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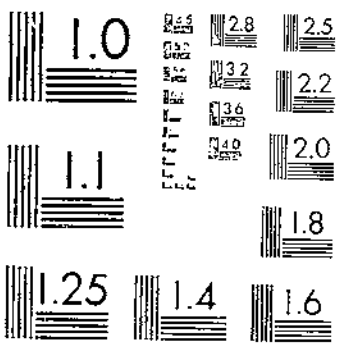

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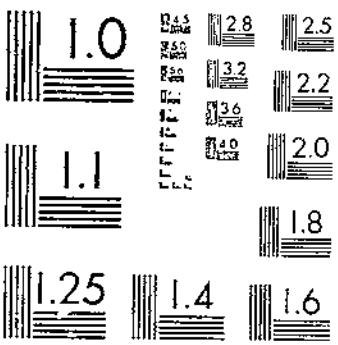

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MICROBIOLOGICAL STUDIES OF SALT IN RELATION TO THE REDDENING OF SALTED HIDES[1]

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INTRODUCTION

Excessive reddening of salted hides and skins has long been familiar to tanners. Hide reddening is usually recognized by the tanner as abnormal and is regarded with suspicion. Consequently reddening has often been the cause of controversy and sometimes of litigation between dealers, ocean carriers, and consignees in connection with claims for allowances and adjustments. The cause and consequences of hide reddening, however, have not been established, and, in fact, only recently has the subject begun to receive the scientific attention that would seem to have been fully warranted long ago.

Confusion frequently occurs in the leather trade as to just what condition is meant when reference is made to stains, discolorations, and other defects of hides and skins. Often different persons will use the same trade term in referring to defective conditions that are apparently very dissimilar. The reddening here referred to is probably best known as "frigorifico reddening", a term applied because of its frequent appearance on hides from South American meat-packing houses. It is not confined exclusively to these hides, how-

[1] Presented in abstract form at the twenty-ninth annual meeting of the American Leather Chemists' Association, Atlantic City, N.J., May 25, 1932.

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ever, having been found and reported on dry-salted skins and on domestic green-salted hides. The condition is also referred to in the trade as "strawberry" reddening and as "heating."

On commercially green-salted hides the condition often appears as a bright-red discoloration on the flesh side, in spots, streaks, and great blotches. It may be observed frequently as streaks and patches on the exposed flesh side of piles of bundled, infected hides. Often, under optimum moisture conditions, the red discolored areas are distinctly wet and slimy and impart a bloody appearance to the flesh side of the hide. When the hide is partly dried out, the discoloration is frequently modified to a rather nondescript brown.

Usually the discoloration appears to be confined only superficially to the flesh surface, and is apparently removed by light scraping with a knife. Actual destruction of fibers and loss of hide substance in the reddened areas is seldom evident on ordinary visual inspection.

An apparently similar reddening of salted fish, particularly salted codfish, has been reported for many years. In the fish trade reddening was early recognized as a matter of economic importance, and its cause, effect, and prevention have been, therefore, the subject of much scientific study.

Although the causes of the reddening of salted codfish and the reddening of salted hides have not been identified as the same, there is a striking similarity between many of the circumstances under which fish and hides are cured and the development and appearance of the reddening in the two cases. This strongly suggests that it may be profitable to consider observations and information from the many investigations of fish reddening in connection with studies of hide reddening.

REVIEW OF THE LITERATURE

The first scientific investigation of reddened codfish is credited to Farlow (*11*, *12*).[3] By examining scrapings from reddened codfish, the wood work of fishing vessels, and the packing houses at Gloucester, Mass., he found a fungus or alga occurring as simple spheroidal masses of cells imbedded in slime, to which he ascribed the reddening. He considered this organism to be identical with *Clathrocystis roseo-persicina*. He found it also as a contaminant of the Cadiz sea salt used in curing the fish, and as preventive measures recommended the use of clean, uncontaminated salt, more sanitary conditions of handling, and temperatures below 65° F. for storage. Farlow also found another organism associated with the *Clathrocystis*, which appeared in fours surrounded by a thin hyaline envelope. This organism bore a strong resemblance to *Gloeocapsa crepidinum* and was described by Farlow under the name of *Sarcina morrhuae*. He did not consider it responsible for the reddening. Farlow's studies were confined apparently to microscopic examinations without cultural or inoculation confirmations.

Poulsen (*25*) found, on mud at Copenhagen, organisms which he named *Sarcina littoralis* and *Micrococcus littoralis*, and to which he attributed the reddening of fish. He found *S. littoralis* to be identical with the *S. morrhuae* of Farlow.

[3] Italic numbers in parentheses refer to Literature Cited, p. 22.

Mégnin, as reported by Bertherand (*3*), gave *Coniothecium bertherandi* as the cause of the reddening of salted fish. Considerable question then arose as to the identity of Mégnin's *Coniothecium* and Farlow's *Sarcina*.

Layet (*21*) in reporting on the *Coniothecium* of Mégnin described it as—

> Round spores of very pale rose color, with granular contents, and a small "kernel" measuring 6 to 10 microns in diameter; the largest of these spores are divided into two or four equal parts, which become new spores; a short mycelium, hardly discernible, in most of these diminutive spores.

Gayon and Carles (*27*) described to the Society of Public Hygiene at Bordeaux a *Bacillus* and a *Micrococcus* which, when mixed, always produced a red color on cod. The individual rôle of each organism in producing the discoloration was not determined. Gayon and Carles stressed as remarkable the ability of these organisms to live on sea salt and to develop on moist crystals of salt.

Mauriac (*24*) in reviewing the public-health aspects of reddened fish concluded that this condition had no direct connection with food poisoning. He considered the salts used in curing as the origin of reddening organisms and recommended mined salts in preference to solar salts.

Edington (*10*) attributed the reddening of salted codfish to *Bacillus rubescens*, which he described as small nonmotile rods from 0.3 to 0.5 micron thick and from 1.5 to 4 microns long. Young cultures did not show red, but when the moisture content of the medium was limited old cultures did. Among the prophylactic measures proposed was the addition of 3 percent of boric acid to the wash water. Edington apparently did not demonstrate that this organism produced reddening on salted codfish or salted media.

Le Dantec (*8*, *9*) refers to healthy reddened codfish with firm muscular flesh beneath the discoloration and to spoiled reddened codfish, characterized by a viscous red matter having an alkaline reaction and a sickening odor. Algae, bacilli, and cocci were associated with the first stage, but in the more advanced condition cocci and a sarcinal organism occurring in groups of four were present. Le Dantec concluded that the algae present were the same as had been reported at various times as *Clathrocystis*, *Protomycetes*, and other algae, and, furthermore, that they were not responsible for development of the reddening.

Le Dantec reported isolation of two red-forming organisms, a coccus and a *Bacillus*. The coccus he called "the coccus of the red of the salted cod." It grew with difficulty on artificial media and failed to reproduce reddening on codfish unless associated with a small coccus with which it is often found. The *Bacillus* was motile with a terminal spore. It was capable of producing red on codfish and of growing in a saturated salt solution with variations in morphology from short to long rods and filaments. This organism was also found on the salts used for curing fish. Le Dantec recommended the mixing of from 10 to 15 percent of bisulphite, nitrate, or hyposulphite of sodium with the salt to prevent development of the reddening.

Høye (*15*, *16*, *17*, *18*), in elaborate studies of the reddening of salted fish, found molds, yeasts, and bacteria not only capable of

growing in high concentrations of salt but also possessing halophilic properties, in that growth was more luxuriant at high salt concentrations. He studied in much detail the influence of high concentrations of sodium chloride upon the morphological characteristics of the organisms originating from sea salts. Høyer associated *Torula epizooa* and red bacteria with the reddening of fish, and stressed that the latter should be regarded with suspicion by curers of fish. He found these organisms, together with a number of others, in many samples of sea salt, including those from Cadiz, Trapani, Torrevieja, Port Said, Ibiza, and Tunis, and in salted fish. The red coccus reported was slightly oval and encapsulated, always growing on the surface in raised, intensive red colonies, and often appearing as a *Diplococcus*.

Beckwith (*1*), using gelose made from brines found in containers of salted codfish, isolated a diplococcus, which he called *Diplococcus gadidarum* and with which he produced typical reddening on tubes of shredded fish. The organism was strictly aerobic and grew in 15 but not in 20 percent salt solutions.

Bitting (*4*) found cocci, occurring as tetrads or as *Diplococci*, a sporulating bacterial rod, and an oidium, as the organisms usually associated with the reddening of salted fish. The coccus averaged about 2 to 2.5 microns in diameter and ranged from pink to bright red. The coccus in artificial media was nonmotile, and no flagella were noted. The organism was strictly aerobic and encapsulated. Its growth was delayed in 5 and 10 percent salt and inhibited by 15, 20, and 25 percent salt media. The *Bacillus* was a large rod, thicker at one end than at the other, owing to the formation of a spore. This organism grew well on laboratory media but without color except for a pink tint on bread paste. Bitting attributed the reddening of fish to the coccus and reproduced red viscous discolorations on fish with inoculations from cultures of it.

Kellerman (*20*), in reporting on two cocci from red codfish, considers one of them identical with *Micrococcus littoralis* (Poulsen), *Sarcina littoralis* (Poulsen), *Clathrocystis roseo-persicina* (Farlow), *Diplococcus gadidarum* (Beckwith). This micrococcus ranges from 1.2 to 1.6 microns in diameter. It occurs in clumps but more frequently in the diplococcus and single coccus formation. Cultures isolated in 1907 and allowed to dry out were revived in 1911. When they were again dried and revived in 1914, it was found that they had retained their typical characteristics. For the other and smaller micrococcus Kellerman suggested that the name *Micrococcus littoralis gadidarum* be retained. This organism measured from 0.35 to 0.5 micron in diameter. Both organisms were aerobic and when associated grew especially well. Both of them grew in media containing as much as 30 percent sodium chloride. Kellerman points out that because of their extreme halophilic properties these organisms cannot be described by reference to the conventional chart of the Society of American Bacteriologists.

Brown (*5*) ascribed the reddening of fish to two organisms, a "spirochaete" and a "bacterium", originating from the sea salt used in curing. Owing to the close harmonious intergrowth of these organisms, whose separation into pure culture was very difficult, the color ranged from pale opaque pink to deep transparent crimson.

Good growth occurred on heavily salted fish, brine, sea salt, and media saturated with sea salt. The organisms also exhibited decided heliophilic and thermophilic characteristics. Both organisms were strictly aerobic and their shape, size, and motility were influenced by the salt concentration. Spherical forms were found in 18 percent or more of salt. Sea salts from all over the world were found to contain *Spirocheta halophilica* and *Bacterium halophilica*, whereas mined and domestic salts seemed to be free of them.

Harrison and Kennedy (*13*) first thought that the reddening was caused by two organisms, which, however, they showed later to be but one of a highly pleomorphic nature, the ability of which to undergo regenerative changes in morphology is attributed to a synplastic stage of growth. The red organism—

> occurs as spheres and also as long rods, the former averaging 2 to 3 microns in diameter and the latter from 1 to 1.6 microns in width and as long as 15 microns. Between these two extremes many intermediate forms may be found, differing in diameter, length, and shape—oval, amoeboid, clavate, cuneate, truncate, pointed, spindle, club, and pear shape.

From examination of brines from Turks Islands they conclude that the spherical form is the first shape encountered in the natural habitat of this organism, which in all probability is sea water. Changes in morphology of the organism with changes in salt concentration are described in detail. They showed that all known solar or sea salts, such as those from Ibiza, Trapani, Torrevieja, and Turks Islands, contain the red organism, whereas they were never able to find it in mined salts. While pointing out certain objections to placing the organism in the genus *Pseudomonas*, they suggested that for the time being the name *Pseudomonas salinaria* be retained for it. They rejected the names *Rhodo-bacter* and *Rhodo-bacillus*, as these had already been used for classifying some of the thiobacteria.

Cloake (*7*) reported the reddening of salted codfish caused by a coccus and an organism designated X. These organisms could grow only in high salt concentrations. Organism X was exceedingly polymorphic.

Tattevin (*32*) made a detailed study of the microflora of sea water, sea salts, and brines and on the basis of his findings and a review of the available literature concluded that the principal organisms involved in the reddening of salted flesh were *Micrococcus littoralis* (Poulsen), *M. gadidarum* (Beckwith), *Sarcina littoralis* (Poulsen), *Bacillus rubescens* (Edington), the red bacillus of Newfoundland (Le Dantec), and *Clathrocystis roseopersicina* (Cohn). He further concluded that the development of the red color was the initial sign of rapid microbic increases of all kinds.

Scientific studies of the reddening of hides and skins begin to make their appearance only in comparatively recent literature. In a letter to the president of the Camara de Sub-Productos Ganaderos de la Bolsa de Comercio en Buenos Ayres,[4] the South American producers of frigorifico hides stated that flesh reddening of hides had

[4] Letter from the American consul at Buenos Aires to the Secretary of State inclosing a letter dated Apr. 4, 1928, to the president of the Camara de Sub-Productos Ganaderos de la Bolsa de Comercio en Buenos Aires from the packing plants in Argentina with reference to claims for alleged damage to hides. (Copy on file in Industrial Farm Products Division, Bureau of Chemistry and Soils.)

been the subject of study by them for more than 10 years, and that it is caused probably by chromogenic bacteria or colored fungi. They found the stain to be confined to the flesh surface and capable of being easily scraped off. The organisms were found on the salts used for curing and were strictly aerobic. They stated that the appearance of red color did not necessarily indicate heating in the pack but was influenced by atmospheric conditions, and that the red organisms had no particular aversion to the hair side but were merely difficult to discern there.

Stather (*23*) termed brick-red to carmine-red spots on the flesh "heating spots." They often cause a loosening of the hair and grain damage. He isolated organisms from such spots and found that salt in low concentrations stimulated them, whereas higher concentrations inhibited their growth. The bacteria isolated were the more common types usually found on normal hides such as *Micrococcus pyogenes aureus*, *Bacterium mesentericus*, a *Sarcina*, a *Corynebacterium*, and two species of *Actinomyces*.

Bergmann (*2*), in reporting on work with Stather, says that the opinion that red discoloration alters only the surface of the flesh side and therefore does no damage coincides with the facts as long as the red discoloration is in its first stages, but is not correct if the condition is in advanced stages. Pure cultures of a number of organisms were isolated from red spots, among which the following were identified: *Sarcina lutea*, *S. auriantica*, *Micrococcus roseus*, *M. tetragenus*, *Proteus* variety, *Actinomyces* variety, and *Bacillus subtilis*. These organisms showed very low salt tolerance, more than 8 percent salt inhibiting growth of even the most resistant.

Lloyd (*23*) attributed reddening of salted hides to red and yellow chromogenic halophilic bacteria that she isolated on 25 percent sodium chloride agar plates from salts and brines used in curing. She considered this type of reddening entirely different from the red stains described by Stather and as evidence of this fact pointed out the marked difference in salt tolerance between the organisms studied by her and the ones reported by Stather. Lloyd stated that the evidence of actual damage to hides by halophilic bacteria is very slight. Atmospheric humidity and temperature were shown to play an important part in the growth of the organisms, which were strictly aerobic. Lloyd brought out that halophilic bacteria are not found commonly on fresh hides or in ordinary dust or dirt, but are introduced through use of salt of marine origin. After a thorough histological study, Lloyd recommended the addition of 1.5 percent of sodium bisulphate to the salt for prevention of reddening.

Stather and Liebscher (*30, 31*) from a continuation of their studies, to which work Bergmann referred, concluded that reddening was caused by microorganisms commonly found on raw hides and skins. The limit of salt tolerance for these organisms they found to be 16 percent. The organisms were able to grow over a wide range of pH, good growth being obtained in some instances at pH 11.0. In advanced stages of reddening the epidermis was often destroyed, and at such spots the hair slipped. The organisms were also found to split fats, and consequently infected hides produced uneven leathers.

Stather (*29*), after a preliminary examination of cultures from Lloyd, stated that Lloyd's cultures were very similar to the organisms reported by him except for a somewhat greater tolerance of salt. He believed no distinction should be made between types of flesh reddening since his work indicated no essential difference beyond that attributable to strain variations of organisms of the same general type.

Robertson (*26*) cultivated halophilic organisms from four varieties of curing salts on agar media containing 30 percent sodium chloride. These cultures were chromogenic and chiefly cocci of sarcinal type. They were compared for salt tolerance with cultures of the organisms reported by Stather and also cultures of "proteus, pyocyaneous, and fluorescens liquefaciens." It was shown that none of the nonhalophilic organisms gave any sign of growth in broth of a sodium chloride concentration as high as 8 percent, whereas with cultures from salt the heaviest growths were obtained at high sodium chloride concentrations and no growth took place at less than 6 percent salt concentration. Robertson concluded that certain brick-red stains on salted hides are produced by halophilic or salt-loving organisms that come from marine salts used in curing.

Hausam (*14*), continuing the work of Stather and of Stather and Liebscher, reported the isolation of *Bacillus prodigiosus* from salted hides and skins, and attributed the development of initial reddening to this organism.

Harowitz-Wlassowa (*19*) reported the isolation of a red chromogen from the intestinal tract of cattle that was capable of causing the flesh reddening of salted hides.

It is thus evident that there is a marked lack of agreement between findings reported and that there is still a great diversity of opinion as to the true cause of hide reddening and its effect upon both the quantity and quality of the resulting leather.

Many investigators agree in attributing the source of the causative organisms to the salts used in curing. Others, however, indicate that organisms causing reddening may be found normally on freshly flayed hides and skins.

Early workers on fish first specified algae as the cause of reddening, but subsequently found bacterial cocci in large numbers associated with reddening and concluded that these were the cause rather than algae. With the introduction of cultural methods in subsequent studies much confusion arose over the identity of the chromogenic cultures isolated on media of high salt content. Some investigators classified them as cocci (obligate halophilic), whereas others who studied such cultures on media of different salt concentrations noted marked variations in morphology. These variations were first attributed to the presence of more than one organism, existing possibly in symbiotic relationship. However, after a thorough study of such cultures, Harrison and Kennedy (*13*) concluded that the different morphological forms belonged to the same organism, the coccus form occurring at high concentrations of salt and the rod form at low concentrations.

Some workers consider that the evidence of damage to hides and skins by reddening is slight, whereas others associate hair-slip and

uneven leathers with the condition. Bergmann (*2*) claims that with the first appearance of the red color there is little or no evidence of damage to hides and skins, but in advanced stages definite damage can be attributed to its development.

SALT AS A SOURCE OF REDDENING MICROORGANISMS

PRESENCE OF RED CHROMOGENIC ORGANISMS IN VARIOUS SAMPLES

Most of the workers on the reddening of salt-cured products have attributed the source of the causative organisms to the salts used. Therefore, a large collection of commercial salts used for curing hides and skins was examined.

For this purpose a modification of the fish-rice medium of Clayton and Gibbs (*6*) was used. A hide-peptone-salt broth was prepared by steaming 1 pound of freshly flayed calfskin in 1,000 cc of water for 1 hour. To the pressed broth from this digestion 0.1 percent of peptone and 28 percent of sea salt were added. Twenty cubic centimeters of this preparation was added to 5 g of polished rice in a 50-cc Erlenmeyer flask, and the whole was sterilized in an autoclave at 15 pounds pressure for 30 minutes. This medium will be referred to as medium A, or the hide-salt-rice medium. Sea salt was used with the idea that it would provide the most natural and favorable salt environment, because of its content of organic and mineral matter other than sodium chloride.

Five grams of each salt examined was added, in solid form, to a flask containing medium A, and for the first 3 days 2 cc of sterile, distilled water was added to each flask daily. All flasks were incubated for 21 days at 37.5° C. Throughout the incubation period an excess of solid salt was present in every flask. All microscopically visible red, pink, rose-red, and crimson growths were tentatively considered as indicative of the presence in the salt of organisms capable of reddening salted hides and skins.

The results of this examination are summarized by types of salt in table 1.

TABLE 1.—*Reddening organisms in commercial unused salts*

Type of salt	Samples examined	Samples with red growth—		
		Present		Absent
	Number	*Number*	*Percent*	*Number*
Solar, crude	35	34	97	1
Solar, kiln dried	12	0	0	12
G.A. open pan	39	25	64	14
Fine, vacuum pan	17	0	0	17
Mined, only	62	0	0	62

[1] All samples were obtained from the same manufacturer and had the same origin.

The samples of salt were not collected directly or under aseptic conditions. Consequently there is the possibility of an occasional error in identification of individual samples and also of accidental contamination. Regarding accidental contamination with red organisms, however, it would seem that this possibility can be elimi-

nated from consideration in view of the consistent absence of these organisms in 62 samples of mined salt included in the collection.

The crude solar salts included samples from South America, Turks Islands, Spain, Utah, and California. Of these, all but one developed the red growth.

The kiln-dried solar salts were all from one source, and none gave evidence of the presence of red chromogenic organisms.

The results with grainer or open-pan, artificially evaporated samples, all of domestic origin, were unexpected and may be exceedingly significant in pointing out possible additional sources of the reddening organisms and their distribution. Their presence raises the question as to whether there may be some connection here with the faint pink blush frequently reported on the flesh of domestic cured calfskins. There has been no opportunity yet to systematically trace the origin of the organisms in any of these grainer salts.

All samples of domestic salt artificially evaporated in vacuum pans failed to show any red growth.

The samples of mined or rock salts were from New York, Louisiana, Michigan, and Russia, but represent only four rock-salt deposits. Without exception, these samples failed to develop a red growth, which is in agreement with the results of several previous investigators and upon which they based their recommendations of mined salt for curing fish to prevent reddening. Mined or rock salt is generally prepared simply by mining, crushing, and screening, without the use of water, another point that may be significant with regard to the source of the reddening organisms.

These results show beyond a reasonable doubt that some salts are contaminated with reddening microorganisms. Almost all crude solar salts are heavily infected with such organisms, and some G.A., or open-pan evaporated salts, also carry them. The freedom of mined salts from these chromogens must not be misinterpreted as proof of their superiority for curing hides and skins. Mined salts come in the class of crude or unrefined salts. They were usually found to carry a rather heavy, mixed nonchromogenic microflora, of as yet undetermined influence upon hides and skins. Yesair [5] has recently shown that such salts offer a source of varied infections in meat packing.

RED GROWTH ON HIDES AS RELATED TO SALT ORGANISMS

To correlate the development of red growth on hides and skins with the results obtained on laboratory medium A, the following parallel sets of experiments were made: A freshly flayed calfskin was thoroughly fleshed and the hair clipped off close. The skin was then cut into small pieces, which were thoroughly washed with water. Twenty grams of the prepared wet skin was then placed in a 50-cc Erlenmeyer flask to which was added 15 g of salt to be tested. The flasks were then incubated for 6 weeks in a saturated humidity at 55° C., after which inspection was made for the development of a red discoloration.

[5] YESAIR, J. BACTERIAL CONTENT OF SALT. Address given at the meeting of the meat section of the National Canners' Association, Chicago, Ill., Jan. 23, 1930. 4 p. [Mimeographed.]

For the tests, summarized in table 2, 25 samples that had developed reddening on medium A, comprising 16 solar evaporated and 9 artificially evaporated salts, were selected. Of these, 23 developed reddening on calfskin at 55° C. Also 25 samples that had not developed reddening on medium A, comprising 1 solar kiln-dried, 12 mined, and 12 artificially evaporated salts, were selected. None of these gave evidence of reddening on calfskin. For controls, 50 separate portions of a sterilized chemically pure sodium chloride were run. All failed to show a red growth on either medium A or the pieces of calfskin.

TABLE 2.—*Reddening produced on medium A and on calfskin by the same collection of salts*[1]

Type of salt	Total samples examined	Samples showing reddening on—	
		Medium A	Calfskin
	Number	*Number*	*Number*
Solar, crude	16	16	15
Solar, kiln-dried	1	0	0
Artificially evaporated	21	9	8
Mined	12	0	0

[1] Controls consisted of 2 sets of 25 flasks each, 1 set containing medium A and the other set calfskin, to which sterilized chemically pure sodium chloride was added. None of the controls developed red growth.

These results show that the Clayton and Gibbs medium or a suitable modification, such as the one described, while possibly not infallible, provides a simple, practical means for testing various salts for the probability of their developing reddening when used on hides and skins.

FACTORS INFLUENCING THE DEVELOPMENT OF HIDE REDDENING

Since Le Fevre and Round (*22*), Stather and Liebscher (*30*, *31*), and others have shown that generally organisms isolated from brines and salted hides prefer alkaline conditions for growth, it was thought that there might be a correlation between the alkalinity or acidity of the salts examined and the development of red growth. Eighteen salts, comprising six samples for each of the three different types, were accordingly tested for their reaction. With boiled, distilled water, solutions of 4 M, 1 M, and 0.1 M, respectively, were made of each salt selected. The pH of these solutions was determined electrometrically with the hydrogen electrode and colorimetrically with isohydric indicators. The results are given in table 3.

The results do not show differences in pH of either sufficient magnitude or consistency to explain the differences in behavior of the several types of salt in developing reddening. However, they do show that the commercial salts used in curing are in general distinctly alkaline. This is particularly interesting in view of existing recommendations for the use of acid salts and brines for hide curing.

Several workers have noted that certain factors exert important influences upon the development of red discolorations on salted hides and skins under conditions of commercial storage. Preliminary

studies were made in the laboratory to determine the extent to which these factors influenced the development of reddening on pieces of salted calfskin when the microorganisms were known to have been introduced through the use of naturally contaminated salt.

TABLE 3.—*pH measurements of different salt solutions at various concentrations*

Crude solar salt							Artificially evaporated salt							Mined salt							
Sample	4 molar		1 molar		0.1 molar		Sample	4 molar		1 molar		0.1 molar		Sample	4 molar		1 molar		0.1 molar	
	H.[1]	C.[2]	H.	C.	H.	C.		H.	C.	H.	C.	H.	C.		H.	C.	H.	C.	H.	C.
A	9.0	8.9	8.8	9.0	8.8	8.7	G	7.6	7.5	8.4	8.1	8.4	8.7	M	7.4	7.3	8.1	8.0	8.3	8.2
B	8.0	7.6	8.3	7.9	8.2	8.0	H	8.0	8.0	8.4	8.1	8.3	8.2	N	7.7	7.6	8.2	8.0	8.4	8.2
C	7.4	7.6	7.6	7.6	7.6	7.4	I	7.8	7.8	8.1	8.1	8.4	8.2	O	8.0	7.2	8.3	8.1	8.6	8.4
D	7.4	7.4	7.5	7.4	7.7	7.4	J	8.0	8.1	8.2	8.1	8.4	8.1	P	7.4	6.9	7.7	7.2	7.5	7.3
E	8.2	7.6	8.5	7.7	8.2	8.6	K	8.1	7.6	8.4	8.0	8.8	7.6	Q	7.7	7.2	8.2	7.9	8.7	8.4
F	7.8	7.6	7.5	7.6	7.2	7.3	L	8.5	7.7	8.2	7.7	8.0	7.7	R	8.0	7.0	8.2	7.2	8.4	7.5

[1] pH values determined with the hydrogen electrode.
[2] pH values determined colorimetrically.

To determine the importance of temperature, humidity, and acidity, pieces of clipped and washed calfskin salted on the flesh side with an excess of a crude solar salt that had been shown by culturing on medium A to be heavily contaminated with reddening organisms, were used.

The effect of temperature was studied by incubating pieces over water at 20°, 30°, 37.5°, 45°, and 55° C. Reddening developed within 2 weeks at all these temperatures. Brick-red discolorations predominated at 37.5°, coral-pink colors at the lower temperatures, and bright-crimson discolorations at the higher temperatures. These results show that reddening will develop on the flesh of salted hides over a wide range of temperature conditions. High temperature is not essential for the development of reddening, although it may intensify the color.

To determine the influence of relative humidity, pieces were incubated at 37.5° C. in relative humidities of 100, 80, 60, 40, and 25 percent saturation. Red discolorations developed only at relative humidities of 60 percent and above. Reddening was distinctly more generalized and intensified at the higher relative humidities than at 60 percent. These results show that relatively high humidities are necessary for the development of flesh reddening.

The effect of acidity was studied by adding $NaHSO_4$ and Na_2CO_3, respectively, to the contaminated solar salt used. In a slightly acid condition, obtained by adding from 0.5 to 1 percent $NaHSO_4$ to the salt, reddening was greatly delayed and of a pale-red shade, as compared with the brick-red discoloration formed when the contaminated salt alone was used. Under alkaline conditions, obtained by adding 1, 2, and 3 percent, respectively, of Na_2CO_3, reddening developed sooner and was more intense than with the contaminated salt alone. The results show that the addition of small quantities of alkali carbonates does not prevent reddening, and they further indicate that the reddening organisms prefer alkaline conditions for growth.

These studies with salts strongly suggest that most cases of flesh reddening result from the use of contaminated salt, although the possibility of contamination with reddening organisms from other sources is not eliminated. They substantiate the general observations and experiences of the trade regarding the influence of temperature, humidity, and acidity on the development of reddening on hides and skins in commerce.

THE MICROBIOLOGY OF HIDE-REDDENING ORGANISMS

METHODS OF ISOLATION

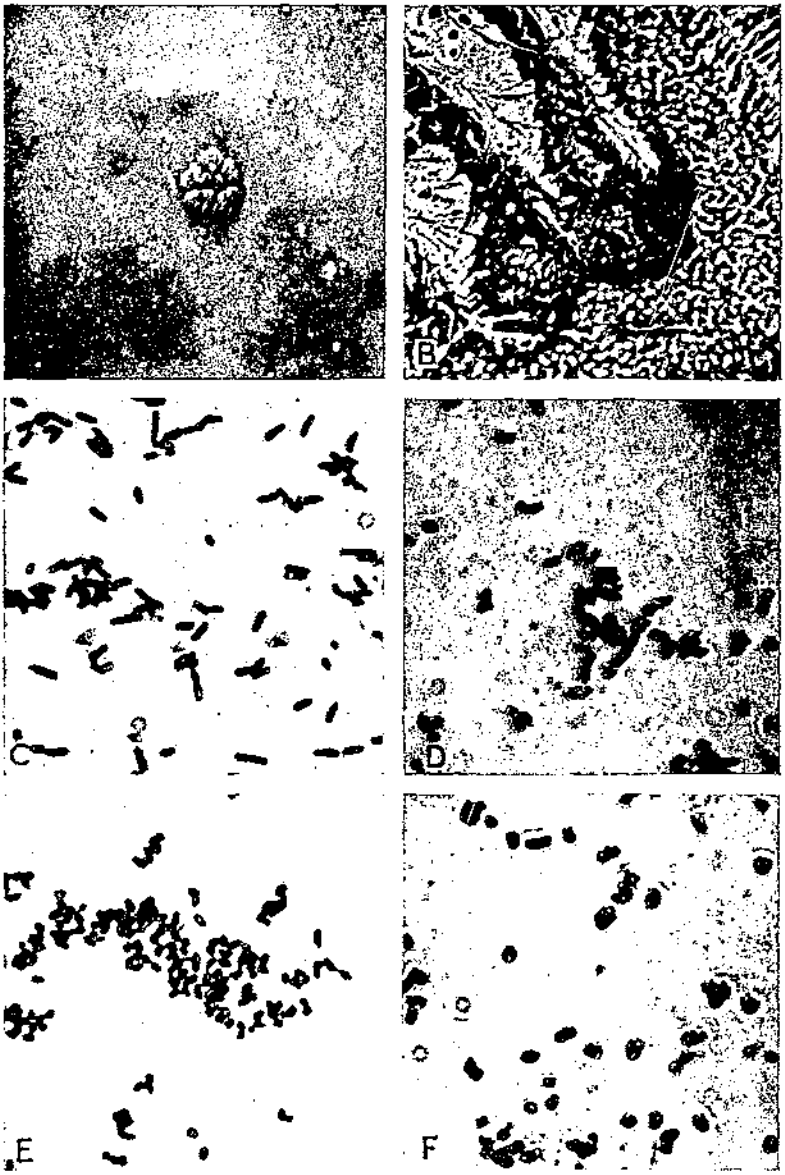
Red growths upon medium A and hides resulting from the use of contaminated salts were invariably found by direct microscopic examination to consist largely of cocci,[6] with either diplococcal or apparent sarcinal arrangements. With these cocci there were usually associated threadlike organisms and other microorganic forms resembling perithecial bodies of fungal growth. There was great variation in size of the cocci even within the same culture. Often the individual cells within a single sarcinalike group showed marked differences in size. These cocci appeared also to be encapsulated. Under low magnification and in wet mounts a red color could often be seen associated with large aggregates of cocci. These masses of cells were usually embedded in a gelatinous slime, making differentiation of the individual cells difficult. The cocci in some of these masses had no evident regularity of arrangement, whereas in others they were arranged in a definite manner. One type of regular arrangement of coccal cells observed is shown in plate 1, A.

When typical red slimy growths from medium A and from salted hides and skins were dried in thin films, amorphous masses adhered to the crystallized salt (pl. 1, B). These masses did not dissolve in dilute hydrochloric acid, but instead became swollen and when crushed under a cover glass were found to consist essentially of cocci similar to those observed in the slime before drying.

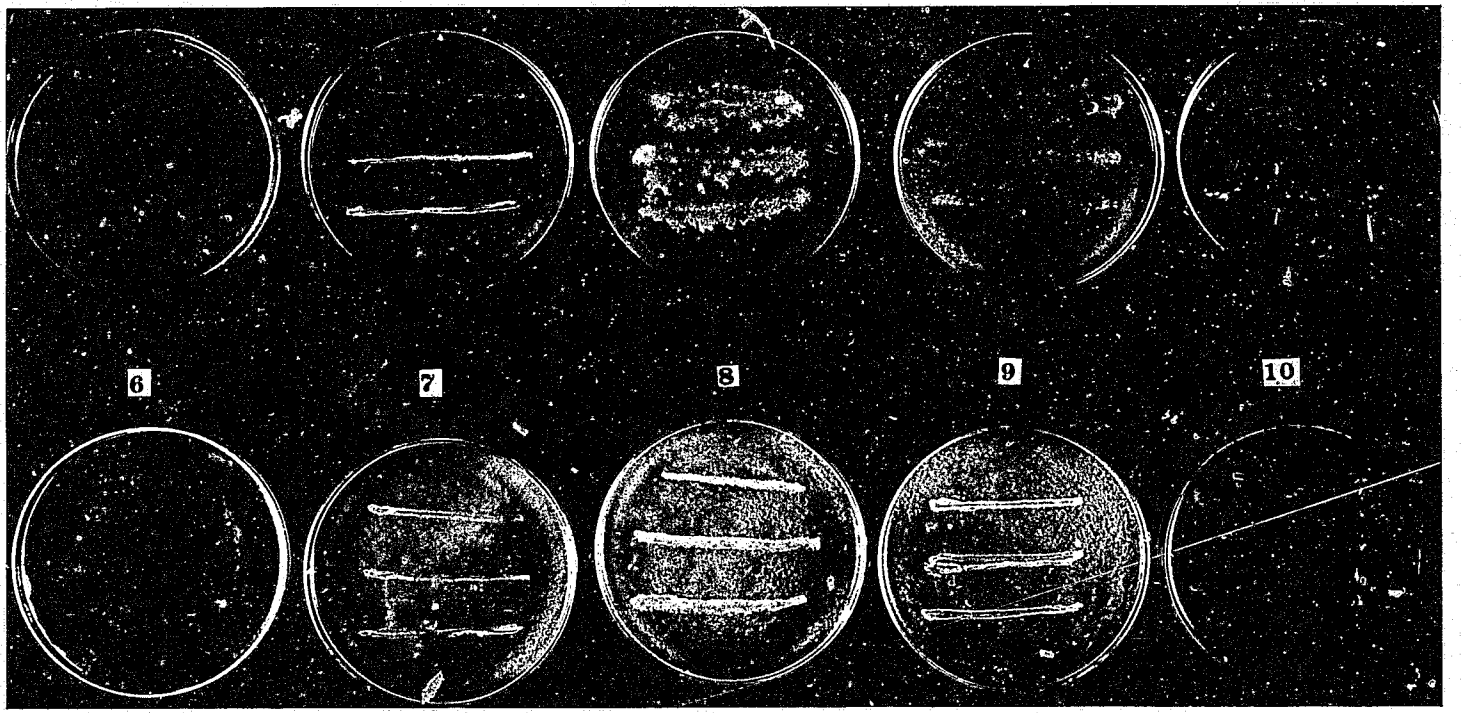
To classify such coccal cells by direct microscopic examination would be hazardous, since in dried and stained preparations they could be preliminarily classified as large-celled bacterial sarcinæ or diplococci, whereas in wet preparations they closely resembled certain of the lower algae, the fragmentation spores of actinomyces, and vegetative resting-spore stages in the life cycles of certain fungi.

Attempts were made to secure isolations from the red slimy growths on hides and on medium A by using a solid medium made of calfskin broth, agar, and 25 percent sea salt. This was called medium B. Most luxuriant red growths were usually obtained at pH 8.0 (pl. 2). By repeated streak transfers it was found possible to maintain such cultures practically indefinitely on this medium, on which they presented a fairly constant morphological picture of cocci with sarcinal or diplococcal arrangements, very similar to the red growths from which they were cultured. These cultures, in general, appeared to be similar to the halophilic cultures reported by Bitting (*4*), Kellerman (*20*), Beckwith (*1*), Lloyd (*23*), and Robertson (*26*).

[6] The terms coccus and cocci in this bulletin refer to spherical cells and not to their more restricted meanings as associated with bacteriological nomenclature.



A, Group of coccal cells found in red growth induced by contaminated salt. Unstained wet mount. B, Amorphous mass adhering to salt crystals. Unstained. C, Twenty-four hour growth of rod form on dextrose agar. D, Forty-eight hour growth of rod form on dextrose agar showing large granulated cell structure. E, Cells from young cap. F, Cells from old dried cap. A and B × 1,000; C, D, E, and F × 1,800.



Growth of mixed red chromogenic cultures on medium B adjusted to pH 6.0, 7.0, 8.0, 9.0, and 10.0, respectively.

It was noted early in this study that, when maintained on a medium containing 25 percent salt, these cultures stained as cocci only. When transferred to media of lower salt concentrations, however, they produced not only cocci but organisms resembling ovoid-bacilli, threadlike bacteria, and mold growths of one kind or another. These cultures were unquestionably mixed. This fact was also recognized by Lloyd (*23*), Robertson (*26*), and Stather (*29*) in isolating and studying cultures on media of high salt content.

The actual nature of the organisms involved in these cultures is a moot question. Many of the investigators would class them as obligate halophilic bacteria. Robertson (*26*) reported an inability to secure growth of such cultures with sodium chloride concentration below 6 percent. Others apparently would interpret these growths as pleomorphic strains of ordinary bacteria. Stather (*29*) reported that from such halophilic cultures he was able to isolate organisms similar to those he found normally on hides and that the halophilic cultures he received from Lloyd could be induced to grow on media containing little or no sodium chloride.

Red cultures developed on medium B were suspended in 25 percent sodium chloride solutions. When brined in these suspensions and resalted with chemically pure sterile sodium chloride, pieces of clipped, washed, fresh calfskin showed a flesh reddening in about 7 days at 37.5° C.

CLASSIFICATION AND IDENTIFICATION

A persistent effort was made to establish the actual identity and nature of these cultures.

Numerous investigators have clearly established marked changes in the morphology of pure cultures when they are transferred to media of increasing concentration of sodium chloride. It, therefore, seemed necessary as a first step in a taxonomic study to induce growth of these cultures, if possible, on standard media, or on media of low sodium chloride content, that is, from 1 to possibly 3.5 percent, for reproduction of comparable forms capable of interpretation and classification.

For preliminary studies a typical mixed red culture from a Turks Islands solar salt was used. This culture, identified as culture 1, had been maintained on medium B by transfer every 2 weeks for more than 6 months. A series of flasks containing medium A with salt content reduced progressively from 33 percent to zero was prepared. Inoculations were made with 0.1 cc of a 25 percent sodium chloride suspension of culture 1 from medium B, and the flasks were incubated at 37.5° C. for 4 weeks.

With salt concentrations of 7.5 percent and above, red growths developed after 7 days, the most intense reddening occurring at the higher concentrations. By the end of 28 days, nonfruiting mold hyphae had developed in the flasks containing 7.5 to 15 percent of added salt. In the flasks containing less than 7.5 percent added salt the medium darkened, and the rice was digested without evident development of reddening. A grayish slimy growth developed in the absence of any added salt. Uninoculated controls remained unchanged.

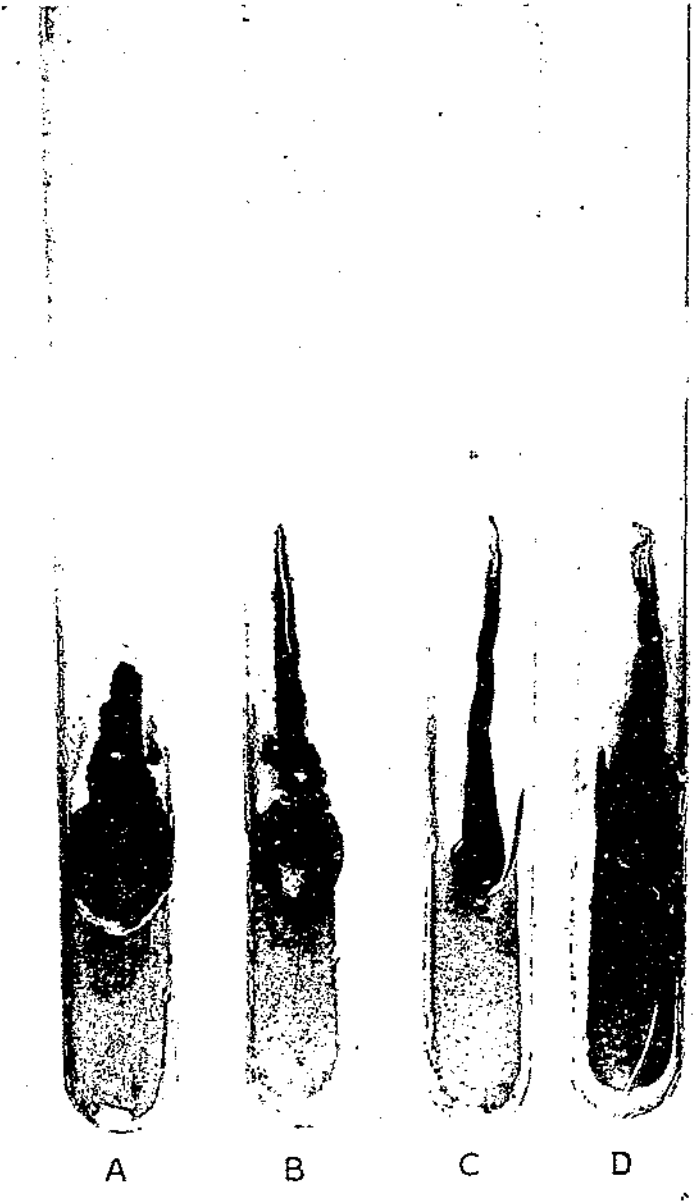
Microscopic examination of smears showed a preponderance of cocci with 15 percent and more of salt. Mold forms appeared first at concentrations below 15 percent, and at the lowest concentrations of salt the cocci were replaced by ovoid bacilli, bacteria, yeast, and mold forms. These results strongly suggest that both bacteria and fungi are involved in the development of such growths.

A number of solid media were tried for the isolation of pure cultures. These may be summarized briefly as calfskin-broth-peptone-agar, alone and with the addition of 2 percent of soluble starch, sucrose, and mannose, respectively. Crude solar salt was used to vary the sodium chloride content from 1 to 25 percent. Inoculations were made with culture 1. All growths appearing on media with high concentrations of salt were immediately transferred to media of the next lower concentration of salt. By a continuation of this system 10 pure cultures were finally isolated that grew well on a dextrose-agar medium containing only 1 percent of added salt. By preliminary examination these were classified as one variety each of a *Bacillus*, a Bacteriaceae, an Actinomycetales, a *Torula*, an *Alternaria*, a Phycomycetes, *Catenularia fuligineae*, and three varieties of *Aspergillus*.

These cultures were experimented with extensively. They demonstrated varying degrees of chromogenesis and of salt tolerance on artificial culture media. However, by direct inoculation on medium A or on salted calfskin none produced red growths which were in any respects similar to those produced by naturally contaminated salts. Negative results were also obtained after attempting to build up salt tolerance in these cultures by propagation in brines and by using mixtures of the cultures to take care of possible symbiotic relationships.

Red growths similar to culture 1 were obtained on medium B from a large number of different salts and when studied in the same manner invariably yielded at least two or three varieties of bacteria and fungi. *Catenularia fuligineae*, aerobic spore-forming bacteria, and varieties of *Alternaria* and of *Aspergillus* were usually found. Actinomycetales, pink yeasts, and gram-negative bacteria were found occasionally. The most common types of *Aspergillus* found belonged to the *A. versicolor* and *A. sydowi* groups.

As a preliminary study to determine whether or not the common fungi isolated from mixed red growths could grow in pure culture in brines of gradually increasing concentrations of salt and were not merely carried in the slime of other organisms as dormant spores, 12 strains of green *Aspergillus*, which had been identified as belonging to the *A. versicolor* group, were inoculated into filtered, nutrient sterilized brines made up to a concentration of 10 percent. These brines were incubated at room temperatures for 8 months, during which time, through slow evaporation, they became saturated, with the formation of large salt crystals. A profuse slimy growth formed beneath the surface of each brine. Direct microscopic examination of the slime showed no forms typical of the normal growth of the cultures inoculated, except occasional hyphae. However, culturing this slime on Czapek agar resulted, in every instance, in the recovery



Cultures of red chromogenes isolated on dextrose agar. A and B, Mealy, C, hard and horny, D, soft and gelatinous.

Caps and encystments developed in mealy strains of red chromogenes. × 32.

only of the organisms inoculated. This indicates that at least certain types of fungi, in a vegetative state, are capable of withstanding conditions which exist during a slow evaporation such as occurs in the deposition of solar salts. This probably accounts for the enormous numbers of fungi found on plating solar salts and shows that not all of them, at least, are carried as aerial spores.

It thus appears that cultures isolated on media containing high percentages of salt may carry, even through repeated transfers, a large variety of microorganisms, either as static spores or as pleomorphic growths, that are unrecognizable by the ordinary methods of staining and direct microscopic examination.

Numerous attempts to develop differential media of low salt content for isolation of the specific organisms causing reddening failed. It was therefore decided to resort to chance isolation, a large number of dilutions and many platings of each being used in the hope of eliminating associated organisms that in lower salt concentrations apparently overgrew the particular reddening organisms. This was done with solutions of many of the salts known to be contaminated with red chromogens. They were cultured in dextrose broth at 20° C. for 14 days and then plated in dextrose agar without added salt. All plates were incubated at 30° for 30 days. Most of the plates were overgrown with spreading bacterial colonies and molds. An appreciable number were obtained, however, in which there were red colonies within clearly defined zones of inhibition between mold colonies.

These red colonies were cultured on dextrose-agar slants containing no added salt. A few of the colonies proved to be pink torulae, but most of them were cocci. The latter were peculiar in that sarcinalike, staphylococcal, diplococcal, and short-chain coccal arrangements were usually found in one and the same culture. Young growths were slimy and sticky, but with age became either mealy, hard, and horny, or soft and gelatinous, depending upon the strain (pl. 3).

These growths were suspended in saline and streaked on dextrose agar, and the subsequent colonies were studied. By repeated transfers in this manner, apparently pure cultures were obtained.

Morphological studies of these cultures on dextrose agar containing no added salt showed that prior to the appearance of the coccus form the organisms developed as short spindle-shaped rods approximately 0.5 by 2.5 microns in size (pl. 1, C).

The coccus form developed within about 48 hours incubation at 30° C. By transferring these cultures every 24 hours the rod form could be maintained to the exclusion of the coccus form. After 48 hours the cultures invariably developed not only the coccus form but also large granulated cells (pl. 1, D).

In strains that became mealy, caps or encystments developed coincident with the appearance of the coccus forms. After about 7 days incubation the caps were well developed and could be picked off readily with a needle (pl. 4).

Microscopic examination of smears from such caps revealed cocci of approximately 1 micron in diameter but no rod forms or large granulated forms (pl. 1, E and F).

RESULTS OF SUBCULTURING THE COCCUS FORMS

Subculturing the coccus forms from the old caps on dextrose agar and incubating them for 24 hours at 30° C. resulted in a preponderance of elongated diplococcuslike forms ranging from apparently true diplococci to elliptical rods in pairs. After from one to three further subcultures, made every 24 hours on dextrose agar, only typical spindle-shaped rods developed in the first 24 hours incubation. Coccus forms began to appear within 48 hours, and upon prolonged incubation caps and encystments were reproduced.

Growth of these cultures upon other substrates and at different temperatures was somewhat modified from that just described. On sucrose agar, growth was profuse but in rod form only. On plain nutrient agar both the coccus and rod forms were present, the former usually occurring in greater numbers. On plain nutrient and dextrose agars containing various quantities of salt the coccus form predominated, being greater at the higher salt concentrations and also greater at 37.5° C. than at 30° and 20°.

These observations indicate that age, comparatively high osmotic pressures, restricted available moisture, and rather high incubation temperatures favor development of the coccus form, whereas under opposite conditions the organisms tend to develop in the rod form only.

Coloration of the cultures was greater at 37.5° C. than at 30° and was intensified by light and age. The pigment produced was insoluble in water, slightly soluble in absolute ethyl alcohol, and readily soluble in strong alkalies, chloroform, ethyl ether, and carbon bisulphide. With concentrated sulphuric acid, the dried ethyl ether extract gave a lipocyanin reaction ranging in the different strains from violet brown to fairly intense blue, indicating a carotinoid pigment. Preliminary spectrophotometric readings made on a carbon bisulphide solution of the dried ether extract from one typically brick-red culture gave a maximum absorption between 460mμ and 500mμ, indicating a pigment closely related to bacteriopurpurin.

Studies of these organisms in differential culture media showed that they grow well on potato agar slants and that over a period of 30 days they slightly reduce nitrates to nitrites and ammonia, do not change litmus milk at 30° C., and do not liquefy gelatine at 20°.

These observations show that the organisms do not belong to the group of *Serratia*, since this group is characterized by gelatine liquefaction and rapid alteration of litmus milk. Also, contrary to the behavior of these cultures, pigment production in *S. marcesens* (*Bact. prodigiosus*) is retarded by higher temperatures and exposure to light. That the organisms are not classifiable as *Pseudomonas* is evidenced by the insolubility of the pigment in water.

Other possible classifications for these organisms are in the Thiobacteriales under the Rhodobacteriaceae, or in the Myxobacteriales. The presence of a carotinoid pigment closely related to bacteriopurpurin and the ability of these organisms to grow in a broth composed of 1 percent each of peptone, potato starch, sodium sulphide, anhydrous sodium carbonate, and sodium chloride, in which they demonstrate family types of growth either with definite membranes inclosing the families or with the cells held together loosely

by a gelatinouslike substance, suggest classification under the Rhodobacteriaceae (Buchanan). While the organisms did not produce definite stalks or fruiting bodies on potato agar, some did develop cystlike resting stages resembling those described for certain members of the Myxobacteriales.

Although the Thiobacteriales and the Myxobacteriales are imperfectly understood, they both demonstrate an approach to the production of specialized cell structures within the gross morphology of their colonies. This characteristic has been observed in the morphological studies with the cultures isolated. Further work with the individual strains will be necessary before they can be definitely classified.

SALT TOLERANCE OF ORGANISMS ISOLATED FROM SALT

Organisms of the type described were isolated from crude solar salts from South America, Spain, Turks Islands, Utah, and California, and from four domestic artificially evaporated grainer salts. Young (48-hour) dextrose-agar cultures of some of these organisms were inoculated on pieces of freshly flayed, clipped, and washed calfskin that had been salted with an excess of sterilized salt (50 percent by weight of the wet skin). For controls an equal number of pieces of calfskin were salted in the same manner and left uninoculated. All pieces were incubated in deep culture dishes over water at 37.5° C. No flesh reddening developed within 30 days.

A similar experiment was made with old cultures prepared by incubating dextrose-agar slants for 2 weeks and then drying them for 2 weeks over calcium chloride. Flesh reddening developed in from 15 to 30 days on all inoculated pieces of calfskin, whereas the controls showed no reddening in this period. It was recognized that these results did not eliminate the possibility of symbiosis, since other organisms carried on the skin itself may have played a joint part in the development of flesh reddening. Therefore, inoculations were made from similarly prepared old cultures to medium A containing 25 percent of salt by weight. After incubation periods of 30 days or more, red growths appeared on this medium. In these experiments with old cultures it will be noted that reddening developed sooner on calfskin than on the sterilized hide-broth-rice medium.

Study of the salt tolerance of these organisms was made on a solid medium composed of hide broth, 1 percent peptone, 0.5 percent potato starch, and 1.5 percent agar, and adjusted with cresol red to a pH of 8.0. The sodium chloride concentration ranged from 0 to 28 percent by weight.

Transfers were made from 24-hour and 48-hour dextrose-agar cultures of the red chromogens, and the slants were incubated at room temperatures (25–30° C.) in desiccators over water for 28 days. Daily readings were taken. The results with a typical culture are shown in figure 1.

In general, the growth of the young culture inoculum was not inhibited by concentrations of from 0 to 10 percent. Growth was delayed, however, by concentrations of from 12 to 16 percent, was markedly inhibited by concentrations of from 18 to 22 percent, and was entirely stopped by concentrations of from 24 to 28 percent.

This tolerance for salt is similar to that reported by previous workers and confirmed in this laboratory for strains of *Staphylococcus aureus*, *S. albus*, *Bacillus subtilis*, and *B. megatherium*.

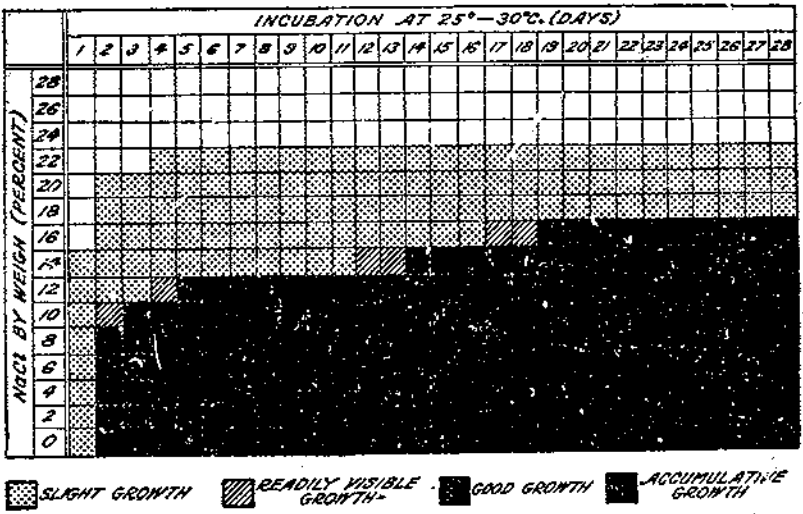


FIGURE 1.—Growth of red chromogen on solid media containing increasing percentages of sodium chloride. Inoculation from young (24-hour) dextrose-agar culture.

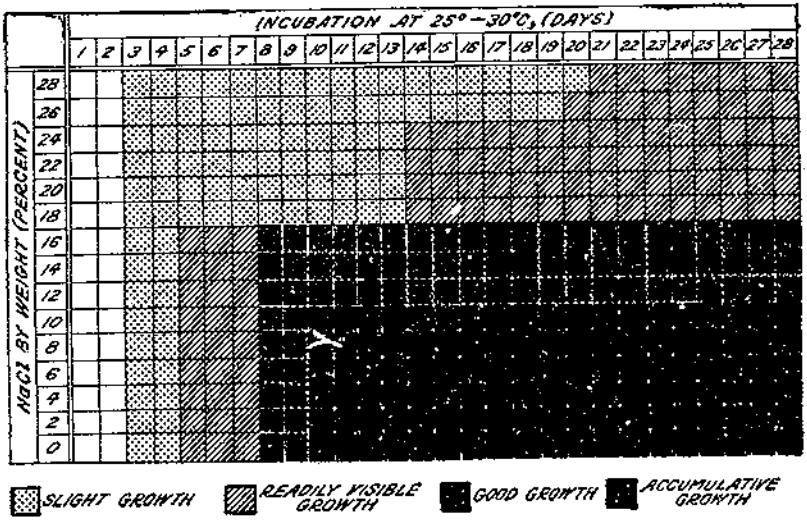


FIGURE 2.—Growth of red chromogen on solid media containing increasing percentages of sodium chloride. Inoculation from old dried dextrose-agar culture.

Another experiment was then made in a similar manner with the same organisms transferred from old (14-day) dextrose-agar cultures that had been subsequently dried over calcium chloride for 2 weeks. Daily readings were made. The results with a typical culture are shown in figure 2.

In this series readily visible growth occurred at all concentrations of sodium chloride. On the slants containing the smaller quantities of sodium chloride good growth was obtained, though it was slower in appearing than when inoculation was made from a young, actively growing culture. On concentrations above 16 percent growths became readily visible after from 2 to 3 weeks' incubation, although they were slower in developing than those on media of lower concentrations. On media containing 24, 26, and 28 percent of sodium chloride pigmentation was retarded, that is, the cultures, even at the stage of readily visible growth, were slower to develop red coloration than cultures at lower concentrations, and in some instances required prolonged incubation periods for the development of this color.

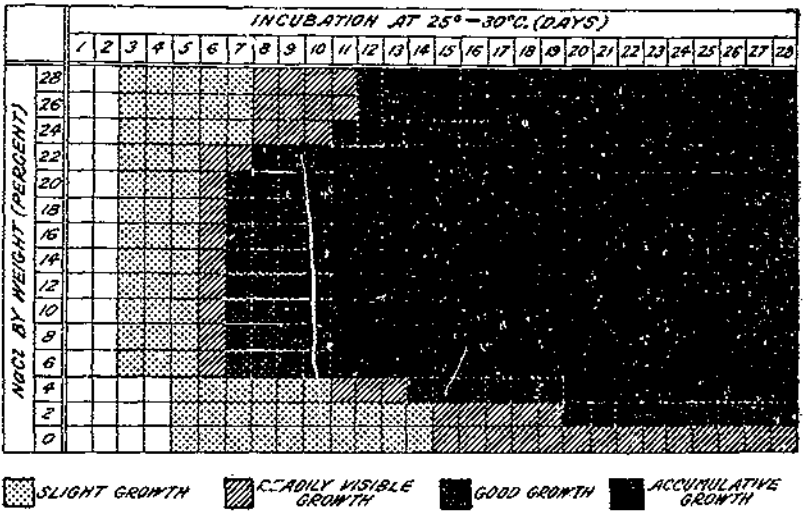


FIGURE 3.—Growth of red chromogen on solid media containing increasing percentages of sodium chloride. Inoculation from vegetative growth on 28 percent sodium chloride agar.

In an attempt to obtain more luxuriant growth at high salt concentrations, cultures of three of these organisms on 28 percent sodium chloride agar were repeatedly transferred to the same medium. In each case the subcultures were transferred only after they had developed red color. Growths gradually became more luxuriant, and after 5 or 6 transfers were typical of the red growths obtained by direct plating of contaminated salts on salt-saturated media.

Transfers from the sixth subculture of each strain were made to the same series of media as was used previously in determining the salt tolerance of young cultures and old dried cultures. Daily readings were made. The results for a typical culture are shown in figure 3.

A comparison of the results given in figure 3 with those given in figures 1 and 2 shows that when the organisms have previously been subcultured upon media of high salt content a marked building up of salt tolerance can be brought about. When transferred from sub-

cultures on a high salt concentration medium, the organisms grew at all concentrations of salt. Good growth, however, first occurred at concentrations of from 6 to 22 percent, inclusive. The accumulative growth in the upper range of salt concentrations is especially significant.

DISCUSSION

From an examination of a large number of salt samples it has been found that organisms capable of causing flesh reddening occur almost universally in crude solar salts and frequently in open-pan evaporated or grainer salts. All samples of kiln-dried, vacuum-pan evaporated, and mined salts so far examined were found to be free from such organisms. It seems probable that most of the flesh reddening of hides is caused by the use of contaminated salts.

On pieces of calfskin salted with contaminated salt, reddening developed at widely different temperatures, ranging from 20° to 55° C. Reddening, therefore, is not necessarily dependent upon heating. Relatively high humidities and slightly alkaline conditions were found to favor reddening.

Chromogenic cultures were isolated from reddened hides and contaminated salts on media containing large quantities of sodium chloride. When transferred progressively to media of lower concentrations of salt these cultures were found to consist of a large variety of microorganisms. Studies of isolations made from these cultures show that they were mixed and included molds as well as aerobic spore-forming and other varieties of bacteria. These studies also indicate that fungi may grow in the presence of high concentrations of salt in forms not readily recognizable by direct microscopic examination.

When these mixed chromogenic cultures were subcultured in dextrose broth and then on dextrose agar, a group of organisms capable of causing reddening were isolated in apparently pure cultures. These have been identified as belonging among the higher bacteria, probably more definitely classifiable in the Thiobacteriales under the Rhodobacteriaceae, or in the Myxobacteriales.

Gelatinous caps and encystments filled with cocci were formed in some of these cultures. These structures may explain the ability of the organisms to survive over long periods of drying and rest, and to adjust themselves to marked changes in salt concentration and other factors of environment. This is indicated by salt-tolerance studies in which transfers from old dried cultures with well-developed caps and encystments were capable of growing over a wider range of concentrations of salt than transfers from young vegetative cultures.

The Thiobacteriales and Myxobacteriales have been isolated largely from sea water, river water, stagnant water, soil, mud, and decomposing vegetable matter, and therefore may be fairly common contaminants of the hide or skin of living animals. Such a wide distribution might account for cases of hide reddening reported under circumstances that would give every reason for the belief that the salt used for curing was not originally contaminated with the organisms. However, the salt-tolerance studies of the organisms described indicate that when infection is from a source other than that of the salt the development of reddening probably will be much slower and

not nearly so intense as when the organisms have had an opportunity to acclimate themselves to a high salt environment. It has been shown that the ability of these organisms to withstand large quantities of salt can be markedly enhanced by repeated propagation on media of a high concentration of salt.

It has not been possible yet to determine definitely the damage to hides and skins resulting specifically from the reddening organisms described. A few cases have been noted of serious damage and destruction of hide substance in the presence of a heavy infection of reddening organisms. On the other hand, reddening has been allowed to develop on pieces of salted hides and skins under favorable conditions in the laboratory, and damage was not evident from a cursory examination. Lloyd says that the evidence of damage due to reddening is slight. Stather, however, associates hair slip and unevenly finished leathers with advanced cases of reddening. It is difficult to see how definite conclusions can be drawn from commercial specimens and samples because of the certainty of their infestation by a highly mixed microflora and the consequent impossibility of fixing upon any one type of organisms the responsibility for any defective conditions manifested.

The possibility of damage from the reddening organisms alone, from associated fungi and bacteria alone, or from the combined action of several types of micro-organisms must be recognized, as well as the influence of the many different environmental conditions. For the present it must be admitted that the responsibility for damage by specific types of organisms is an open question pending findings from controlled and specific studies.

SUMMARY

A large collection of commercial salts for curing hides and skins was examined for the presence of micro-organisms that cause reddening. Thirty-four out of thirty-five samples of crude solar-evaporated salts and 25 out of 39 open-pan evaporated grainer salts were found to be contaminated with red chromogens. All the kiln-dried solar-evaporated salts (12 samples from 1 source), all the vacuum-pan evaporated salts (17 samples), and all the mined or rock salts (62 samples) were found to be free from contamination with these organisms.

A modification of Clayton and Gibbs fish-salt-rice medium, in which the fish broth was replaced by hide broth, was found to be reliable for culturing salts to determine the presence of red chromogenic micro-organisms that cause flesh reddening on salted hides and skins.

No correlation was found between the pH of the salts and the presence of the chromogenic organisms. In general, commercial salts for curing hides and skins are distinctly alkaline.

Flesh reddening developed on pieces of clipped washed calfskin salted with naturally contaminated salt. Development was favored by high relative humidities and an alkaline reaction.

Cultural studies of red chromogenic growths on media of high concentrations of salt showed them to consist usually of a highly mixed microbial flora.

By subculturing mixed red chromogenic growths obtained on media of high concentrations of salt in dextrose broth, and then employing a large number of dilutions and many duplicate platings of each in dextrose agar with no added salt, red chromogenic micro-organisms have been isolated in apparently pure culture. These isolations have been made from crude solar salts from South America, Spain, Turks Islands, Utah, and California, and from four domestic artificially evaporated grainer salts. Under specified conditions these organisms are capable of producing flesh reddening on salted hides and skins.

Studies of the salt tolerance of these organisms showed that their ability to withstand high concentrations of salt is markedly enhanced by repeated propagation on media of high salt content.

These organisms have been identified preliminarily as belonging to the higher bacteria, either in the Thiobacteriales under the Rhodobacteriaceae, or in the Myxobacteriales.

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