ABNORMAL CONDITIONS IN ANIMAL SKINS WITH PARTICULAR REFERENCE TO THE EFFECT OF BACTERIAL ACTIVITY.

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The manufacture of leather from sheep skins comprises a large and important part of the leather industry. The sheep pelt, that is, the skin plus the wool, is the source of greater revenue than is the pelt of any other domestic animal. We find that in the past decade there has been an average approximate yearly world production of 50,000,000 calf skins, 90,000,000 cattle hides and kips, 120,000,000 goat and kid skins, and 175,000,000 sheep and lamb skins.

The leathers made from sheep skins have always been used for many purposes. Formerly the leather was very poor, but within recent years, improvements in the process of manufacture have produced some of the better types of leathers from sheep skins. But even so, there still remain numerous defects on the finished leather which decrease its value and prohibit its employment in the manufacture of those articles requiring strong and flawless leather. These defects may be on the skin when it is taken from the carcass or they may be damages resulting from the various processes through which the skin passes during its conversion into leather. Such defects as shear marks, tick marks, scabs and other skin diseases, and seeds (the presence of sharp seeds and thorns in the skin) are unquestionably present when the skin is taken from the carcass. On the other hand, broken grain, bacterial damage, buckiness, loss of skin substance, and heat and acid burns result from the manufacturing processes. The origin and cause of such defects as low tensile strength, pin holes, ribs, loose grain, and cockle have long been debated. It is probable, as we shall point out, that each of these is either present when the skin is taken from the carcass or the tendency towards it is evident at that time, in which case it ultimately appears during the process

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of manufacture.

It is particularly necessary to bear in mind the differences which exist between the manufacture and uses of sheep skin leathers and the manufacture and uses of the other major types of leathers. The following points are characteristic of the sheep skin and its conversion into leather:

(1) Economically, the sheep skin itself must be considered as a by-product of the wool industry but as such yields from 20 to 40% of the revenue derived from the entire pelt.

(2) Industrially, it is found that the beamhouse work and pickling are usually separate from the tanning and finishing processes. That is, the former are generally performed at the pullery and the latter at a distant tannery.

(3) A large number of sheep skins are processed without curing.

(4) When curing is carried out by the salting method, the heavy wool coating makes proper curing very difficult.

(5) The ratio of hair (wool) weight to skin weight is relatively high.

(6) Due to seasonal variations in the amount of wool and variations resulting from type of breed, there is a large variation in the area of skin per unit weight of pelt.

(7) Sheep skins contain much lipoidal material.

(8) The inherent weakness of sheep skins as compared to calf skins or goat skins necessitates great care in their handling.

The experimental work of this study has been divided into the following three sections.

I. Histological Examination of Sheep Skins.

II. Bacteriological Examination of Sheep Skins.

III. Soaking of Sheep Skins.

I. HISTOLOGICAL EXAMINATION OF SHEEP SKINS.

Recent studies on the histology of animal skins have shown that the microscopic examination of the skins is a great aid to the leather technologists. Recent researches by Wilson,(1) Kuntzel,(2) Turley,(3) and McLaughlin and O'Flaherty(4) have served to review the procedures and the basic principles of animal skin histology. In the present work, we have made a histological examination of the following abnormalities of sheep skins:

(a) Low tensile strength.

(b) Pinholes.

(c) Rib.

(d) Loose Grain.

(e) Cockle.

(a) Low Tensile Strength.

Wilson(1) and his associates have compared the structure of the sheep skin to the structure of the calf skin by microscopical examination of cross sections. They conclude that "sheep skin cannot be substituted for calf skin, where firmness and substance are desired. The collagen, or leather-forming, fibres of the sheep are extremely thin and not closely interwoven and tend to run parallel to the skin surface, which in itself makes for looseness of texture. Moreover, in the thermostat layer, there are numerous sweat glands and fat cells which leave empty spaces in the finished leather and make it very spongy".

Printer - Insert Figure 1.

Figure 1 tends to verify these conclusions. We see further that there is much less corium proper, in relation to the total thickness of the skin, in the sheep skin than in the calf skin. The

tensile strength of a piece of animal skin is a property primarily of the corium, since the loose, open epidermal system offers very little resistance to stress. This small amount of corium together with its character, that is, short and almost parallel fibre bundles, causes the sheep skin to be inherently weak as compared to calf skins. Furthermore, it is probable that almost any of the various processes through which the skin passes as it is being converted into leather may markedly decrease the tensile strength of the skin.

(b) Pinholes.

A large number of very small "holes" often appear on the grain side of the leather and although these holes do not extend completely through the leather, they do penetrate to a considerable depth and render such leather unsuitable for many purposes, particularly for rollers or skivers. It is generally stated that these pinholes or "open-grain", as the defect is also called, occur more particularly in fine-wooled skins (especially merinos) and are absent in the case of coarse-wooled or hair skins. It has been suggested that the pinholes result from bacterial damage; but if this be the case, it is difficult to understand why the pinholes should appear on only the fine-wooled skins or be confined usually to one particular area of a skin.

The appearance of the grain of any skin is dependent primarily on the hair follicles - their size, arrangement, and number in a given area. The size of the hair follicle is in turn dependent upon the size of the hair root. Consequently, it follows that fine-wooled skins should show a better grain than coarse-wooled skins. But this is seldom the case. Pinholes, ribs, and other defects bring about a course, open-grained appearance to fine-wooled skins.

Printer - Insert Figures 2 and 3.

In Figures 2 and 3, we see horizontal and vertical sections of coarse- and fine-wooled, green-salted sheep skins. We note that in the coarse-wooled skin the individual wool fibres are well isolated but that in the fine-wooled skin, the wool fibres are distinctly grouped. As a result of the drastic unhairing action to which sheep skins are almost invariably subjected, we believe that the small amount of skin substance separating the individual wool fibres of these groups is weakened and that in pulling the wool the narrow band of skin substance is also removed, causing a large hole. Figure 4 shows an enlarged section of a pinhole in a pickled sheep skin. Five or more individual wool fibres are seen to lead directly into the pinhole pictured in the vertical section. Fragments of individual hair follicles may be seen along the edge of several of the pinholes pictured in the horizontal section. Furthermore, it can be pointed out that a large portion of the wool roots remain in the skin - a condition which has been evident in all of the many sections of pickled sheep skins which we have examined.

Printer - Insert Figure 4.

(c) Ribs.

In practically all of the fine-wooled sheep skins, and in some of the coarser-wooled, folds or ridges are noticeable. These ridges often appear as true ribs showing through the skin and hence the term "ribby" has been used to designate such a condition. Ribs may be of two varieties; first, ribs practically invisable on the surface of the skin and evident only when the skin is viewed by transmitted light and second, heavy, raised ridges on the surface of the skin. The first type has been called "blind rib" by the trade and is seen mainly on the neck and shoulders; the second is called "lap rib" and may be on any

part of the skin, usually running in more or less parallel ridges from the back bone out to the flanks.

Printer - Insert Figure 5.

The presence of the ribs has been attributed to "the weight of the wool pulling the structure of the corium out of its normal pattern"(5). Figure 5 shows vertical and horizontal sections through a rib on a green-salted, fine-wooled sheep skin. These sections have been stained with Scharlach R which has the property of staining neutral fats and cholesterin-esters a dark orange. In making the photographs, of these sections, the light filters used were such that orange would photograph black. An extremely heavy deposit of fat is seen at the upper edge of the corium and immediately below the raised or rib part of the skin. Such a fatty deposit was found in the case of both blind rib and lap rib and was evident after each stage of the process examined, that is, from the fresh through to the pickled state. It is believed at the present that the primary cause of blind and of lap ribs is the same, and that lap rib is only an exaggerated case of blind rib. The cause of the deposition of the fat in such ridges is not know and consequently we do not know the primary cause of ribs. It is evident, though, that the weight of the wool pulling on the skin of the living animal is not likely to bring about such a deposition and hence cannot be considered as the sole cause of ribs.

(d) Loose Grain.

Under certain conditions the fat is apparently laid down in a fairly uniform manner at the upper edge of the corium instead of being deposited in ridges. This condition has been investigated to some extent by Wilson and Seymour-Jones(6). The fatty deposit decreases the amount of fibrous material at the junction of the two principal layers

of the skin and hence weak as the skin. Mechanical processes tend to separate the epidermal system from the corium proper because of this weakened state and bring about a condition known to the trade as "loose grain". Figure 6 shows a section of a loose-grained pickled skin stained with Scharlach R so that the fatty deposit may be clearly seen. As in the case of ribs, the cause of this deposition of fat is not understood. It is probable that the amount of fat so deposited will vary with the breed of sheep and possibly be affected by the character of the food.

Printer - Insert Figure 6.

(c) Cockle.

During the winter season, sheep skins from some districts show the presence of dark, raised spots well distributed in no particular order over the grain surface of various parts of the skin but primarily on the shoulders and flanks. These spots, known as cockle spots, become hard and brittle after tanning, refuse to take most dyes, and constitute a serious defect. The most complete study of cockle up to the present time has been the work of Seymour-Jones.(6)

It is most likely that the cockle spots are present in the skin at the time of its removal from the carcass, although it is very difficult to positively demonstrate their presence because of the heavy wool coating. Cockle is best seen in the pickled skin. Figure 7 shows two sections through cockled spots on a pickled skin. When stained with haematoxylin-eosin the cockle spot shows a much greater affinity for the eosin than does the surrounding normal tissue. In the section stained with Scharlach R a congestion of the vessels leading directly to the cockled area is plainly evident. In both sections the cockled area itself shows an intense cellular congestion.

Printer - Insert Figure 7.

Previously it has been thought that the cockle condition was connected in some way with the fatty metabolism of the sheep. A large number of sections of cockled sheep skins stained with Scharlach R, Sudan III, Nile Blue, and Osmic Acid, each of which should stain fat, have failed to show the presence of any abnormal amount of fat in the cockled area.

The significance of these findings is not yet known. Experiments are now under way which it is hoped will throw more light on the exact nature of cockle as well as its cause and possible cure.

II. BACTERIOLOGICAL EXAMINATION OF SHEEP SKINS.

The wool fibres on sheep skins are long, small in diameter and not very close together; hence, as has been pointed out above, the ratio of the weight of the wool to the weight of the skin is high as compared to the corresponding ratio for either steer hides or calf skins. Furthermore, this heavy wool covering furnishes a large surface suitable for the accumulation of bacteria and materials such as manure which are favorable for bacterial growth. Hence it is not surprising that we find fresh sheep skins heavily contaminated. Quantative examinations of a number of fresh sheep skins show from 2,000,000 to 200,000,000 viable organisms per gram of pelt of which well over 95% are usually found on the wool. The large variations in the number of organisms on different pelts is probably due to the wide variations in the amount of the wool and the foreign matter it contains.

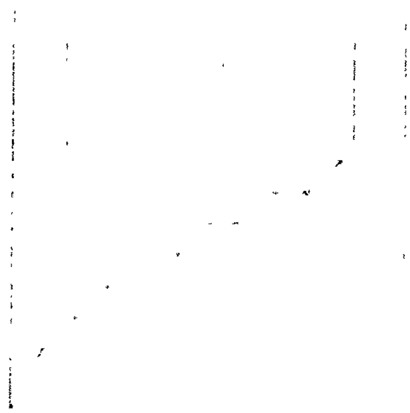
Sheep skins are cured by placing the skins in a pile, flesh side to wool side, with a layer of salt (NaCl) between the skins. The heavy mat of wool prevents the salt from coming into proper contact with the grain side of the skin and a poor cure results as has been shown by McLaughlin and Rockwell.(7) The existing method of curing leaves much to be desired but the present study has not been concerned with this particular problem. Salted sheep skins show even a greater variation in flora than do fresh pelts. From 150,000 to 260,000,000 viable organisms per gram of pelt have been found on green-salted skins. A single pelt has shown a variation as great as 230,000,000 organisms per gram on different areas. The average inoculation of fresh skins is much higher than that of salted skins.

The organisms on sheep skins are similar in character to those on steer hides and calf skins as reported in previous papers.(8) There seems to be a greater number of chromogenic organisms on sheep skins than on other skins. The growth of mixed cultures from both fresh and green-salted sheep skins in the presence of salt always follows very closely the curve shown in Figure 8. The variation in the salt tolerance of mixed cultures from the two types of skin which was evident in the case of steer hide, as discussed previously by McLaughlin and Rockwell,(9) was not evident in the case of sheep skins. Only new salt is generally used in the curing of sheep skins and organisms on salted sheep skins have not become tolerant to salt. The method for making the salt broths for the determination of salt tolerance may be found in a previous paper.(10)

Printer - Insert Figure 8.

III. SOAKING OF SHEEP SKINS.

The practice of soaking, the first process to which sheep skins are subjected in the pullery, has never been standardized. The charact-er and duration of the soak has been dependent upon those particular



conditions which exist at a pullery and the judgment of the pullery superintendent. A plant situated on the sea coast may from necessity use sea water for soaking; a plant may have deep artesian wells as their water source and thus maintain a low temperature in the soaks; chemicals, such as NaCl, may be added to the soak water at the discretion of the superintendent; the soak water may be changed several times during the soaking period or not at all; the soaks may be "still soaks" or the skins may be reeled during part of the soak; no uniformity exists as regards the amount of water used for a definite weight of skins; the duration of soaking varies; and, finally, pulleries are at a loss how to change the soaking process when working fresh, green-salted, or dried skins.

Each of these factors affect bacterial activity in the soaks. When the bacteria multiply, the skin proteins serve as one of the sources of foodstuffs for the organisms. Consequently, bacterial growth in the soaks will probably damage the skin. Previous experiments on the soaking of animal skins (11) showed that, from a bacteriological standpoint, the maximum soaking period should not be longer than the minimum lag period. It will be remembered that the lag period has been defined as that period which elapses between the time when the skins are put into soak and the time when bacteria begin to actively multiply. Skins are soaked in order to accomplish certain chemical and physical objects, such as, removal of curing salts, albuminous substances, blood and dirt, and softening and hydration of the skin. It may be found that under existing methods of soaking, the objects are not accomplished until long after active multiplication of the bacteria begins, that is, the minimum lag period is exceeded. If such conditions exist, the soaking procedure should be altered so as to increase the lag period and thus accomplish the desired physical and chemical changes within the lag period.

The factors, affecting bacterial growth, which may be altered in order to increase the lag period and which will be considered in subsequent pages, are:

(a) Temperature.

(b) Character of Soak Water.

(c) Proportion of Skin to Water.

(d) Milling.

(e) Change of Water.

(f) Delayed Soaking of Fresh Skins.

The bacterial growth has been followed by the dilution plate method of counting. We realize the inadequacy and inaccuracy of this method as has recently been brought out by many investigators, particularly Wright and Thornton (12) and Mudge and Lawler. (13) But we have chosen this method, since we feel that it is the most suitable of any of the available methods known and we have taken as many precautions as possible to insure uniformity in the counting procedure. Nevertheless, the following objections to this method must be borne in mind. First, when a soak water contains from 1,000 to 100,000 organisms per c.c., this method will probably give a much truer picture of the conditions as they actually exist than when there are present several million organisms per c.c. Second, this method gives only the living organisms present in the soak water at a given time, but we lose sight of those organisms which have multiplied and used up skin proteins as food and have died previous to the time of making the count. Third, the method makes no attempt to determine the number of obligatory anaerobes present.

Bearing in mind the above objections, as well as the fact that the original inoculation in the soaks varies over a wide range, how may we best interpret the data obtained? Let us assume we are investigating two soaks of sheep skins, A and B. Soak A shows an original inoculation of 1000 organisms per c.c. of soak water and soak B, 2000 organisms per c.c.; otherwise all conditions are the same. At the end of four hours, we find 2000 organisms per c.c. in soak A and 4000 organisms per c.c. in B; at the end of six hours, 4000 in A. and 8000 in B. How do the two soaks compare in respect to growth? If we disregard the original inoculations and consider only the number of organisms present after six hours soaking, we would erroneously conclude that there had been twice as much growth in B as in A. If, however, we say that the organisms have undergone one generation when the total number doubles, we see that in both soaks there have been one generation at the end of four hours and two generations at the end of six hours, and hence the rates of growth in the two soaks are the same. Thus, if we calculate the total counts as generations, we may compare the rates of growth of two soaks independently of the original inoculations and consequently we are able to observe the effect of any particular factor upon the bacterial activity of a soak.

But conclusions drawn from the rate of growth alone may also be erroneous. In the example cited above, even though the rates of growth are the same, more damage would be done to the skin by soak B than by soak A, since there have been twice as many new organisms formed in soak B than in soak A. Consequently, it cannot be too strongly emphasized that the number of generations, the number of organisms taking part in a generation, and the character of the organisms must each be considered before conclusions can be drawn.

There are a number of factors, which influence the increase of the number of the organisms in the soaks, and which cannot be arbitrarily controlled by the experimenter. When all conditions of growth are held constant, the rate at which any one particular species of organisms multiplies is probably an inherent characteristic of that organism, that

TABLE I.

Showing the Effect of Temperature on the Rate of Growth of Soak Water

Organisms 1 Part Skin Soaked in 10 Parts Distilled Water.

Temperature	Original Inoculation per Gram of Skin	Generations after				Gms. Nitrogen per 100 c.c. 48 hr. Soak Water
		12 hrs.	24 hrs.	36 hrs.	48 hrs.	
10° C. (40° F.)	3,300,000	0.6	1.6	2.3	4.8	0.0347
20° C. (68° F.)	2,600,000	1.6	4.4	6.1	7.5	0.0371
30° C. (86° F.)	1,200,000	3.7	7.3	7.5	7.3	0.0545
37° C. (98° F.)	3,000,000	5.8	7.3	7.8	6.6	0.0918

is, two species of organisms under identical environmental conditions may multiply at different rates.

Various species of organisms require various types of food. Waste products from the growth of one particular species may serve either as a foodstuff or as a poison for a second species and hence the rate of growth of the second species will be stimulated or retarded by the growth of the first. Growth is dependent to some extent upon the concentration of available foodstuff per organism and as this concentration decreases, within limits, as a result of the multiplication of the organisms, the rate of growth also decreases.

(a) Temperature.

One of the characteristics of any species of organism is that there is an optimum temperature for its growth, i.e., a temperature at which its rate of reproduction reaches a maximum under otherwise constant conditions of environment. As the temperature is raised above this optimum, the rate of reproduction decreases until a point is reached at which the organisms are actually killed - the thermal death point of the organisms. Below the optimum, a decrease in temperature decreases the rate of reproduction.

The effect of temperature upon the rate of multiplication of organisms in the soaking of green-salted sheep skins is shown in Table I and Figure 9. These data represent the average of over fifty determinations.

Printer - Insert Table I and Figure 9.

Since in this particular case the average original inoculations were all of the same order with the possible exception of the 30° C. soak, we may compare the growth rates themselves, disregarding the original inoculation. We see that at the end of 12 hours soaking, the 10^{o} C. soak had not yet completed one generation. In each of the other soaks, the growth exceeded one generation at the end of 12 hours. At 30^{o} C., the rate of growth is almost as great as at 37^{o} C. During the later hours of the soaking period in the case of both the 30^{o} C. and 37^{o} C. soaks, the death rate exceeded the rate of multiplication and hence the curves drop.

Temperatures of 20^{o} C. (68^{o} F.) and even higher, at certain periods of the year, are frequently found in pullery soak pits. Green-salted skins are often soaked at this temperature for as long as forty-eight hours. Under such conditions, it is seen that the lag period is far exceeded. Sheep skin pullers have pointed to the fact that if shorter soaking periods are used, the pulling process cannot be properly carried out. It is doubtful if this is the case, but if so, it is likely that the fault lies with the depilating process and not with the soaking.

Experiments on the effect of temperature on the soaking of sun-dried skins tend to show results very similar to those for the soaking of green-salted skins. In the case of the former, the rates of growth exceed slightly the rates of growth for salted skins which results no doubt from the retarding effect of the small amount of salt in the soak waters in the latter type of soak. This slight increase in the case of fresh skins is probably of considerable importance when we consider the fact that the original inoculation of fresh skins is considerably greater than that of green-salted skins. The soaking of dried skins is a separate problem, necessitating a different line of attack and will be more thoroughly studied in later work.

(b) Character of the Soak Water.

There are a number of pulleries situated close to a supply of sea water and use this water for soaking. The advisability of such a practice has been debated for some time. A number of factors, both chemical and bacteriological, come into play in such a case and it is not an easy question to answer.

From a bacteriological point of view, we know that micro-organisms require for their metabolism small quantities of such elements as phosphorous, sulphur, calcium, potassium, magnesium and iron, in addition to carbon, hydrogen, oxygen and nitrogen. Small concentrations of these elements, in various salt combinations, stimulate growth, but larger concentrations retard it. All of these elements are found to a greater or less extent in sea water. But one could not prophesy just what effect the sea water would have upon the growth of the organisms present in the soak. If samples of skins are soaked in sea water along with distilled water controls and quantitative examinations of the bacterial content of the soak waters made, we may determine the effect of sea water on the rate of multiplication of the organisms present.

Printer - Insert Table II and Figure 10.

Table II and Figure 10 show the average results of a large number of such experiments, using green-salted sheep skins. We see that sea water slightly retards bacterial growth throughout the entire soaking period. The amount of total nitrogen in the forty-eight hour soak water is slightly less in the case of sea water - a further indication of less growth. The sea water used in these experiments contained 3.45 gm. total solids per 100 c.c. and a total chlorine content equivalent to 2.98 gm. NaCl per 100 c.c. The distilled water soaks

TABLE II.

Showing the Effect of the Use of Sea Water on the Rate of Growth of Soak Water

Organisms. 1 Part Skin Soaked in 10 Parts Water at 20° C. (68° F.)

	Inoculation per Gram of Skin	Generations after				Gms. Nitrogen per 100 c.c. 48 hr. Soak Water.
		12 hrs.	24 hrs.	36 hrs.	48 hrs.	
Distilled Water	2,600,000	1.6	4.4	6.1	7.5	0.0371
Sea Water	1,600,000	1.2	5.5	5.6	7.5	0.0351

contained an average of 1.5 gm. NaCl per 100 c.c. at the end of the soaking period resulting from the solution of the curing salt.

(c) Proportion of Skin to Water.

As we increase the amount of water in which a given weight of green-salted sheep skin is soaked, we likewise increase the total amount of salt and dissolved nitrogenous materials; but nevertheless, the concentrations of both the salt and the nitrogenous materials as well as the number of organisms per cubic centimeter is decreased. By thus decreasing the concentration of the salt in the soak water, we have removed a factor which would have retarded growth. Likewise, a decrease in the concentration of nitrogenous materials available for food may retard growth. But it is possible that even at the lowest concentration of nitrogen substances (the greatest proportion of water to skin), there is sufficient foodstuff available to permit maximum growth. Also, it has been found that in very heavily inoculated soak waters - as may occur when poorly cured sheep skins are soaked in small amounts of water - the rate of multiplication, as determined by measuring the bacterial content of the soak waters at different times, appears to decrease. This results, no doubt, from an increased death rate (due, doubtless, to the accumulation of toxic products of bacterial metabolism) rather than a decreased rate of growth.

Because of the bulkiness of the heavily wooled skins, a larger proportionate amount of water is necessary to completely cover sheep skins than is necessary in the case of heavy hides or calf skins. For laboratory experiments, it was found inadvisable to use less than five parts of water to one part of skin (by weight) and in some cases this did not completely cover the skins.

Printer -- Insert Table III and Figure 11.

TABLE III.

Showing the Effect of Varying the Proportion of Skin to Water on the Rate of Growth of Soak Water Organisms at 20° C. (68° F.)

Proportion of Skin to Water (by Weight)	Original Inoculation per c.c. of Soak Water	Generations after				Gms. Nitrogen per 100 c.c. of 48 hr. Soak Water	Gms. Nitrogen per 100 gm. of Skin
		12 hrs.	24 hrs.	36 hrs.	48 hrs.		
1 to 5	890,000	1.2	5.2	7.2	8.1	0.0896	0.4480
1 to 10	260,000	1.6	4.4	6.1	7.5	0.0452	0.4520
1 to 15	370,000	1.2	5.1	6.3	7.9	0.0322	0.4830

Table III and Figure 11 show the effect of varying the proportion of skin to water on the rate of growth of the soak water organisms. It is seen that in the early hours of soaking the growth is less in the case of a 1 to 5 soak and 1 to 15 soak than in the 1 to 10 soak. After fifteen to eighteen hours the growth is least rapid in the case of the 1 to 10 soak. These variations in the rate of growth result from the varying percentages of salt and available foodstuffs as explained above. It is further seen that while the concentration of nitrogen in the soak water varies in the order expected, that is, the least in the 1 to 15 soak and the most in the 1 to 5 soak, the nitrogen loss per gram of original skin remained practically constant. We understand that the majority of pulleries use a ratio of skin to water even less than 1 to 5.

(d) Milling.

The practice of milling sheep skins followed by changing the water at the beginning of or during the soaking period varies in the different pulleries of the United States. Changing the water during the soaking of green-salted sheep skins, whether preceded by milling or not, serves to remove a large number of bacteria and much of the salt. The operation is probably more efficient in this respect if milling precedes the change. However, it is probable that milling may cause the wool to mat and consequently be considered poor practice.

The efficiency of the milling process as regards its removal of bacteria from the skin will be dependent upon a number of factors, as for example, speed of milling, amount of agitation given to the skins, proportion of skin weight to water weight, character of the soak water, etc. Hence, the value of milling must be determined according to each individual case. In the laboratory, we milled 50 gm. pieces of skins with 500 c.c. of sterile distilled water in quart jars, rotated at an average speed of 75 R.P.M. We have examined two factors in the milling process. First, how long should the skins be milled? And, second, what is the value of milling well cured skins (small original inoculation) as compared to the value of milling poorly cured skins (high original inoculation)?

To determine the rate at which organisms were removed from the skin during milling for various lengths of time, pieces of green-salted sheep skin were milled as above for ten minutes and the soak water counted. The pieces were immediately milled again for ten minutes in the same soak water and the water counted again. This process was repeated a third time. The entire operation was carried out in less than one hour so that no appreciable growth could have occurred. If the number of organisms in the soak water after the first ten minute milling is taken as 100%, the percentage at the end of a total of twenty minutes milling was found to be 119 and after thirty minutes milling 131.

To determine the value of milling for well and poorly cured skins, pieces of skin were milled for ten minutes, the water counted and then discarded, an equal amount of sterile distilled water added, milled again for ten minutes, and counted. The count after the second milling divided by the sum of the two counts is taken as the percentage remaining after the first milling. Table IV shows the results of such an experiment.

Printer - Insert Table IV.

From Table IV it will be seen that as the original inoculation increases the percentage remaining after a ten minute milling decreases.

TABLE IV.

Showing the Effect of Milling and Changing the Water on the Number of Organisms Remaining in the Soak. 1 Part of Skin in 10 Parts of Water.

Inoculation per c.c. after 1st Milling	Inoculation per c.c. after 2nd Milling	Percent Remaining after 1st Milling
68,000	62,000	47.7
88,000	38,000	30.0
99,000	40,000	28.0
270,000	90,000	25.0
274,000	85,000	23.7
6,800,000	460,000	6.5

Furthermore, we see that regardless of large variations in the original inoculation, the number of organisms remaining after milling and changing the water is fairly constant. This would indicate that milling may be particularly advantageous when working poorly cured skins which have a high original inoculation.

(c) Changing the Water During Soaking.

The majority of pulleries find it advisable to change the soak water from one to four times during the soaking period. This means that a single soak water may remain in contact with the skins only eight hours or it may not be changed until after twenty-four hours contact with the skins. It has been shown above that, after the first change of water, which was necessarily preceded by a 10 minute milling,(x) the bacterial counts of the second soak waters are all of the same order independent of the original inoculations. Hence, in this case we may make a comparison of the bacterial activity of the soaks by comparing the total increase in the number of organisms in a soak in which the water is changed, to the increase in the number of organisms in a soak of the same length of time but in which the soak water is not changed. A soaking period of forty-eight hours was chosen. A soak in which there was no change in the soak water was taken as a control for the comparison of soaks with one change of water after twenty-four hours, and soaks with three changes of water, that is, once every twelve hours. The soak waters were counted at the beginning, immediately before, and immediately after each change. Thus we were able to determine the total increase in the number of organisms during each period. The results of such an experiment on green-salted sheep skins are shown in

(x) A ten minute milling previous to counting was a uniform procedure throughout all of this work.

Table V.

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It is seen from Table V that changing the water materially decreases the total growth during soaking. This results from several factors. The total number of organisms present is of course decreased considerably after the first change of water. The rate of reproduction is decreased during the early hours which follow each change - a condition which at present is not completely understood. The concentration of nitrogenoous materials in the soak water is decreased by changing the water. However the total loss in nitrogen is increased when the water is changed as is shown in Table VI. This excess in nitrogen loss results from chemical solution of skin proteins and not from excessive bacterial growth.

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(f) Delayed Soaking of Fresh Sheep Skins.

The sheep skin industry is the only major branch of the entire leather industry which processes a large number of skins without previous curing. In processing fresh skins we meet an additional factor which influences bacterial growth and which has not been considered in our study of the soaking of green-salted skins. Conditions in the industry necessitate the lapse of an interval of time varying from one to twenty-four hours between the flaying of the animal and the time when the skin is put into soak. As soon as the animal is killed, its skin may be attacked by bacteria. Placing the skins in soak water does not prevent this attack by bacteria, but the temperature of the skins is reduced and they are kept from direct contact with the air,

TABLE V.

Showing the Effect of Changing the Water on the Rate of Growth of Soak Water Organisms. 1 Part of Skin Soaked in 10 Parts of Water at 20° C. (68° F.)

	Increase in Millions of Organisms per c.c. of Soak Water during							Total Increase
	First 12 hours		Second 12 hours		Third 12 hours		Fourth 12 hours	
No Change	7.3		11.1		34.6		14.5	67.5
One Change	7.3		11.1	Water changed	8.7		9.9	37.0
Three Changes	7.3	Water changed	5.1	Water changed	0.6	Water changed	4.4	17.4

TABLE VI.

Showing the Effect of Changing the Soak Water on the Nitrogen Loss During Soaking. 1 Part of Skin Soaked in 10 Parts of Water at 20° C. (68° F.)

	Grams Nitrogen per 100 c.c. Lost after Soaking for			
	12 hrs.	24 hrs.	36 hrs.	48 hrs.
No Change				0.0403
One Change		0.0443		0.0525
Three Changes	0.0367	0.0450	0.0490	0.0514

both factors tending to decrease bacterial growth. Since at the present time we can see no advantage in allowing bacterial growth to take place, it would seem advantageous to put the skins to soak as soon as possible after their removal from the carcass.

Experimentally, it is very difficult to determine just how much growth takes place in an interval of time which the skins may stand before soaking. With our present method of counting, it is necessary to immerse the skin in water, mill a definite length of time and count the number of organisms in the soak water. Thus it is impossible to obtain counts on the same piece of skin both before and after allowing it to stand. But if we determine the number of organisms present on different pieces of skin at varying intervals of time following flaying, we may find that there are fewer bacteria on the skin after allowing it to stand for several hours than there were at the beginning - a condition which in itself would indicate that the death rate had exceeded the rate of multiplication. It is likely, however, that in such a case, those samples which were counted in the late hours had fewer organisms originally. When pieces are chosen at random there is no uniformity to the original counts and if several determinations are made and the results averaged, we may gain some knowledge as to what happens when soaking is delayed. Such data are presented in Table VII. The skin samples were held at 65° F. to 75° F. previous to soaking.

Printer - Insert Table VII.

It is seen from Table VII that if the skin is allowed to remain exposed to the air for seven hours after it is taken from the carcass and before being soaked, as frequently happens in industry, there are nearly twice as many bacteria on the skin as were originally

TABLE VII.

Showing the Effect of Delayed Soaking on the Number of Organisms on
Fresh Sheep Skins at the Beginning of and During Soaking
1 Part Sheep Skin Soaked in 10 Parts Water at 20 ^{o}C. (68 ^{o}F.)

Counts in Ten Million Viable Organisms per Gram of Skin

Hours Delayed	1	3	5	7	23
Count on Entering Soak	9.56	9.28	12.28	15.23	55.00
Count after 48 hrs. Soaking	99.00	99.00	96.00	125.00	178.00

present. Likewise, the number of organisms in the soak water at the end of the soaking period is materially increased.

CONCLUSIONS AND SUMMARY

The most common and troublesome abnormalities in sheep skins are cockle, ribs, low tensile strength, pinholes and loose grain. In each case, either the defect itself or the tendency towards it seems to be present in the skin when taken from the carcass. Cockle is a cellular and vascular congestion, the cause of which is as yet unknown. The ribs are characterized by a heavy deposition of fat at the upper edge of the corium. A similar deposition of fat which, however, is not laid down in ridges, is evident in skins showing loose grain. The presence of this fatty layer weakens the skin at the junction of the corium and the epidermal system, and the mechanical working of the skin brings about loose grain. Pinholes develop during the unhairing process when wool fibres are removed in clumps instead of singly. The tendency towards low tensile strength results from the small amount of fibrous material and large open glandular spaces present in normal sheep skins and may be aggravated by any of the beamhouse or tannery processes.

Sheep skins are heavily contaminated with bacteria. Excessive growth takes place in the majority of pullery soak pits and probably damages the skin. A low temperature, the use of sea water, and changing the soak water at frequent interval decreases the amount of bacterial growth during soaking. Milling previous to changing the water increases the number of organisms removed. Fresh skins should be put into the soak water as soon as possible after the skin is removed from the carcass so that bacterial growth will be minimized.

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