THE PHYSIOLOGY OF BACTERIA IN RELATION TO THE SOAKING OF GOAT SKINS.

Thesis for the Degree of
Ph. D.

from

The University of Cincinnati,

June, 1926.

By

JOHN HENRY HIGHBERGER

Taft Fellow in Bacteriology,

Department of Leather Research,

University of Cincinnati.

CONTENTS.

INTRODUCTION.

Tanning, or the process of making leather from the skins of animals, is perhaps one of the oldest arts in the history of humanity. It had its birth when man discovered that the skins of the animals which were his victims in the chase were of no use to him unless they were treated in some way to preserve them. Although of such ancient origin, the leather industry has progressed but slowly, and has been peculiarly reluctant to make use of the applicable parts of our modern scientific knowledge. It is only within comparatively recent times that the art has been anything but an empirical, rule of thumb process.

While a superficial consideration may not reveal the fact, the process of tanning is nevertheless one which is peculiarly concerned with bacteriological problems. The very object of the procedure, which is to so modify the skin that it will not be destroyed by micro-organisms, is an expression of an empirically realized bacteriologic fact, namely, that certain bacteria have the power of digesting proteins. If there were no proteolytic bacteria, it would hardly be necessary to subject hides to such elaborate procedures in order to make them usable. The whole question of tanning, then, is seen to be resolved into the one fundamental bacteriological problem which may be expressed: to what degree is it necessary to modify hide substance in order to make it absolutely resistant to bacterial action, and how may this be accomplished with the retention or improvement of the elasticity and impermeability of the skin? These considerations of course take no account of the more mechanical problems of suitably modifying the density or porosity of the hide.

But this is not the only role that bacteria play in tanning. All through the process, from fresh hide to finished leather, micro-organisms of one sort or another constantly endanger the quality of the product. It is possible that in certain stages their activities may be helpful rather than injurious. We do not know with any degree of exactitude, for instance, what part the soluble bacterial enzymes play in the so-called "mellow lime liquors."

In all these stages of the process a knowledge of the physiology of bacteria may be expected to assist in controlling their activities, with consequent improvement of the quality of the finished leather. Our knowledge of the physiology of bacteria is by no means in an advanced state, but certain laws and principles have been evolved which give us the power of controlling to a large extent their growth and activities. It is the purpose of this study to investigate the bacterial phenomena of a particular phase of the conversion of goat-skins into leather, namely, the soaking process, and to show how these phenomena are affected by the application of the above-mentioned principles.

I.

THE SKINS BEFORE SOAKING.

A. The Types of Goatskins.

Before investigating the bacterial phenomena of the soak, it is necessary to examine the condition of the skins as they are received at the tannery. Most of the goatskins tanned in this country are imported, and it is therefore necessary that they be cured in some way before shipment. The manner in which this curing is carried out has a great bearing upon the bacterial growth in the soak, as will be shown later.

Depending upon the method used in curing, goatskins may be classified in three main groups, which are: (1) Sun Cured, (2) Dry salt cured, and (3) Green salt cured. In the following work examples of each class were investigated, namely:

1. Sun cured skins.
2. Dry salt cured skins.
3. Green salt cured skins.
    a. Deccan skins.
    b. Cawnpore skins.

The first two classes are stiff and brittle, and in a highly dehydrated condition. Both of these types of skins are dried in the sun, but thd dry salt cured skins are rubbed with a native salt (Khari Salt) which consists chiefly of sodium sulphate. The two types of green salted skins examined are very similar. They come from the districts of India indicated by the names. The salt used in curing these skins is largely sodium chloride, as the material scraped from their surfaces contains about 50 percent of this salt.

When ten-gram pieces of each of these skins are shaken for ten minutes in two hundred cc. portions of sterile distilled water, and the water then examined for those factors which influence bacterial growth, the results shown in Table I are obtained. The figures represent the average of sixteen different skins of each kind, many times repeated. It should be noted that the number of bacteria varies widely from skin to skin, but the figures represent very approximately the comparative numbers on the different types.

Table I.

Type of Skin.	Bacteria per gram of Skin.	Grams Surface Nitrogen per 100 cc.	Grams NaCl per 100 cc.	pH	Percent Moisture in Original Skin.
Sun Cured	91,000	0.00748	0.0283	7.21	12.00
Dry Salt Cured	4,763,000	0.00715	0.0465	7.44	12.40
Deccan Green Salt Cured	379,000	0.0493	1.2160	7.24	30.74
Cawnpore Green Salt Cured	133,000	0.0481	0.8095	8.65	31.09

It is seen that the dry salt cured skins are the most heavily contaminated with bacteria; the sun cured skins the least, while the green salted skins occupy an intermediate position. The soaks of the green salted skins contain far more nitrogen and sodium chloride than those of the dry cured, as is to be expected. The reason for the sun cured skins being the least contaminated is obvious, since they are the most highly dehydrated, and since the direct rays of the sun are among the most affective bactericidal agents known. The question arises, however, why the dry salt cured skins, which are subjected to the same general treatment, should be so heavily contaminated. A bacteriological examination of the salts used in curing the skins gives interesting information.

Table II.

Bacterial Content of the Salts Used in Curing Goatskins.

Type of Salt.	Bacteria per Gram of Salt.
Khari Salt (Unused)	297,800
Cawnpore Salt (Used)	121,600
Deccan Salt (Used)	152,400

It should be noted that the Khari salt was a fresh sample, while both the Cawnpore and Deccan salts were those scraped from skins. It thus appears that the native salt used on the dry salted skins is itself heavily contaminated with bacteria, and when it is rubbed into the skins in the process of curing, the latter are actually inoculated with millions of bacteria which were not present originally, and which, furthermore, are well adapted to growing in the presence of the salt. Many of these organisms are actively proteolytic, as is shown by the fact that when coagulated blood serum slants are inoculated with mixed cultures from the salt, the coagulated protein is almost completely digested in the course of 48 hours at 37°C.

This particular salt (Khari), as noted above, consists chiefly of sodium sulphate, which, aside from its dehydrating capacity, has practically no toxicity for skin bacteria. This is shown in Figure 1, in which is plotted the growths of mixed cultures from sun and dry salt cured skins in sodium sulphate broth at 37°C. It is seen that growth occurs in one day through 10 percent of anhydrous sodium sulphate, and in three days through 16 percent, the limit of solubility. It is evident that sodium sulphate inhibits growth, other than by its dehydrating action, very slightly, if at all.

It seems not improbable that the sodium sulphate actually exerts some protective influence on the skin bacteria, by reason of its great dehydrating capacity. It is well known that the increased resistance to destructive influences of spores over vegetative forms is to be attributed largely to their dehydrated condition. It seems reasonable to assume that the sodium sulphate exerts its dehydrating power upon the skin bacteria, as well as upon the skin itself. If such is the case, one would expect that the bacterial flora of skins treated in this way would be more resistant to destructive agencies, such as sunlight, than that of other skins not so treated. Attempts to demonstrate this experimentally, however, gave negative results, owing to the lack of a suitable method. One experiment may be cited, however, as it demonstrates in a striking way the intense germicidal power of the sun's rays. Three tubes were prepared, containing ten ccs. of 15 percent solutions of the following salts: sodium chloride, magnesium sulphate and sodium sulphate. These were sterilized, together with one of physiological salt solution, and each of the four was inoculated with 1,200,000 Staphylococci per cc. They were then

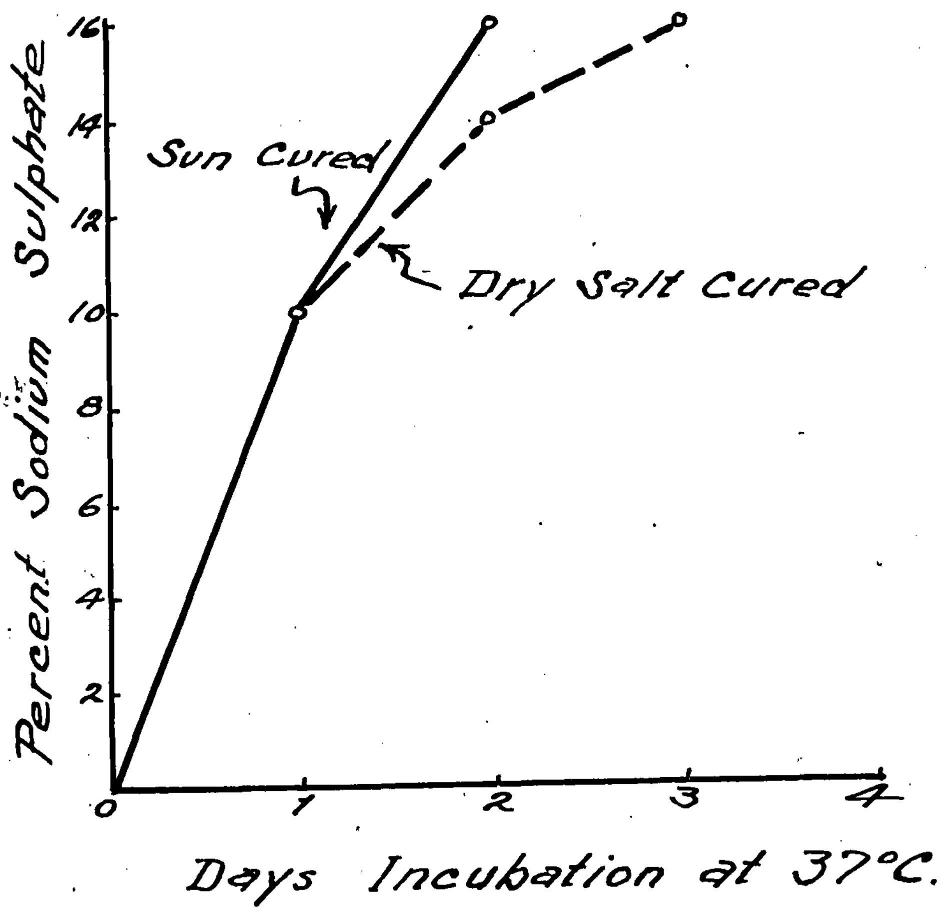

Percent Sodium Sulphate
16
14
12
10
8
6
4
2
0
1
2
3
4
Sun Cured
Dry Salt Cured
Days Incubation at 37°C.

Fig. I.

exposed to the direct rays of the sun for two hours, at the end of which time they were all found to be sterile.

Aside from the bacteriological evidence presented above, certain technical difficulties contra-indicate the use of sodium sulphate in the curing of skins. The precipitation of calcium sulphate in the skins during liming may be cited as an example.

B. The Bacterial Flora of Cured Goatskins.

While it was not considered profitable to attempt to identify all of the bacteria found on the skins, certain strains were isolated and studied. Brief descriptions of a selection of these which may be considered as fairly representative of the bacterial flora of goatskins are given below.

Strain 1. Isolated from sun cured goatskin.

Agar colony: White, spreading, branched, viscid.

Morphology: Slender Gram negative bacillus, rather large; many forms slightly curved. Rounded ends. Occurs singly and in pairs. Actively motile.

Cultural: Uniform turbidity in broth. Blood serum rapidly liquefied. Obligate aerobe. Non-fermentative.

Strain 2. Isolated from sun cured goatskin.

Agar colony : Round, white.

Morphology: Short, thick Gram negative bacillus, occurring singly, in pairs and short chains. Rounded ends. Spores sub-terminal, smaller than rod. Actively motile.

Cultural: Broth turbid with some sediment. Blood serum rapidly liquefied. Obligate aerobe. Dextrose and sucrose fermented with acid production.

Strain 9. Isolated from dry salt cured goatskin.

Agar colony: Round, lemon yellow.

Morphology: Short, thick Gram negative bacillus. Many forms almost coccoid. Occurs singly and in pairs.

Cultural: Uniform turbidity in broth. Blood serum not liquefied. Non-fermentative. Culturally inert. Obligate aerobe.

Strain 10. Isolated from sun cured goatskin.

Agar colony: Spreading, branched.

Morphology: Slender Gram negative bacillus of variable length. Square-cut ends. Arranged in a parallel fashion. Occurs singly and in pairs.

Cultural: Uniform turbidity in broth, with later pellicle formation. Blood serum liquefied. Obligate aerobe. Non-fermentative.

Strain 11. Isolated from sun cured goatskin.

Agar colony: Round, lemon yellow.

Morphology: Very short Gram positive bacillus. Arrangement very similar to Staphylococcus.

Cultural: Broth uniformly turbid. Blood serum not liquefied. Non-fermentative. Culturally inert.

Strain 13. Isolated from sun cured goatskins.

Agar colony: White, round, raised.

Morphology: Medium sized, slender Gram positive bacillus. Rounded ends. Some long filaments. Occurs singly and in pairs. Spores sub-terminal, same diameter as rod.

Cultural: Broth turbid with heavy pellicle. Blood serum liquefied. Facultative aerobe. Dextrose and sucrose fermented with acid production and reduction of litmus.

Strain 14. Isolated from sun cured goatskin.

Agar colony: Irregular, fimbriate.

Morphology: Medium sized Gram negative bacillus. Rounded ends.

Cultural: Broth uniformly turbid, becoming clear with heavy pellicle formation. Blood serum liquefied. Obligate aerobe. Non-fermentative.

Strain 1CG. Isolated from Cawnpore green salted goatskin.

Agar colony: Round, raised, irregular. Yellowish, opaque with darker center.

Morphology: Gram positive bacillus, rounded ends. Spores central, same diameter as rod. Occurs singly, in pairs and short chains.

Cultural: Uniform turbidity in broth. Blood serum rapidly liquefied. Obligate aerobe. Indol negative. Ferments dextrose and sucrose with acid production.

Strain 2CG. Isolated from Cawnpore green salted goatskin.

Agar colony: Irregular, raised. Center opaque and structured.

Morphology: Gram positive bacillus, rounded ends. Occurs singly, in pairs and chains. Spores terminal to sub-terminal, same diameter as rod.

Cultural: Broth turbid with heavy pellicle. Blood serum liquefied. Indol negative. Facultative aerobe. Dextrose, maltose and sucrose fermented with acid and slight amount of gas.

Strain 3CG. Isolated from Cawnpore goatskin.

Agar colony: Flat, spreading, fern-like. Opaque and creamy.

Morphology: Gram positive bacillus with rounded ends. Occurs singly. Spores sub-terminal, same diameter as rod.

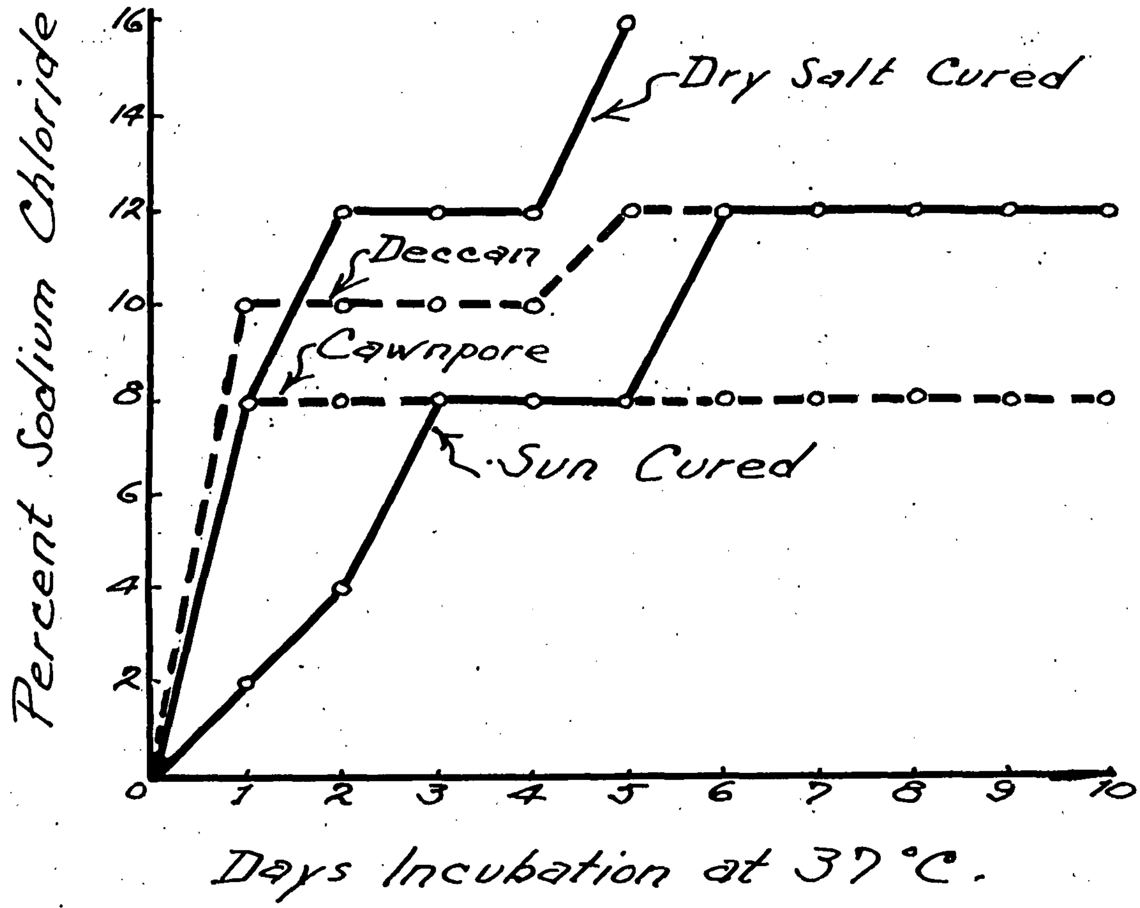
Cultural: Uniform turbidity in broth. Blood serum liquefied. Indol negative. Obligate aerobe. Ferments dextrose with slight acid production.

Strain 4 CG. Isolated from Cawnpore green salted goatskin. Agar colony: White, irregular, flat, spreading, cottony. Morphology: Large Gram positive bacillus occurring in chains. Ends slightly rounded. Spores sub-terminal, same diameter as rod. Cultural: Broth uniformly turbid. Blood serum liquefied very slowly. Indol positive. Facultative aerobe. Dextrose and maltose fermented with acid production.

In considering as a whole the flora of the skins, the points that are important are: (1) their salt tolerance, and (2) the relative proportion of their number which are proteolytic.

In Figure 2 is shown the growths of mixed cultures from the various skins, in sodium chloride broth at 37°C. The bacteria from the dry salt cured skins are inhibited least by the salt, probably because they are best adapted to growing in a highly dehydrated medium. Those from the sun cured skins are inhibited most, during the earlier period of incubation, since they have had no chance for salt adaptation.

The percentage which are proteolytic of the total number of organisms, of course varies widely from skin to skin. It is certain, however, that a large percentage of the bacteria found on goat skins are actively proteolytic, since from 30 to 70 percent of those on the dry cured skins were found to be so, and from 37 to 80 percent of those on green cured skins. Proteolysis was judged by the digestion of coagulated beef serum.



Percent Sodium Chloride
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
Dry Salt Cured
Deccan
Cawnpore
Sun Cured
Days Incubation at 37°C.

Fig. 2.

II

THE SOAKING OF GOATSKINS.

The first tannery process to which skins are subjected is termed "soaking". This consists in allowing the skins to stand in water or a solution of some chemical for a certain period of time, which varies considerably with the individual tanneries. The object of the process is to remove the blood, dirt, salt, etc., which are on the hide, and to properly soften and rehydrate it for the unhairing treatment. It is obvious that the bacteria which are on the skins will multiply when placed in this environment, and it is the study of this growth with which we are at present concerned.

Relatively few investigations of the soaking process have been carried out from a bacteriological standpoint. Various observers have isolated and identified certain organisms from soak waters, but the first quantitative bacteriological study of the soak is that of McLaughlin and Rockwell (1924), who investigated the soaking of heavy hides. They later (1925) extended this work to calf skins.

In order to appreciate the influences which govern the growth of bacteria in soak waters, it is necessary to understand the principles which have been discovered to control the growth of pure cultures. Let us then consider briefly the factors which influence the growth of bacteria in fluid cultures.

A. The Growth of Bacteria in Fluid Media.

1. General Considerations.

When a nutrient medium, held at a favorable temperature, is inoculated with bacteria, and their numbers enumerated at definite intervals thereafter, at least four definite stages are easily distinquishable in the growth of the culture. These are:

(1) The lag period, or the period during which the organisms are not growing at their maximum rate.

(2) The period of logarithmic increase, during which the organisms are growing at their maximum rate, according to the law of geometric progression.

(3) The period of maintenance, during which the number of organisms remains constant.

(4) The period of decline, during which the number of organisms decreases.

The mechanism of bacterial growth is best studied by considering the relations of these various stages in the life history of a culture, together with another value, namely, the generation time. This may be defined as the time necessary for one complete generation, A little consideration suffices to show that this value varies with the stage of growth, changing during the period of lag from an infinitely great value to a minimum one during the logarithmic period, then to infinity again during the maintenance period, and finally to negative infinity during decline. For this reason the generation time of a given culture is usually taken during the period of logarithmic increase, or at its minimum value.

In recent years the mechanism of bacterial growth has been studied in this way by several workers. The first accurate measurements of the rate of growth of bacteria were undertaken by Buchner, Longard, and Riedlin, (1887), working with the Cholera vibrio. They made, however, no allowance for lag. This phase of growth was first demonstrated by Müller in 1895. Some years later, Hehewerth (1901) showed that the length of the lag period varied with the species and age of the culture employed.

Barber (1908), after the development of his method of single cell isolation, used it to study the growth and multiplication of a single organism, and showed that the period of lag could be completely abolished, provided the organisms used for inoculation came from cultures in their maximum growth stage. This observation has been confirmed by later investigations.

Buchanan (1918) extends the number of distinquishable periods in a culture to seven, which are: (1) initial stationary phase; (2) lag phase or positive growth acceleration phase; (3) logarithmic growth phase; (4) phase of negative growth acceleration; (5) maximum stationary phase; (6) phase of accelerated death; (7) logarithmic death phase.

During the maximum or logarithmic growth phase, the growth of a pure culture of bacteria is expressed by the equation:

$$b = a\ 2^n$$

where b represents the number of organisms at the end of a period T, a the number at the beginning of the period, and n the number of generations during the period. Since this is a geometric progression, it will be represented by a straight line when the logarithms of the bacterial counts are plotted against time.

The nature and cause of lag have been investigated by several workers, without, however, any very decisive results. Coplans (1910) has shown that the length of this period differs on different media and under different conditions. Penfold (1914) has given nine different hypotheses as to the cause of lag, none of which he regards as adequate. Ledingham and Penfold (1914) have given a mathematical analysis of the lag phase, which was subsequently elaborated by Slator (1916, 1917). The former authors showed that the lag phase was adequately represented by the equation:

$$X^n = k \log Y$$

where Y is the number of bacteria after a time X, the initial seeding being one. n and k are constants. Slator showed that this equation could be put in the form

$$Z = aK^nX^{n-1}$$

where Z is the "constant" of growth $(\frac{dY}{dX}/Y)$ at any time X. suitably adjusting the constants a and n this equation can be made to represent any period of growth.

The rather extensive experiments of Chesney (1916), working with the Pneumococcus, led him to the conclusion that "lag is an expression of injury which the bacterial cell has sustained from its previous environment." Sherman and Albus (1923) consider that lag is a period of "biological rejuvenescence".

Whatever may be the true cause of lag, it is at least certain that the measure of its length, together with that of the time required for one generation, provides one of the best means of studying the effect of various factors on the growth of bacteria.

2. Factors Affecting The Growth of Bacteria in Fluid Media.

The principal factors influencing the growth of bacteria in liquid cultures may be summarized as follows:

(1) The amount and type of the inoculum. It has previously been mentioned that the length of the lag period and generation time has been found to vary with the species of bacteria, as is to be expected, and also with the previous history of the culture used for inoculation. The influence of the amount of inoculum on resulting growth is not so obvious, but may be demonstrated by suitable experiments. In Table III and Figure 3 are shown the growths of a

Staphylococcus in meat extract broth at 20°C, with various initial seedings.

Table III.

Hours After Inoculation	Viable Cells per cc. 1	2	3
0	171,500	18,600	2,100
4	180,500	19,000	2,550
6	185,500	18,600	2,150
10	253,000	23,600	2,300

It is evident that the larger the initial inoculation, the sooner active growth begins, or the shorter the lag period.

(2) The amount of nutrient material available. The following quotation is credited by Graham-Smith (1920) to McKendrick and Pai (1911), who worked with B. coli: "Every living organism employs the nutriment which it has absorbed for two objects; first, the maintenance of the individual; and, second, its reproduction. As, however, in the case of those micro-organisms with which we shall deal, the rate of multiplication is very fast, we may, for all practical pruposes, consider that the amount of food-stuff utilized for their upkeep is negligible, and assume that the whole of it is employed in reproduction. If we accept this simplifying assumption we may say that organisms in a test tube multiply, by a simple conversion of the available foodstuff into other organisms, and that the rate of multiplication is proportional to the concentration of the foodstuff."

Penfold and Norris (1912) have studied the relation of the concentration of peptone to the generation time of bacteria, using

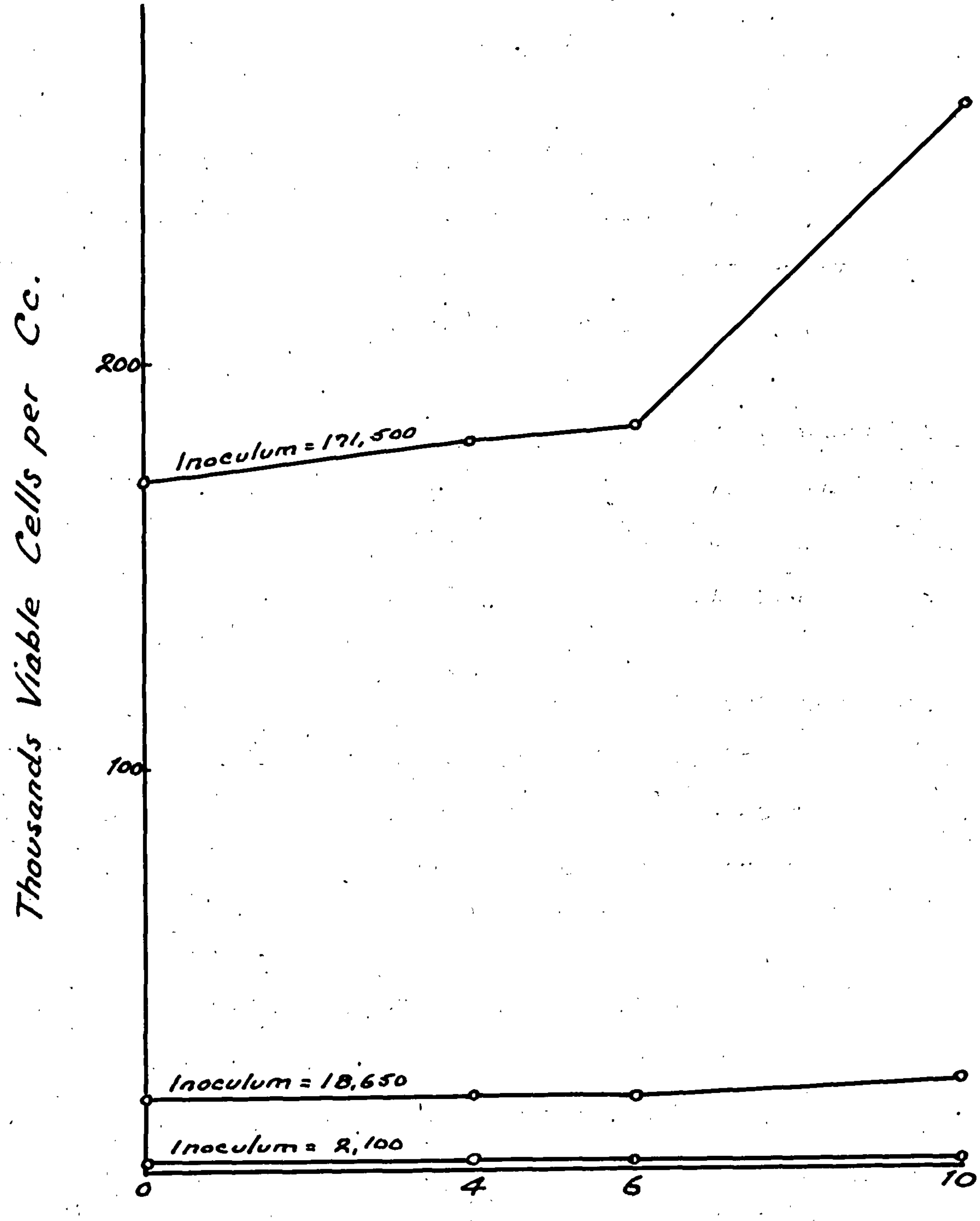

Thousands Viable Cells per Cc.
200
100
Inoculum = 171,500
Inoculum = 18,650
Inoculum = 2,100
0
4
6
10
Hours at 20° C.

Figure 3.

B. typhosus. They found that below a certain limit (0.2%) the generation time is inversely proportional to the concentration of peptone used.

(3) The temperature of incubation. Lane-Claypon (1909) working with B. coli, B. typhosus and B. enteritidis, and Graham-Smith, (1920), working with a Staphylococcus, have made quantitative studies of the effect of temperature upon bacterial growth. They showed that increased temperature, up to a certain point (the optimum temperature) shortens the lag period and the generation time.

(4) The amount and type of inorganic salts present. The inhibitive action of salts such as sodium chloride upon bacterial growth is well known. Hotchkiss (1923) has recently shown that most salts exert also a stimulating effect, if present in small enough concentration.

The above may be taken as the principal factors important for this discussion which influence the growth of bacteria in fluid media. Other factors of course enter in, such as the gaseous environment, the presence of light, etc., but these will not be considered here.

B. The Growth of Bacteria in Goat Skin Soaks.

1. Methods of Investigation.

In the experiments to be described below, the soaking medium, unless otherwise stated, was sterile distilled water. The containers used for the experiments were ordinary Mason fruit jars, and were sterilized in the autoclave before each experiment.

All bacterial counts were made by the dilution plate method, in duplicate. The medium used was meat extract agar, of reaction + 0.5 to phenolphthalein. One cc. of a protein enrichment, obtained by filtering an infusion of meat through a Berkefeld filter, was added to each plate. The plates were incubated 24 hours at 37°C and 24 hours at 25°C.

2. The Rate of Growth.

As has been pointed out above, the growth of bacteria is best studied quantitatively by observations of (a) the length of the lag period, (b) the generation time. In soak waters, however, it is impossible to use the same criteria in judging lag as have been explained above for pure culture work. There are many different species of bacteria present, each with a different rate of growth, and each of these tend to obscure the clear cut picture which is obtained in pure culture. For this reason, the period of lag has been considered in the following work, as the length of time which elapses after the skins are put into the soak, until one complete generation has taken place, provided steady growth ensues thereafter. The generation time was always taken over a period of about eight hours immediately following the end of the lag period as thus defined.

In Table IV these factors are given for various types of skins, when soaked in the weight proportion 1:20 at 20°C.

Table IV.

Kind of Skin	Length of Lag Period in Hours	Generation Time in Hours
Sun Cured	14	2
Dry Salt Cured	9	2½
Cawnpore Green Salt Cured	20	4
Deccan Green Salt Cured	20	4

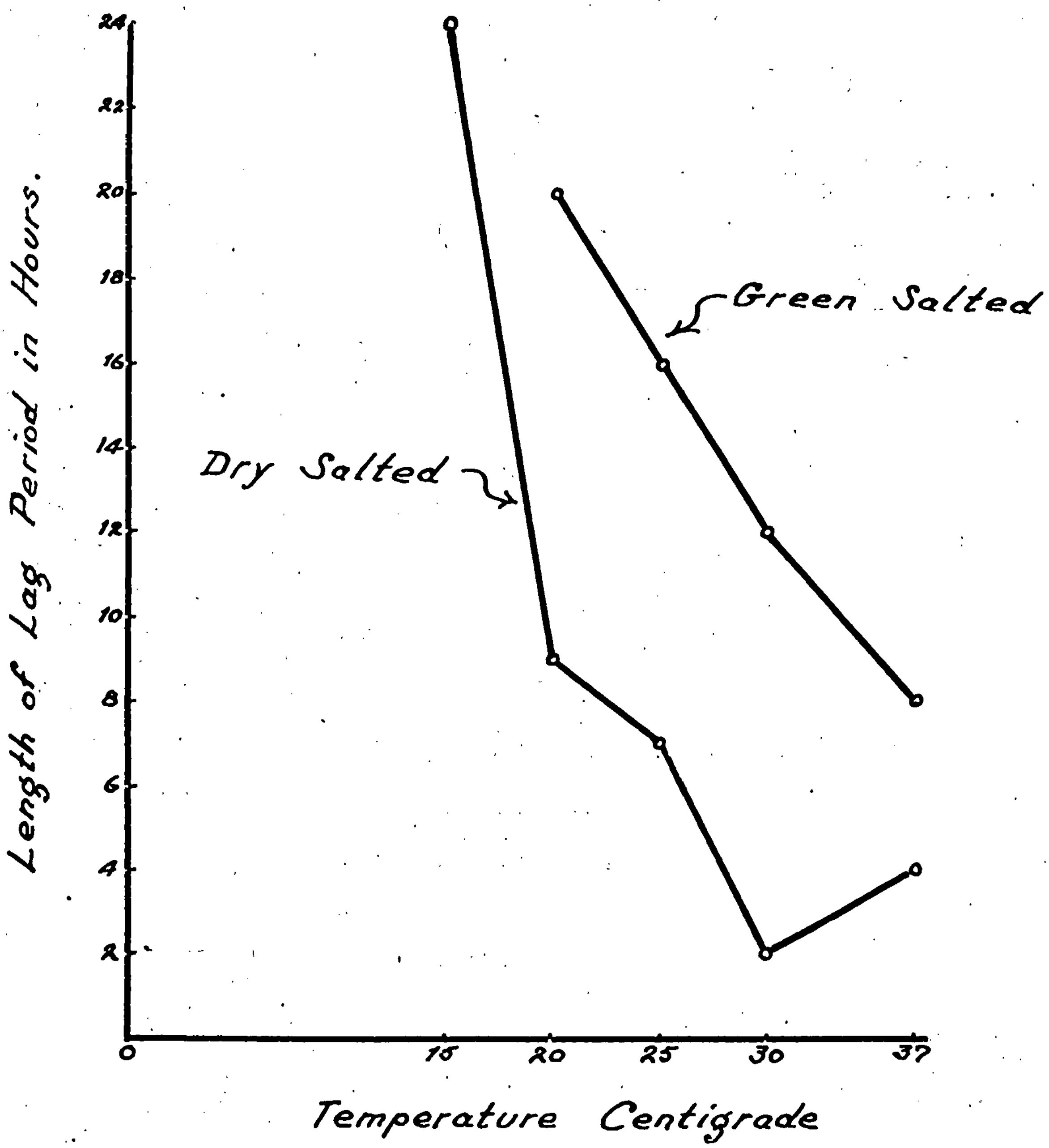
For the purposes of comparison, the lag periods and generation times are given for all of the skins at the same weight proportion, 1:20. This is experimentally the most convenient in the case of the dried skins, and a higher proportion of skin to water is very difficult to work with, owing to their large surface area in comparison to weight. Higher proportions are more conveniently obtained with the green cured skins, and will be dealt with later. In the present comparison, it is to be noted that, when soaked at the same weight proportion (1:20), the green salted skins have the longest lag period; the sun cured skins the second longest, and the dry salted skins the shortest. Since the green salted skins contain the larger percentage of moisture (30 percent), it is obvious that a shorter soaking period will be required.

3. The Effect of Temperature.

In Table V is given the lengths of the lag periods at different temperatures for each class of salted skin.

Table V.

Temperature		Length of Lag Period in Hours Proportion 1:20	
		Dry Salt Cured	Cawnpore Green Salt Cured
15 °C	59 °F	24	--
20 °C	68 °F	9	20
25 °C	77 °F	7	16
30 °C	86 °F	2	12
37 °C	99 °F	4	8



Length of Lag Period in Hours.
24
22
20
18
16
14
12
10
8
6
4
2
0
15
20
25
30
37
Green Salted
Dry Salted
Temperature Centigrade

Figure 4.

The length of the lag period is seento be greatly affected by change in temperature, incréasing temperature, up to a certain point, shortening the lag period. This is illustrated in Figure 4.

Table VI shows the effect of temperature upon the generation time.

Table VI.

Temperature		Generation Time in Hours Proportion 1:20	
		Dry Salt Cured	Cawnpore Green Salt Cured
15°C	59°F	4½	--
20°C	68°F	2½	4
25°C	77°F	1½	2½
30°C	86°F	1	2
37°C	99°F	1	1½

The data of Table VI is shown graphically in Figure 5.

The effect of temperature is also seen in modifying the inhibitive action of salt. This is shown in Figure 6, where the growths in sodium chloride at various temperatures of mixed cultures from dry salted cured skins are plotted. It is seen that increasing temperature counteracts the inhibitive effect of the salt.

4. The Effect of Changing Proportion of Skin to Water.

Skins of each class have been studied to determine the effect of varying the proportion of skin to soak water. No definite variation in the length of the lag period with changing proportion is found in the case of the dried skins. This is probably because the surface

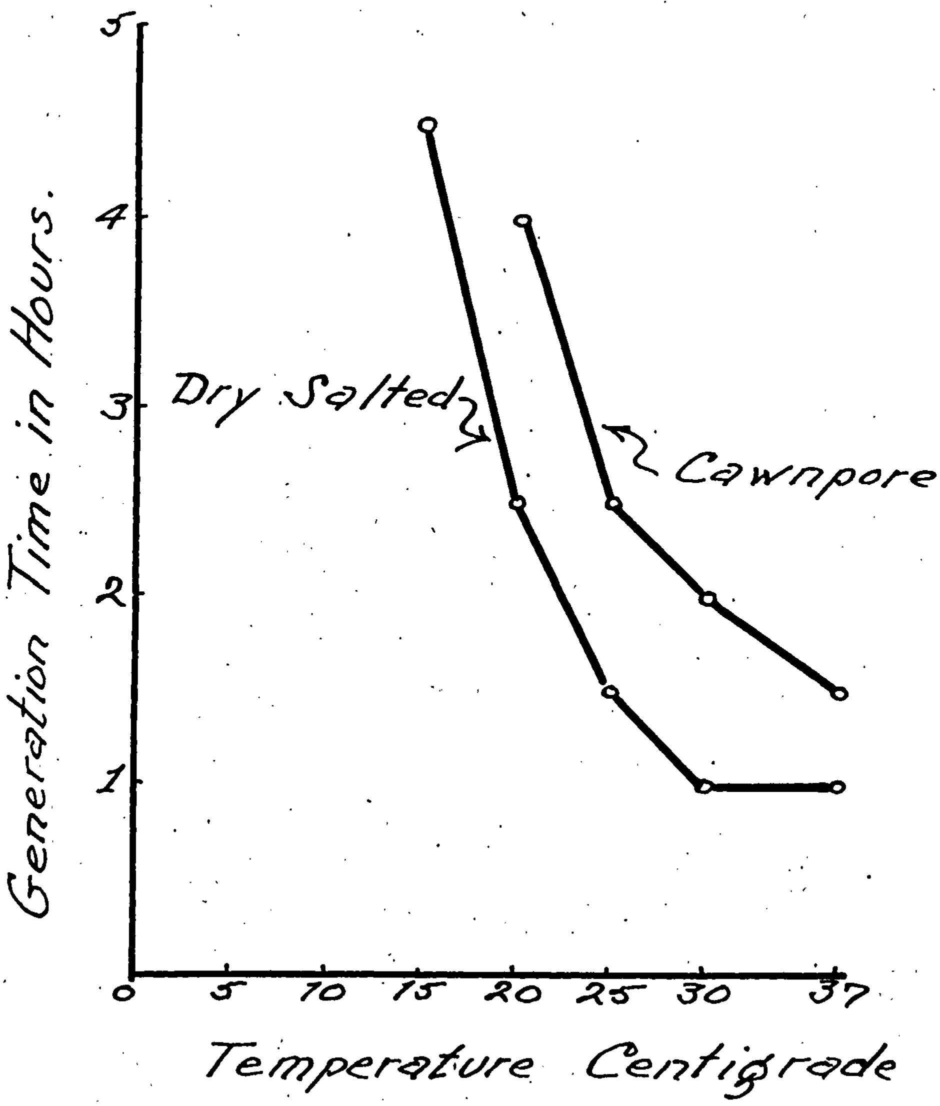

Generation Time in Hours.
5
4
3
2
1
0
5
10
15
20
25
30
37
Dry Salted
Cawnpore
Temperature Centigrade

Fig. 5.

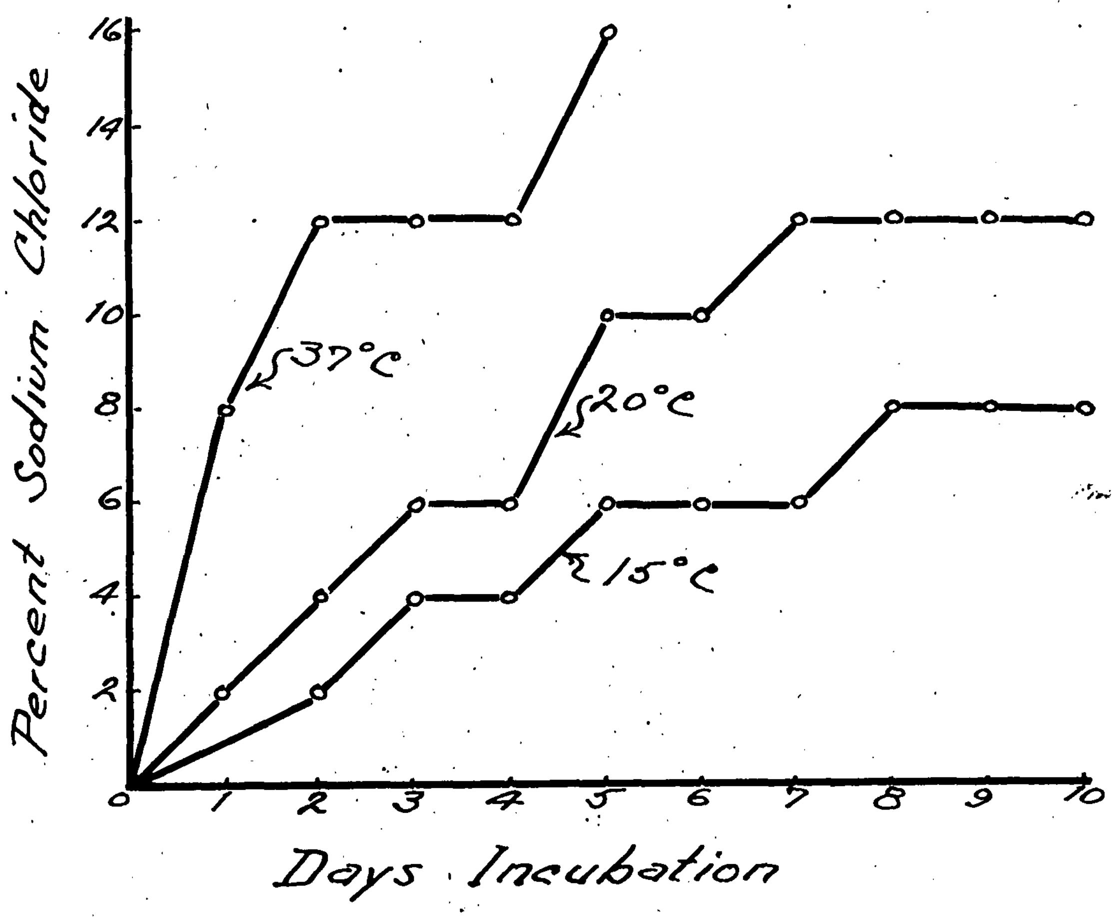

Percent Sodium Chloride
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
37°C
20°C
15°C
Days Incubation

Fig. 6.

nitrogen is so small. (See Table I) Table VII gives the variation in surface nitrogen with changing proportion, for this class of skins.

Table VII.

Variation in Surface Nitrogen with Changing Proportion of Skin to Water.

Sun Cured Goat Skin.

Proportion Skin to Water.	Grams Surface Nitrogen per 100 cc.	Grams Nitrogen Dissolved per 1 gram Original Skin.
1 : 30	0.0044	0.00132
1 : 20	0.0075	0.00150
1 : 10	0.0084	0.00084
1 : 5	0.0185	0.00092

Table VIII gives the variation of surface nitrogen and length of lag period in the case of the green salted skins.

Table VIII.

Proportion Skin to Water.	Grams Nitrogen per 100 cc.	Length of lag period in hrs.	Grams Nitrogen Dissolved per 1 Gram Original Skin.
1 : 30	0.0085	20	0.00255
1 : 20	0.0481	20	0.00962
1 : 10	0.1445	18	0.01445
1 : 5	0.2030	16	0.01015

The length of the lag period is seen to be materially influenced by the weight proportion change, increasing the ratio of skin to water resulting in shortening the lag period. This is the effect of increasing the concentration of nutritive substances (surface nitrogen).

5. The Effect of Milling with Change of Water.

Dry cured goatskins are commonly milled before soaking. The following experiments were devised to ascertain the effect of this milling and change of water on the subsequent bacterial phenomena of the soak. Pieces of dry salt cured goat skin were milled for the lengths of time shown, after which the water was changed and the skins allowed tosoak at 20°C. The results are given in Table IX.

Table IX.

Effect of Milling Dry Salt Cured Goat Skins
With One Change of Water.

Before Soaking, 20°C, 1:20

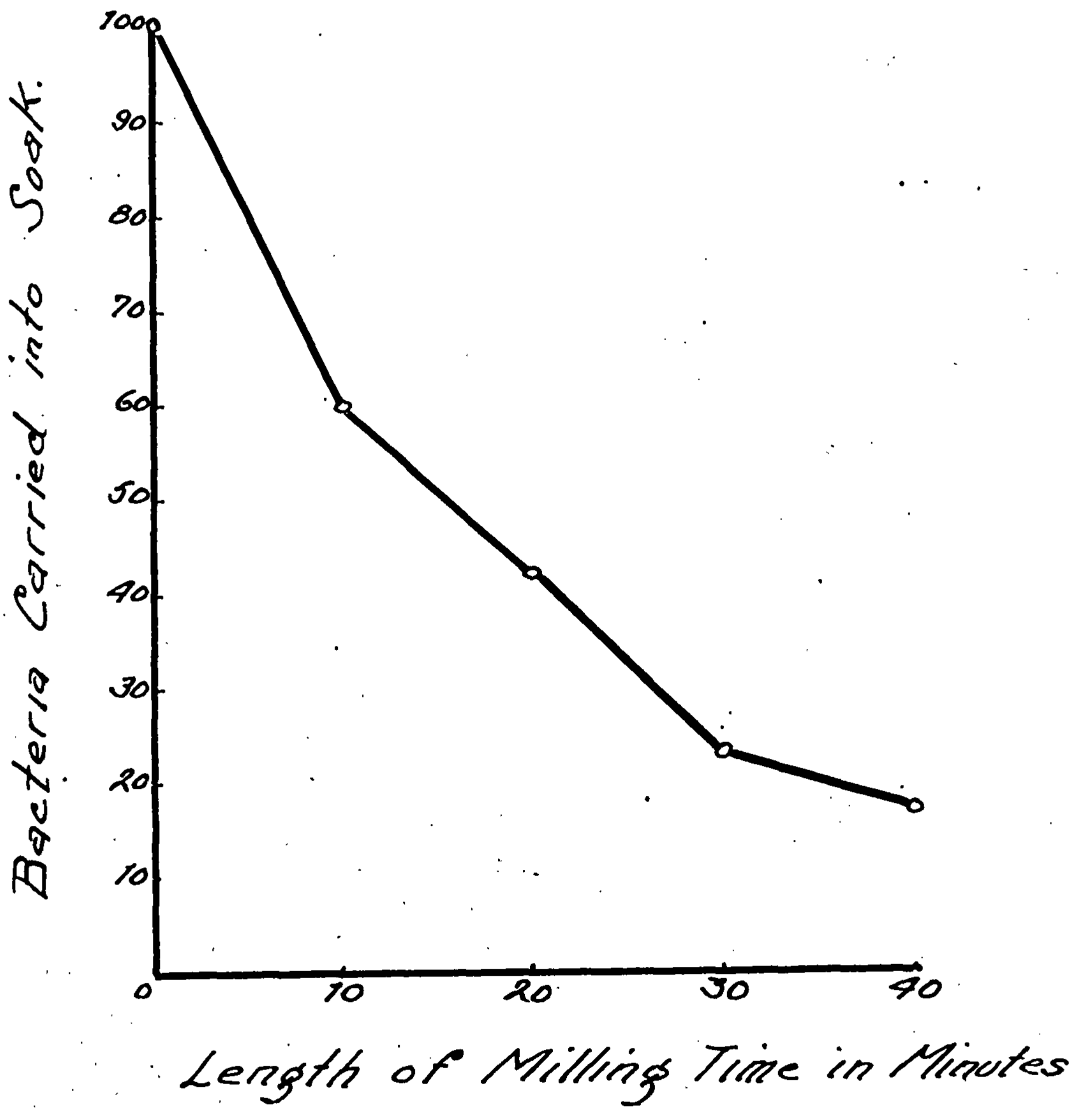
	Control	Milled 10 min.	Milled 20 min.	Milled 30 min.	Milled 40 min.
Percent Bacteria carried into soak.	100	60	44	24	17
Bacteria after 20 hours.	100	27	16	8	3
Grams Nitrogen removed per 100 Grams Skin.	0	0.269	0.273	0.355	0.423
Length of Lag Period in Hours.	9	12	12	14	16

Inspection of the data in Table IX shows that milling the skins with change of water before soaking,greatly decreases the subsequent bacterial growth. This is the resultant of two causes; first, the washing off of the bacteria originally present on the skin, and, second, the decreasing of the available protein matter. Figure 7 shows the relation between the length of time the skin is milled and the number of bacteria carried into the soak. Figure 8 shows the effect of the milling time on resulting bacterial growth. The relation between the growth and the amount of protein washed out of the skins is also shown.

It thus appears that, from a bacteriological view-point, milling is entirely advantageous; that damage in milling can consist only of physical injury to the skin, and that the longer the milling the smaller will be the resultant bacterial growth in the soak. On the other hand, it has been pointed out that the presence of salt in the soak assists in the proper swelling and conditioning of the skin. It is obvious, of course, that the longer salted skins are milled prior to soaking, the lower will be the concentration of salt in the soak.

6. The Effect of the Type ofWater Used.

Water used for soaking in tanneries varies widely from place to place, and it, therefore, seemed best to study the effect of the individual salts which are most commonly encountered. TableX shows the bacterial growth after 24 hours at 20°C. of sun dried goatskins soaked in the proportion 1:20 in the various waters shown. Table XI gives similar data for the green salted skins. The growths are calculated on the basis of a pure distilled water control as 100.



Bacteria Carried into Soak.
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
Length of Milling Time in Minutes

Fig. 7.

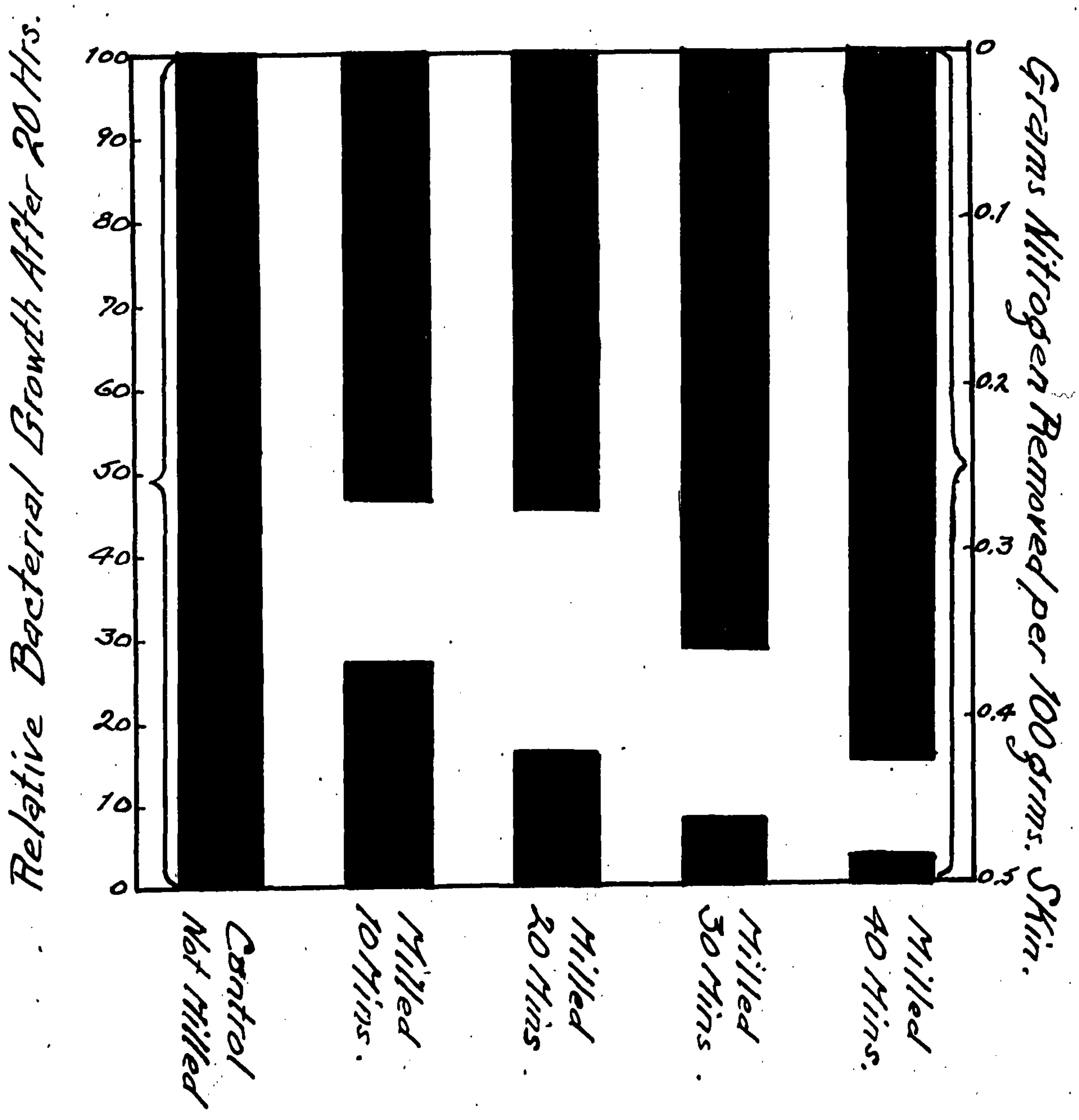

Relative Bacterial Growth After 20 Hrs.
0
10
20
30
40
50
60
70
80
90
100
Grams Nitrogen Removed per 100 grms. Skin.
0
0.1
0.2
0.3
0.4
0.5
Control Not Milled
Milled 10 Mins.
Milled 20 Mins
Milled 30 Mins
Milled 40 Mins.

Fig. 8.

Table X.

Showing Effect of Various Salts on the Growth of Bacteria in Soaks of Sun Cured Goatskins.

24 Hour Soak at 20°C, Prop. 1 : 20.

All salts used in concentration of 500 ppm. with the exception of $MgCo_3$, which was 100 ppm.

	Bicarbonate	Carbonate	Sulphate	Chloride	Sulphide
Mg.	225	127	100	111	---
Ca.	121	---	100	116	---
Fe.	---	---	0.5+	3	---
Na	---	---	---	---	9

( ) Markedly alkaline reaction.

(+) Markedly acid reaction.

Table XI.

Showing Effect of Various Salts on Growth of Bacteria in Soaks of Deccan Green Salt Cured Goatskins.

24 Hours Soak at 20°C. Prop. 1 : 20.

All salts used inconcentration of 500 ppm. with the exception of $MgCo_3$, which was 100 ppm.

	Bicarbonate	Carbonate	Sulphate	Chloride	Sulphide
Mg.	170	120	110	117	---
Ca.	105	---	100	100	---
Fe.	---	---	1	15	---
Na.	---	---	---	---	2

( ) Markedly alkaline reaction.

(+) Markedly acid reaction.

It is to be observed that magnesium salts greatly stimulate growth, while iron salts reduce it. Sodium sulphide is also a marked deterrant to growth. It is apparent from the two tables that the salts do not have as much stimulatory effect in the case of the green salt cured as in the case of the sun dried skins. This is probably due to the so-called "antagonistic" effect of the salts already present.

Summary and Conclusion.

1. From a bacteriological point of view, judged on the bacterial phenomena of the soak, the sun cured goatskins are the best cured, the dry salt cured the worst.

2. The Khari salt has little or no germicidal effect upon skin bacteria.

3. The following factors are shown to be important in the soaking of goatskins.

(a) The amount and type of inoculum.

(b) The weight proportion of skin to soak water (in the case of the green salted skins).

(c) The temperature of the soak water.

(d) The milling of skins before soaking.

(e) The character of the water used for soaking.

Bibliography.

Barber, M. A., (1908) J. Infect. Dis., 5, 379.

Buchanan, R. E., (1918) J. Infect. Dis., 23, 109.

Buchner, H., Longard, K., and Riedlin, G., (1887) Centralbl. f. Bakteriol. u. Paristenk. II, 1

Chesney, A. M., (1916) J. Expt. Med., 24, 387.

Coplans, M., (1910) J. Path. and Bact., 14,1.

Graham-Smith, G. S., (1920). J. Hyg., 19, 133.

Hehewerth, F. H., (1901) Arch. f. Hyg., 39, 321.

Hotchkiss, M., (1923) J. Bact., 8, 141.

Lane-Claypon, J. E., (1909) J. Hyg. 9, 239.

Ledingham, J. C. G., and Penfold, W. J., (1914) J. Hyg., 14,242.

McKendrick, A. G., and Pai, M. R., (1911) Proc.Roy.Soc.Edin.,31,649.

McLaughlin, G.D., and Rockwell, G.E., (1924) J.Am.Leath.Chem. Assoc.. 19, 369.

(1925) J. Am. Leath. Chem. Assoc.,20,312.

Müller, M., (1895) Z. F. Hyg., 20, 245.

Penfold, W. J., (1914) J. Hyg. 14,215.

Penfold, W. J., and Norris, D., (1912) J. Hyg. 12,527.

Slator, A. (1916) Trans. Chem. Soc., 59, 2
(1917) J. Hyg., 16, 100.