where a considerable quantity of iron may pass into solution either from the soil or from the cement lining. According to the same authors the subsequent precipitation of iron under ærobic conditions may or may not be dependent upon microbial activity, while at the same time iron combined with organic radicals may become precipitated owing to action of organisms on the radicals; in which case an oxidation before precipitation might not be necessary.

In light of our own observations the process of stain-formation may be pictured as follows:—The micro-organisms find a rich medium in the hide for their growth, and due to their metabolic activities the oxygen tension in the medium is reduced, with simultaneous production of acids which tend to bring the insoluble iron into solution; this then probably combines with the hide substance. Now, accompanying the decomposition of protein matter there is an appreciable production of hydrogen sulphide which might combine with the soluble iron compound to form ferrous sulphide. Ferrous sulphide so formed, does not precipitate itself either in cures or in soaks, because the reaction is slightly acid, *i.e.*, less than P_H 6.6. In lime liquors, however, the reaction becomes sufficiently alkaline to precipitate the ferrous sulphide on the skin. The distribution of the stain on the grain surface is uneven, and may vary from pin points to large continuous patches. In many cases the stain gives an impression of bacterial colonies. The appearance of stains in such different patterns suggests that the solubilised iron becomes localised by the dense mass of hair or the close packing of the hides, which prevents its distribution, and it then combines with the hide substance as explained by Abt (*loc. cit.*). Whether the iron exists merely as ferrous sulphide in the stains, ferrous sulphide adsorbed on the hide substance or combined chemically with the latter, is at present difficult to say, but if iron exists as free ferrous sulphide in solution, it ought to be washed away while the hides are being soaked. Since it is not removed by washing, it is possible that it may have combined with the hide substance, or may have been adsorbed by it.

That stains are caused by ferrous sulphide has been proved. Pieces of sheep-skin were cured in pure brine for a month, when they were washed and limed; on de-hairing the surface was unstained. They were then immersed for three days in a weak solution of ferric chloride from which they were washed free, showing faint yellow patches resembling the colour of the ferric

chloride solution. On remaining several days in lime the patches retained their colour. One piece was kept as control and others were removed to fresh lime containing a very small quantity of sodium sulphide ; immediately the yellow patches became deep blue, much resembling the colour of the stains observed. The evidence thus points to the conclusion that stains may be caused by ferrous sulphide and not by ferric hydroxide.

Suggestions for prevention of stain-formation.

From the experiments described in a previous section it will be apparent that, using identical materials, stains developed only under conditions favouring the various transformations of iron. On the basis of our observations we suggest the adoption of the following procedure to lessen the incidence of stains :—

(1) Curing in pits should be discontinued, or practised with greater cleanliness so as to avoid laterite soil being associated with the hides or the curing salt.

(2) The godowns should be provided with adequate drainage and better ventilation to diminish the humidity.

(3) A better grade of common salt should be used for curing. The salt used in dry operations must be free from iron as well as magnesium salts which impart hygroscopic properties. The application of once used salt adulterated with soil should be discouraged.

These precautions if carefully observed, will, in our view, reduce the percentage of stained hides to a large extent. The question of de-staining has already been discussed (Patwardhan, *Chem. Ind.*, 1931, **50**, 722).

Summary.

The conditions of curing operations in Malabar leading to stain-formation have been described. The experimental evidence so far points to the following with regard to the origin and the nature of the stains :—(*a*) the stains are not due to direct bacterial action on the skin, (*b*) the presence of iron in the cures leads to stain-formation, and (*c*) stains are caused by precipitation of iron in the hide as ferrous sulphide.

A few suggestions for preventing stain-formation have been made.

The work is still in progress and the various problems arising out of the present investigation will form the subject of later communications.

The authors have great pleasure in thanking Prof. V. Subrahmanyan for his many useful suggestions and kindly interest in the work, and Mr. A. Guthrie, Special Officer on Salt Stains with the Madras Government, for supplying extensive information on the curing operations in Malabar and helping with various suggestions. They also thank the Director of Industries, Madras, through whose kindness one of them (M. S. S.) was enabled to take part in the investigation.

[Accepted, 4-12-31.]

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2797-31 Printed at the Bangalore Press, Mysore Road, Bangalore City.