THE RED DISCOLORATION OF DRIED COD FISH

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By

Margaret E. Kennedy.

Macdonald College,

McGill University.

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THE RED DISCOLORATION OF CODFISH.

The codfish industry provides for the Dominion of Canada a revenue of no inconsiderable amount - according to government statistics, a revenue which surpasses that derived from all other fish enterprises, salmon alone excepted. In figures, the total quantity of cod caught and landed during 1919 was 2,606,770 cwt., which, when marketed, yielded a return of $9,987,612.

The fish is marketed in a variety of ways, such as, - fresh, green salted, smoked fillets, smoked, boneless, canned, cod liver oil, - but by far the greatest amount as dried cod. From the total catch of cod in 1919, 605,135 cwt. was sold as dried codfish, providing a return of $6,811,315 - an amount which exceeds two-thirds of the total revenue from cod industries.

Practically all of the dried fish prepared in Canada is exported. About one-eighth of it finds a market within the British Empire, while the remainder goes to foreign countries, where it is purchased in amounts varying from 6 cwt. to 186,933 cwt. It is unnecessary to enumerate the various countries to which this product is shipped; suffice to say they are numerous and widely scattered. Incidentally, greater amounts are sent to United States than any other one country.

Recently dried cod along the Atlantic Coast has become infected in such a way that the surface of the fish acquires a

distinctly red color. This, naturally, detracts from the wholesome and palatable appearance of the fish and causes an unmarketable product, which, obviously, is a loss to the trade. Complete figures for such loss have been unobtainable, though individual dealers estimate their personal deficits from two and a half to forty percent. The infection, though comparatively new to the Canadian trade, has existed at various times, and in different countries for, at least, the last forty years, during which time investigations as to the nature of the discoloration have been carried on rather from a scientific than from an economic standpoint. In the past the fish was marketed, usually, during the colder months of the year, so that there was not so large a percentage of spoilage as prevails to-day; and whatever loss dealers did experience from reddening was regarded more or less as incidental and unpreventable. Not so to-day. Progressive civilization demands not only increasing attractiveness in food displayed for sale, but also a product procurable at all seasons of the year - preferably out of season. This necessitates infinite care in preparation, requiring additional labor, and adding to the expense of production; but more than that, fish marketed during the warmer months of the year seems more susceptible to the red infection than that marketed during the colder months. To-day, dealers are alarmed at the loss occasioned from this source, and anticipate a satisfactory remedy. Consequently, we are confronted with a problem of considerable economic importance.

A brief review of the investigations carried on to determine the cause of this discoloration of dried codfish, and the nature of the organism or organisms concerned has, at least, historical significance, and may not be out of place in this report. According to all available literature on the subject, the first work was done in 1878 by Farlow,(1) who studied this peculiar reddening at Gloucester, Massachusetts, during the summer of the year mentioned. Farlow maintained that the discoloration was due to Clathrocystis roseo-persicina, belonging to the Schizophytae. The plant consisted of very minute cells, filled with red coloring matter, and imbedded in a mass of slime. He also observed the presence of another organism, which he called Sarcina morrhuae - with the following description - "cells colorless, 5-8 μ in diameter, united in fours, and surrounded by a thin, hyaline envelope. Colonies 10-20 μ, heaped together in irregularly shaped, lobulated masses. Habitat on putrefying codfish in company with Clathrocystis roseo-persicina, Gloucester, Massachusetts, 1878."

Writing from Gloucester in 1884, Mr. H.A. Clark(2) stated that boneless fish packers at Gloucester were using a preparation called preservaline, which appeared to be quite effective in preventing reddening. When this substance was analyzed it proved to be nothing but a solution of borax and common salt.

In 1884, Dr. S. Bertherand(3) published an account of poisoning among French troops in Algiers, due to codfish which

had a red color along the spine. This condition was attributed by M. Megnin to a fungus which he named Coniothecium bertherandi. In 1885, the editor of the "Revue Mycologique," M. Casimir Roumeguere, raised the question of the identity of the Coniothecium bertherandi and the Clathrocystis of Farlow.

Appearing almost at the same time as Farlow's note on Sarcina morrhuae was a paper by Poulsen, in which he described a new species, Sarcina litoralis, found on mud of salt marshes, near Copenhagen. These Sarcinae, despite micrometric differences, were thought by Poulsen to be the same organism, and later Saccardo and Berlese recognized the species on the surface of codfish sent from Algiers. Saccardo and Berlese considered Coniothecium bertherandi of Megnin to be identical with Sarcina litoralis, Poulsen, which they said Zopf considered a condition of Beggiatoa roseo-persicina, under which name Zopf included Clathrocystis roseo-persicina as a Zoogloea form. Farlow refused to believe that the two forms belonged to the same species, contending that neither size of cells nor conformation supported the view that sarcina was a stage of Beggiatoa.

In subsequent work by Farlow (3) a third species was recognized which he called Oidium pulvinatum, but later abandoned for Oidium morrhuae, as the former name had already appeared. He found it difficult to decide whether the organism was a torula or an oidium, as the color and texture of spores resembled

torula, while other characteristics were more like oidium. In an article by Saccardo and Berlese this species was said to occur in Algiers in company with clathrocystis and sarcina, and they considered it a torula rather than an oidium - calling it Torula pulvinata.

In 1885 the French Minister of Commerce, believing that red codfish had caused several cases of poisoning, issued a circular prohibiting its sale. This proclamation led Mauriac[4] to make some investigations as to:- (1) the history of the cases of poisoning caused by spoiled codfish; (2) the physical characters of codfish which produced these cases of poisoning; (3) the nature of red color in codfish - its character, development and prevention; (4) the nature of the poisonous substance contained in putrefied codfish. The history of the cases showed symptoms similar to cholera - some cases were very light, while others suffered more severely, and one patient died. In four out of seven cases of poisoning the codfish did not show any red color, while a putrid odor and crumbling flesh were observed in all cases. In the one instance where symptoms were so violent as to cause death the codfish had a deep yellow color, bad odor, and bad flavour, its flesh crumbling to pieces - very definitely showing signs of putrefaction. These facts, to Mauriac's mind, proved conclusively that the poisoning should be attributed to the poisonous substances produced by putrefaction of animal matter. He believed the red was due to a fungus - the name of which varied with the investigator, while Carles and Gayon,

who worked on the cultivation of these organisms, ascribed the red on cod to two organisms - a bacillus and a micrococcus. They also found that the color could be produced on certain kinds of salt crystals. Under the fourth heading Mauriac concluded that putrefied codfish, or any spoiled food of animal origin, contained ptomaines, or poisonous substances, but that these were in no way connected with the organisms which produced reddening; or, in other words, that the reddening had nothing to do with the putrefaction of the codfish.

In 1886, "Red" cod was observed in Scotland, where J.C. Ewart, M.D.,[5] and Alexander Edington, M.B.,[6] both of the University of Edinburgh, carried on a number of experiments. Although Dr. Edington found several forms of bacteria and micrococci present on the fish he examined, he believed the production of red color was due to a form, which he called Bacillus rubescens; which also existed in salt used for curing. The description of this organism was - "small rods, non-motile, measuring 0.3 μ - 0.5 μ thick and 1.5 μ - 4 μ in length, also in the form of Leptothrix measuring 5 μ - 25 μ long; spores 1 μ in length and 0.5 μ in diameter". It did not grow well at ordinary temperatures, but when nutrient gelatine after being inoculated was kept liquid and incubated it grew readily, forming a thick, globose pellicle on the surface, showing after a few weeks a pink color on its lower surface, and in some tubes small fluffy points in the depths of the gelatine. On agar

it did not grow well at ordinary temperature , but when incubated formed a large greyish film over the whole surface, and gave rather a cracked and blistered appearance. On bread paste it grew well, from a pale pink to a deep red. The toxic effect of red cod was tried upon white mice with no fatal results - which led these investigators to conclude that red cod, in itself, was not especially injurious.

Dr. Alexander Layet's[7] observations, in 1886, on the reddening of codfish were directed more particularly to the hygienic side of the question than to the nature of the causal organisms; and from that standpoint he considered that the red in itself was not the cause of indisposition produced by spoiled codfish, that the poisonous character of codfish depended entirely upon the state of its putrid decomposition. Regarding the nature of the organism producing red codfish, Layet found one which was spherical in appearance, where there was frequently transverse segmentation, and sometimes one or both, hemispheres separated, dividing the entire sphere in fours. In no instance was there the slightest trace of a mycelium, which discredited the theory that the discoloration was due to a fungus. Layet felt certain it was an alga, but doubted if it were a Beggiatoa, like Clathrocystis Farlow.

M. le Dr. le Dantec,[8] in 1893, found several chromogenic organisms associated with reddening of codfish - a yellow coccus, a golden yellow coccus, a rusty colored coccus, and three organisms which he believed capable of producing red on codfish -

a bacillus, a coccus, and a yeast. The bacillus varied from 4 μ - 10 μ or 12 μ in length and sometimes more, and was about 2 μ in diameter. It was motile and formed terminal spores. Apparently le Dantec had little difficulty in isolating and cultivating the rod. It caused rapid clouding in bouillon, liquefied gelatine slowly, but did not grow well on potato. At room temperature it produced better color than at incubation temperature. The coccus was very difficult to obtain in pure culture. It developed very slowly and formed very small colonies. Experiments upon dogs, rabbits and guinea pigs confirmed previous conclusions that the organism was not pathogenic - when injected in pure culture nor when taken directly from fish. Unlike Farlow, le Dantec did not find any proof that the red originated from Cadiz salt.

Høye's[9][10] first two papers in 1901 and 1904, on dried codfish, dealt more with moulds, especially Torula Epizoa, than organisms causing red discoloration. He was particularly interested in the various kinds of salt, the infection of storage houses and methods of disinfection. In his third[11] paper he described a number of organisms found on dried codfish - Torula epizoa, Torula minuta, Sarcinomyces islandicus, (which did not affect the fish nor produce a disagreeable taste or odor), Sarcinomyces niger, and Sarcina sporigenus, also three species of yeast, varying in size from 3-6 μ, growing well on salt media - especially about 10%, cells becoming larger as the percentage of salt increased, but no color produced. In addition to these, three species of bacteria were described -

a coccus, producing yellow to yellowish red pigment, a second coccus of waxy yellow color, and a bacillus, which was non-chromogenic. All three organisms were halophylic, their optimum salinity being from ten to fifteen percent. In concluding, Høye stated that under the name of red bacteria there were at least two species concerned - a medium sized sarcina and a micrococcus; in fact, there were probably three species, but he did not know which was the more dangerous.

In 1908, Høye[12] examined thirty-six samples of salt to study Torula epizoa, but found also a red oval coccus, 1 μ in diameter. This organism formed slimy red colonies on 17% salted meal. From thirty samples of fish Høye found nine containing red bacteria, which, microscopically, were slightly oval, capsulated cocci, about 1 μ in diameter, often appearing as diplococci, whose growth at room temperature was exceedingly slow.

Working at Gloucester during the summer of 1907, Beckwith[13] found, from numerous microscopical examinations, the presence of Clathrocystis roseo-persicina, various sarcinae, and bacterial forms, but, particularly, a diplococcus. After unsuccessful attempts to culture the organism on laboratory media, two special media were devised - the first made from shredded cod and distilled water, to which two percent agar was added, and the second made from "pickle" diluted with an equal volume of water, and again adding two percent agar. Red colonies developed in four days on such media at a temperature varying from 20°-32°C. Upon microscopical examination the red

form appeared as a diplococcus, 0.4-0.5 μ - and after two years' cultivation about.1.0 μ in diameter, the adjacent sides being slightly flattened. It stained readily with all common stains, was Gram positive, non-motile, but showed marked Brownian movement, no capsule visible. After repeated transfers the colony showed some loss of color. The organism was strictly aerobic. Attempts made to culture it on ordinary laboratory media with the addition of sodium chloride showed growth on standard Beef agar to which from seven to ten percent salt had been added, but no growth on agar containing as much as twenty percent salt. As this organism conformed to no previous description Beckwith called it Diplococcus gadidarum.

In 1910, Bitting (14) made a more or less exhaustive study of the reddening of codfish, and in this connection found three organisms concerned - a coccus, a bacillus, and the cells of a mold like fungus. He found the red growth very viscous, especially when mixed with a drop of water. Bitting attributed the reddening to the coccus, which occurred in pairs and tetrads, and less frequently singly. It varied in size from 2-2.5 μ in diameter, and sometimes as large as 3.5 μ in diameter. The organism stained readily with ordinary stains and showed the presence of a capsule in both stained and unstained preparations. On artificial media the growth was very slow, 72°-75°F. seeming to be the optimum temperature. The bacillus was a motile rod, thicker at one end than the other, due to the formation of a spore. It produced no color on any media

except bread, where a pink tint developed. It grew under aerobic conditions, and stained readily with ordinary stains. No viscosity was observed. The source of the infection Bitting was unable to ascertain, but offered several suggestions as to its control.

G.J. Pierce[15], writing under the title "The Behaviour of Certain Micro-organisms in Brine", in collaboration with D.T. MacDougal on "The Salton Sea", about 1914, added to the list of red chromogenic bacteria, a very short and small bacillus, 3.2 μ - 3.3 μ wide and 3.4 μ - 3.6 μ long. He found two types of growth on agar plates - colonial and diffused. Suspecting that this small bacillus might be the cause of red color developing on cod, he tried two experiments:- (1) inoculated sterilized salt cod from a pure culture of the pink organism; (2) inoculated sterilized agar brine from pink salt cod. Colonies appeared on the agar plates of more concentrated brine - but none on the more dilute. Experiments on the fish were only partly successful, but Pierce explains that failure may have been due to some preservative used in curing the fish.

In 1915, Cobb[16] reported that the reddening of cod was not as prevalent on the Pacific Coast as on the Atlantic Coast, and attributed the fact to the lower temperature of the former.

In a bulletin on "Salt Resources of United States", published in 1919, reference was made to the red color exhibited by many alkaline salt lakes; and Lunge[17] was quoted as attributing

this to organic substances present in solution rather than to any form of animal life. However, subsequently, investigators have held opposite opinions, and at the present time, the cause of this reddening is generally believed to be due to chromogenic bacteria.

The most recent investigations on this discoloration have been carried on by Brown,(18) who believes the color to be due to two organisms - a bacillus and a spirochete, whose probable origin is the seasalt. Just what part each organism plays in developing color, or the cultural features of either have not been determined, because of the fact that it is practically impossible to obtain either in pure culture.

By way of historical summary, the following table is given:-

Author.	Organisms producing redness.	Halophylic growing in at least 10% Salt Media.	Inoculation Experiments on Salted Cod.	Solar Salt indicated as Cause.	Optimum Temp. °C.
Farlow	Clathrocystis roseo-persicina	No	None	Yes	-
Farlow and Poulsen	Sarcina litoralis	No	None	No	-
Megnin	Coniothecium bertherandi	?	None	No	-
Carles and Gayon	Micrococcus and a Bacillus together	No	None	Yes	-
Layet and others	Sarcina	No	None	No	-
Edington	Bacillus rubescens	No	None	No	30
Le Dantec	Red Bacillus of Newfoundland	No	No	No	20
Høye	Sarcina, medium sized	Yes	No	Yes	
John Olsen	Sarcina rosacea	?	?	?	-
Beckwith	Diplococcus gadidarum	Yes	Yes	-	?
Bitting	Coccus	No	Incon-clusive	Incon-clusive	23
Pierce	Bacillus	Yes	Incon-clusive	Incon-clusive	-
Browne	Spirochete Bacillus	Yes	Yes	Yes	50-55

Last September the Biological Board of the Dominion of Canada received notification of serious losses amongst the Atlantic cod dealers, due to a reddening of the flesh of the dried cod. This information was communicated to Dr. F.C. Harrison, of Macdonald College, under whose direction and assistance work was, at once, commenced to ascertain the cause, and, if possible, to offer a means of prevention. In proceeding with this work the following aspects of the subject were attempted:-

1. A description of the discoloration on the fish itself.

2. The nature of the specific organism or organisms producing red discoloration on codfish - including their experimental culture, morphological features and communicability.

3. The possible origin of the organism concerned.

4. Suggestions as to the prevention of this infection.

5. Other organisms isolated from fish and able to grow in high percentages of salt.

Through the co-operation of the Marine and Fisheries Department at Ottawa, and the courtesy of various fish inspectors throughout the Maritime Provinces, samples of reddened cod were obtained from various localities, such as - Digby, Antigonish, West Pubnico, Pictou, Annapolis, Harbourville, and Arichat in Nova Scotia, Grand Manaan and Campobello in New Brunswick, and Souris in Prince Edward Island. In all twenty samples of fish were received. Some, however, showed

definite signs of putrefaction - which occasionally had advanced so far that the red discoloration was not distinguishable from the rusty brown color of the putrefied flesh. Such samples were not kept for investigation, but immediately discarded as unedible fish. Of the fish examined the red appeared in varying intensities and amounts, from a sample where the flesh presented a very delicate pink mosaic appearance, to one where the surface of the fish was entirely covered with a dark rose-red growth - and even the salt crystals adhering to the fish were pink in color.* Most of the samples submitted were pieces cut off the fish, but in the majority of cases where the whole fish was sent the color was more pronounced along the backbone. In no instance did the color penetrate into the flesh of the fish, except where the skin was cut or broken apart, and then, did not extend beyond the surface of the fissure. It was very definitely surface growth, which developed equally well on either the white flesh or on the skin of the cod; upon the latter it was particularly noticeable in folds of the skin, where there was a certain amount of moisture, and here, the color was invariably a clear cherry red collecting in drops. On the front of the fish or on parts of the skin, where there was not so much moisture, the color was more pink than red. The fungus Torula epizoa, described by Høye, was present on a number of fish, occurring as black spots on the skin, and on the thinner upper parts of the fish.

PLATE 1.

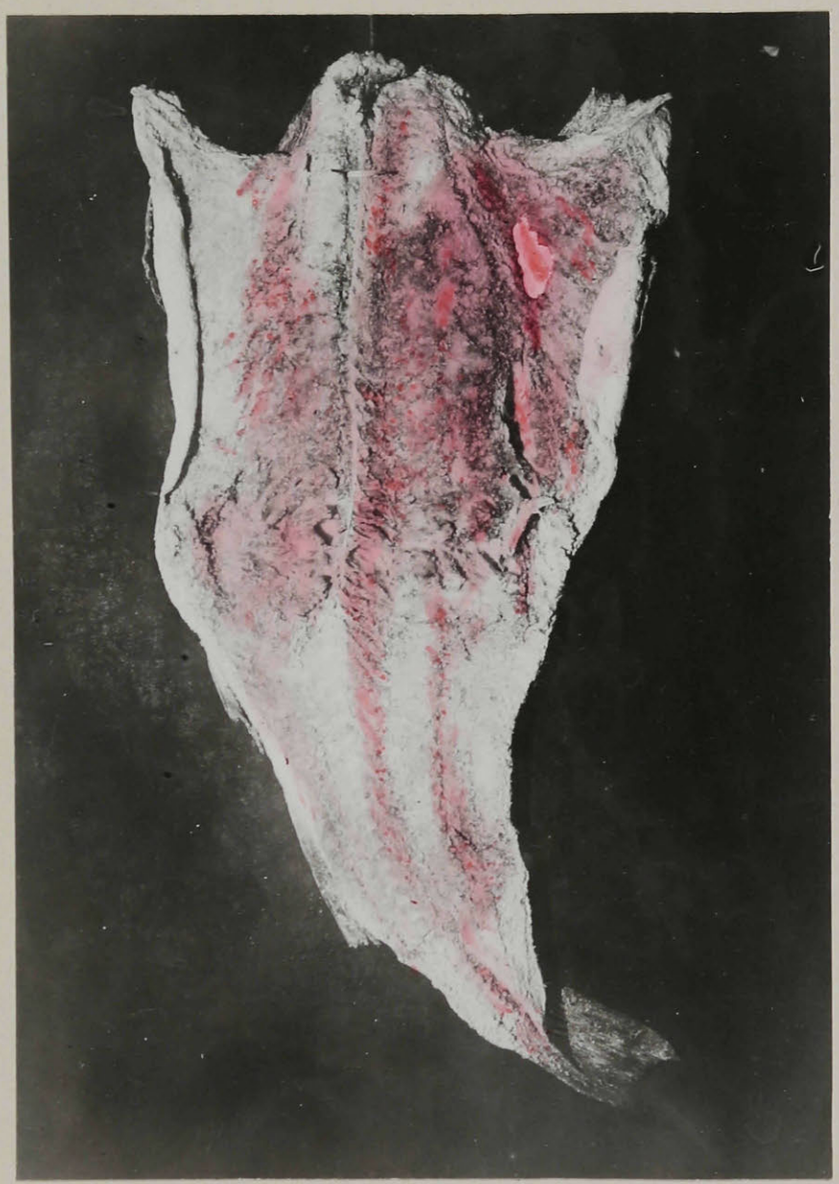
Sample of fish received, the darker parts showing surface discoloration.

Plate 1.

PLATE 2.

Same as plate 1, colored, and closely approximating the appearance of the fish when received.

Plate 2.



Microscopical examinations were made directly from each sample of fish received, and although various organisms were found, the preparations showed more or less similarity. Occasionally small cocci, averaging less than 1 μ in diameter, and without exception, rods of varying size were seen. Frequently the rods were long and slender, measuring from 3 μ - 7 μ in length, and 0.5 μ in width, some slightly bent, while, again shorter and thicker rods about 2 μ in length and 1 μ in width, appeared, but the average rod measured about 3 μ. Practically all of the slides showed the presence of torula-like or amoeba-like forms, and many irregularities in shapes and sizes, such as, oval, egg-shaped, pear-shaped, or lemon-shaped, and varying in size from 1 μ - 4 μ, but averaging about 2 μ. Very often they appeared in pairs, the longer axes being parallel, and the adjacent sides flattened. Two or three fish showed bodies, which resembled Placoma (Engler-Prantl)[19]. The cells were as large as 5 μ or 8 μ in diameter. They were generally rounded in shape, showing one segmentation, which divided the cell into two hemispheres, separated by a clear zone. Occasionally another segmentation, at right angles to the first, making three, and not infrequently four divisions, separated by clear spaces was observed. The fresh preparation showed no coloring matter, so that, evidently, it was a species of Schizophyceae. These bodies resembled in shape, but not in color content, those illustrated by Le Dantec, and thought by him to be the same as Clathrocystis Farlow.

None of the organisms found, except cocci, stained satisfactorily with the ordinary stains, Gram's method no better than any of the others. Heated methylene blue gave the best results until Jenner's, Leishman's, Wright's and Giemsa's stains were tried, when Giemsa proved to be much better than any of the others. Rose bengal in 5% carbolic acid gave fair results. The difficulty in staining was largely due to the impossibility of making preparations on the slide. When the material was transferred to a drop of water it became slimy and sputum-like. After fixation with heat the stain did not take hold, and the preparation washed off. Various substances were tried instead of water, such as ether, xylol, chloroform, alcohol, acetic acid, and so forth. Of these, the last gave the best results, but had a tendency to precipitate the material. The most satisfactory preparations were obtained with 16% solar salt solution, dried, fixed in equal parts of ether and absolute alcohol, or in absolute methyl alcohol, and stained with Giemsa. These preparations were often spoiled by the salt crystals, either from their remaining on the slide, or from the organisms collecting in great masses immediately around the crystals. Fixation with heat not only gave poor results, but the organisms were noticeably smaller. With Rose bengal, some very clear preparations were obtained; the organisms were, however, appreciably smaller when compared with similar preparations made with Giemsa. These remarks on staining are equally true of the conditions experienced when making preparations from cultures from the various media employed.

The first sample of fish received came from the Digby office of the Maritime Fish Company. The pink color was in spots over the entire surface of the fish, and even the salt crystals adhering to the colored parts were decidedly pink. Two pieces of fish, where discoloration had started, were cut off and placed in moist chambers, one kept at 22°C, the other at 37°C. In both cases color spread over the entire surface, the only difference being that at 37°C. the color was much deeper than at 22°C. Numerous attempts to culture the organisms on artificial media, however, were, at first, discouraging. Ordinary laboratory media, such as nutrient agar, sugar agars, nutrient gelatine, beef broth, sugar broths, cider, milk, and potato slants in varying percentages of salt were inoculated directly from the fish, and also from dilutions of the pink in 16% solar salt solution, but no growth developed. Fish slants similar to potato slants were tubed in varying percentages of solar salt - (2, 4, 6 to 18), sterilized, and then inoculated, one set left at 22°C., the other at 37°C. At the latter temperature growth was slow, no color developing in less than a week, and at 22°C. growth was still slower, as there was none earlier than two weeks. Even at the end of this time no color was present on fish in tubes which contained less than 6% Brine, and here color was very pale. As the percentages of salt increased, deeper color developed, so that on 16% it was rosy red, while on 18% it was a more vivid red. The effect of

temperature seemed to be a difference more of degree than of kind, as there was no growth on fish in the lower percentages of salt, while there was growth on both tubes containing 6% salt, for instance, but the tube kept at 37°C. showed deeper color than the tube kept at 22°C.

Fish agar was made up in the proportion of 100 grams salted codfish to a litre of distilled water, 2% agar, and varying percentages of solar salt. Using this medium, slants were inoculated, and three methods of plate culture tried - (1) plates made in the ordinary way by inoculating melted agar, and pouring the plate, (2) by pouring the plate and, after media had hardened, washing the surface with a suspension of the pink in salt water, and (3) by making streak cultures on the hardened agar with a platinum needle, directly from the infected parts of the fish. No growth appeared when the plates were made in the ordinary way, nor when the surface was washed with a suspension. Streak cultures on either fish agar slants or fish agar plates were only fairly successful, for no growth developed on agar containing the lower percentages of salt, and, on the higher percentages, when growth did appear, there were no isolated colonies; so that it seemed impossible to get pure cultures.

An observation which suggested the optimum salinity was that the red color which developed on 16% salt media most nearly approached the color found on the fish itself - on 14% it was a paler pink, while on 18% it was more red. Consequently, new media was made up, using ½ lb. shredded salt cod to a litre of

distilled water, 2% agar, and 16% solar salt. The cod was digested overnight in the water, and then cooked in the Arnold steamer for twenty minutes or half an hour. After straining this through a coarse cloth, the salt and agar were added to the broth, and the whole ~~thing~~ heated until salt and agar were thoroughly dissolved, then tubed. At first very little of the cod was removed in straining the broth, but as it made a very dense medium, and was very awkward to get into test tubes, it was thought advisable to use a finer strainer. This medium proved to be entirely successful for the development of color, and consequently has been used extensively. Possibly a deeper color developed when less of the codfish was removed in straining, or the deeper color which seemed to be present may have been due to the density of the medium. However, as the growth was quite as abundant, and the color a very satisfactory red on the clearer codfish agar, and as mechanical difficulties were overcome by its use, the fine straining seemed the better method.

In order to get isolated colonies, Dr. Harrison suggested using brush plates by the following method:- a small water color brush was sterilized; 6 petri plates were poured, using fish agar, which contained 16% solar salt, and allowed to harden; a suspension of the pink growth from pork was made in solar salt solution, and thoroughly mixed. Then the brush was dipped in the suspension, well "charged", and the surface of each plate brushed one after the other without "recharging" the brush.

PLATE 3.

Photograph of 16% salt codfish agar plate showing small colonies of the organism concerned. Natural size.

Plate 3.

The plates were incubated at 37°C. for at least four days before any red color developed, and sometimes as long as six or eight days. To prevent plates from drying out, about 15 cc. to 20 cc. of the fish agar was used for each plate. It was only to be expected that Brush plate No. 1 would be so covered that there would be no distinct colonies; but the later plates were more successful. The best one varied to some extent because of the fact that sometimes the suspension was more heavily inoculated than at other times. As a rule Brush plate No. 4 was the first of the series where the colonies were well separated, though not infrequently No. 5, or No. 6 even, was the only one where the colonies were sufficiently isolated to permit transfers from single colonies. On these plates various coloured colonies developed, in addition to the desired red ones, such as - sulphur yellow, waxy yellow, luteus, orange, Isabellinus, black, flesh, salmon pink, and two different white ones - one a punctiform colony, and the other a small round one. All of these will be referred to later in detail, but at present we shall continue the methods adopted to culture the organisms producing red coloration.

From single red colonies, transfers were made to sloped fish agar. After four days at incubation temperature, red growth developed along the line of inoculation - never spreading, except at the lower part of the slant, where the condensation water gathered, and here red was noticed around the edge. In an ordinary preparation, one might hope to have a relatively

pure culture on such a slant - but not so, in this instance. Microscopical examination showed the presence of two organisms - a rod, and a torula-like form. Another series of brush plates were made, inoculating from the tube just described, and the single red colonies, which developed, were transferred to sloped fish agar. Apparently the two organisms possessed so great an affinity for each other, that, it was only after repeated transfers from single colonies to sloped agar - and then brush plates again made from the growth developing on the slant, that pure cultures could be obtained. Very often the colonies which developed on the brush plates were white at first, later became pink, and then deepened in color as the culture became older. As the media dried out there was a tendency on the part of the red organisms to become more pink than red, and gradually to become paler in color. Amazing as it seems, at the end of eleven, and also twelve weeks, two or three plates, made from the codfish agar where very little of the fish was strained out, were still slightly moist, and numerous dark red colonies were growing among the salt crystals and dried cod.

Another experiment to obtain a pure culture of the red organism was executed concurrently with the brush plate method. So far, 18% solar salt had been the greatest amount of salt used in making up media for the growth of red organisms. It was suggested that codfish agar containing solar salt in

varying percentages, 5, 10, and so forth, up to concentration, or 35%, might eliminate one or other of the organisms. On salted codfish agar, to which no solar salt had been added, absolutely no color developed; on codfish agar containing 5% solar salt, there was still no color; on codfish agar containing 10% solar salt the color was very faint; on 15% the color was distinctly red, and on each succeeding percentage the color increased in intensity. The amount of growth, from 10% up, also increased in direct proportion to the increasing amounts of salt. As the agar dried out the salt crystallized at the top of the slant - and these crystals even were coloured red. From this series one may reasonably conclude that the organism or organisms causing red discolouration are halophylic - apparently preferring a saturated solution. Instead of eliminating one of the organisms it was found that both forms developed on codfish agar containing 35% salt - or even on a saturated solution.

The suggestion that the two organisms might have different Thermal Death Points offered another possible means of obtaining pure cultures. A suspension was made from red growth on an agar slant, in 16% solar salt solution, and heated in a water bath for ten minutes at 50°C. With a 1 mm. loop drops were placed on the surface of salted codfish agar plates. Other suspensions were made in the same way, one heated for ten minutes at 65°C., a second for ten minutes at 80°C., and the third for ten minutes at 100°C., and plates were inoculated

the same as the first one. Growth developed on only the first plate, where the two forms were found together. This same method and procedure was again followed at 50°C., 55°C., and 60°C., but there was no growth at either of the last two temperatures, and again the two forms developed together when heated at 50°C.

Still another attempt was made to secure a pure culture. A fairly heavy suspension was made from the red growth in 16% solar salt solution, and well shaken. Several successive dilutions were then made until a wet preparation under the microscope contained not more than one form in a field. Using a 1 mm. loop platinum needle, drops were made from this last dilution on salted codfish agar plates. This method, however, proved no more successful than preceding experiments in isolating a single form.

The task seemed hopeless until a certain measure of success was achieved through the brush plate method, as shown from numerous microscopical examinations of cultures from Digby fish. From these cultures transfers were made to various media in order to study the cultural characteristics of the organism.

On beef peptone agar, without salt, incubated at 37°C. for seven days, the growth was very scant, developing only at the lower part of the line of inoculation, raised, glistening, smooth, translucent. There was no color produced and no odor. The consistency was viscid. The medium itself was not changed.

On beef peptone agar, without salt , incubated at 22°C. for seven days, the growth was in every way the same as when incubated at 37°C., except at the former temperature, it was less abundant.

Stab cultures in beef peptone gelatine, without salt, did not develop at all.

In beef broth, without salt, after seven days' incubation at 37°C., and also at 22°C., there was no change in the medium.

In neither milk nor litmus milk incubated for seven days at 37°C., and also at 22°C., was there any change produced in the medium.

On potato slants incubated at 37°C. for seven days, there was no growth at all.

No gas was produced in dextrose, lactose, maltose or saccharose.

Modifications of ordinary laboratory media - chiefly through the addition of salt - were also tried out. After seven days' incubation at 37°C., the growth on beef peptone agar, containing 18% solar salt, was very scant, developing only at the lower part of the line of inoculation. The growth was slightly raised, glistening, smooth, translucent, with very little color, and no odor. The concistency was viscid, and the medium was not changed. When incubated for seven days at 22°C. on this medium, the growth was entirely the same as at 37°C., except that it was less abundant.

Codfish gelatine was made with the addition of 16% solar salt, but was not very successful, as the gelatine did not remain solid unless kept at a low temperature.

Beef broth containing 18% solar salt was inoculated, but no change developed in the medium. Beef broth with varying percentages of solar salt - 5, 10, 15, and so forth, up to 35%, were also tried, but no change took place in any of the series.

Potato slants were tubed in brine, made from varying percentages of solar salt - 5, 10, 15, up to 35%, but there was no growth on any of the slants.

In seven days, at 37°C., the growth on salted codfish agar, containing 16% solar salt in addition to salt present in the codfish, was abundant, filiform, slightly raised, glistening, smooth, translucent, bright red in color, and possessed an unpleasant odor. Its consistency was viscid and the medium uncharged. Growth on the same medium incubated at 22°C. for seven days was the same in every respect as when incubated at 37°C., except that it was scanty.

On salted codfish agar to which varying percentages of solar salt, such as 5, 10, 15, up to 35%, were added, the growth varied with the amount of salt. After incubation for seven days at 37°C., there was no growth on sloped agar to which no salt or 5% salt had been added. On tubes containing 10% solar salt the growth was very scanty, and pale pink in color; on 15% growth was moderate, slightly red in color;

on 20% it was abundant and bright red in color; on 25% there was still more growth, which was a slightly deeper red. Growth on 30% and 35% was practically the same as on 25% in amount, though at times the color appeared slightly deeper on the higher percentages. The other characteristics of growth have not been mentioned specifically because wherever there was growth it was identical with the description of growth on salted codfish agar containing 16% solar salt.

Pieces of dried salt cod were cut and tubed in 16% solar salt solution in a manner similar to the preparation of potato tubes, sterilized, and then inoculated. After incubation at 37°C. for seven days, the growth was abundant, spreading, slightly raised, glistening, smooth, and of slimy consistency. The effect of lower temperature, 22°C., for the same length of time, differed from 37°C. only in the less abundant growth produced.

Small pieces of fresh cod were also tubed in 16% solar salt, sterilized and inoculated. After seven days' incubation at 37°C. there was no growth - not even at the end of four weeks. Presumably the fish was not sufficiently impregnated with salt.

Cooked finnan haddie was treated in the same way as the dried salt cod and fresh cod. After an incubation period of seven days at 37°C. growth was abundant, spreading, slightly raised, glistening, smooth, very bright red color, disagreeable odor, and slimy consistency. In some tubes the red pigment covered the surface of the absorbent cotton and invariably settled in a mass in the bottom of the tube.

Larger pieces of dried salt cod, fresh cod and finnan haddie were placed in flasks containing moistened crystals of solar salt, and then inoculated. Abundant growth, and bright red color developed on the dried salt cod and on the finnan haddie at both 37°C. and 22°C., but there was no growth on the fresh cod. In any of the flasks where there was growth the salt crystals became very pink. This suggested another experiment - solar salt crystals in flasks were moistened, sterilized, and then inoculated, but there was no growth.

Again, pieces of dried salt cod were tubed in a solution of 16% solar salt and 3% boracic acid, sterilized, and inoculated. No growth developed in these tubes.

Fish broth was made up from shredded salt cod, which was digested in distilled water over night, then cooked well, and strained through a fine cloth. Varying percentages of solar salt, 5, 10, 15, and so forth , up to 35%, were added to the filtrate, dissolved, sterilized, and inoculated. In tubes containing 5% and 10% there was practically no clouding, on 15% there was some clouding, on 20% there was more, on 25% there was a very great deal, on 30% less clouding, and on 35% slightly less than on 30%.

Brine agar, in the proportion of 16% solar salt, 1% peptone, and 2% agar, was inoculated in sloped cultures. The growth at 37°C., after seven days' incubation, was very scant, appearing only at the lower part of the line of inoculation, raised, glistening, smooth, translucent, slight red color, no odor, viscid consistency. At 22°C. after seven days' incubation

there was less growth, but the appearance of it was, in other ways, similar to that at 37°C.

A liquid medium made from 16% solar salt, ½% peptone and distilled water was also tried; but there was no color or change of any kind produced.

Shredded salt cod was used in making two other media - the first one, made from one part fish to three parts cooked potatoes, and 10% solar salt with just enough milk to moisten and hold together; the second made from 66 grams, by weight, of egg white, 100 grams, by weight, of fish, and 10% solar salt. These two were tubed as slopes, sterilized and inoculated, but no growth developed on either medium.

Høye found that Torula epizoa and also the red organisms developed well on a special medium he made from thirty parts flour to thirty five parts salt and as little water as possible to make a stiff paste. Consequently, it also was tried in these laboratories, but the attempts were unsuccessful,- although massive inoculations were made.

On fish agar brushed plates the growth was slow, developing in from three to four days at 37°C., while the colonies did not reach their maximum size until about the seventh day. Colonies were small, and round in form, averaging at full growth 1 mm. in diameter, with surface smooth and glistening, raised elevation, entire edge, and the internal structure coarsely granular. The color varied from clear pale pink to transparent cherry red. When examined under the low power of

the microscope there was a round area in the centre of each colony, which was pale in color. This was surrounded by a very dark ring, which extended half way to the outer edge. The outer area was not so dark as the dark ring, but not so pale as the centre. When growth was collected on the end of a needle the color was distinctly red and transparent. The colonies were all surface colonies, and showed very definite aerobic tendencies when a cover glass was dropped on the surface of the agar, as there was absolutely no growth underneath the cover glass, while immediately around the edges of it there was considerable growth.

The red pigment was soluble in solar salt solution, absolute methyl alcohol, ethyl alcohol, acetone, and slightly soluble in water, but was insoluble in sodium chloride, ether xylol, chloroform, or weak acetic acid.

The maximum temperature at which the organism grew was 50°C., and here growth was slight, with very little color produced. The lowest temperature obtainable was 7°C., which was not too low for slight growth or the production of pale red color. The optimum temperature, however, seemed to be between 37°C. and 40°C., where growth was abundant, and bright red color developed. The thermal death point was between 50°C. and 55°C. for ten minutes, which is rather remarkable in that, this temperature is not far above the maximum temperature, at which the culture was kept for four days.

PLATE 4.

Two photographs of salt codfish agar plates, showing small round colonies of the red organism, and also salt crystals. The upper one reduced, lower one natural size.

Plate 4.

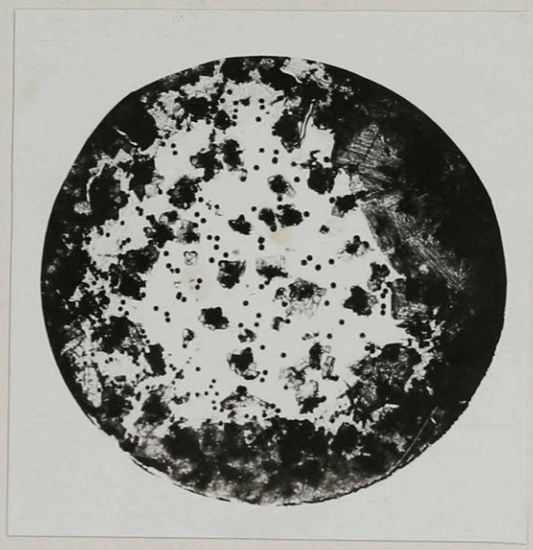


Fig 1.

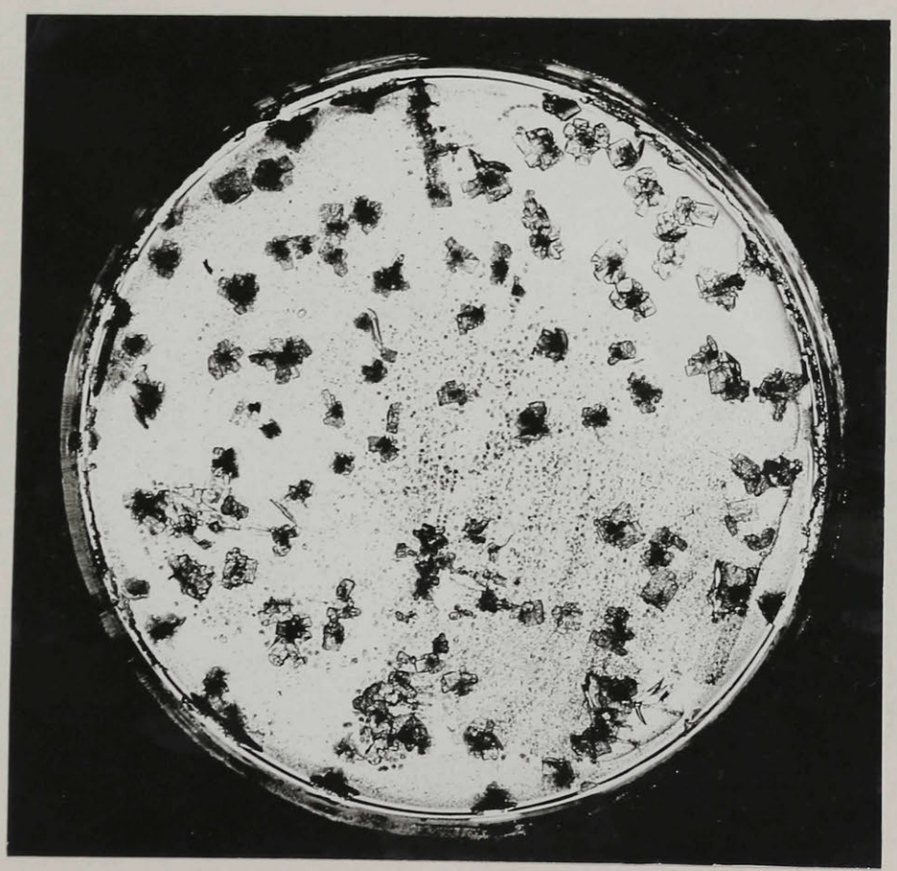


Fig 2.

The ability of the organism to grow on media containing very high percentages of solar salt, or even on saturated salt media, together with the fact that it did not grow on fish agar to which no salt had been added, suggested the influence of osmotic pressure. Ignoring dissociation and also the permeability of the membrane, which latter has not been determined, 15% salt at 37°C. exerts an osmotic pressure of 65.2 atmospheres, while 35% salt at 50°C. exerts an osmotic pressure of 152.2 atmospheres. In order to confirm or disprove the possibility that the organism was growing because of the osmotic pressure and in spite of the salt, other substances, such as glucose, and saccharose were added to fish agar in place of solar salt, in corresponding proportions, according to molecular weight. On both glucose and saccharose fish agar growth developed on all tubes inoculated, but without color . Unfortunately, these experiments have not been completed except for the lower percentages, and these only at 37°C., but it is hoped to obtain more complete results from these experiments in the near future.

The morphology, though rightly placed before the cultural features, purposely has been left until now, so that in describing preparations made from cultures on the various media the reference to cultures, and also to media, would convey something tangible to the reader. Many of the stained preparations made from cultures on artificial media showed the presence of both rod-like and torula-like forms, which, at first, were thought to be two different organisms; but after making numerous microscopical

examinations from cultures of various ages, and from media containing different percentages of salt, it was concluded that the great diversity of form was due partly to the salt content of the medium upon which the culture was growing, and partly to the age of the organisms. Certainly it possessed many varied shapes and sizes. In order to note the effect of salt on the morphology of the organism, several series of fish agar and fish broth, each containing varying percentages of solar salt, were inoculated from pure cultures of the red organism, and the results on a complete series noted at the one time. Microscopical preparations showed a marked similarity in form between cultures on solid media and those in liquid media containing corresponding percentages of salt . Transfers made from growth on solid media, after five days' incubation at 37°C., spread in 16% solar salt solution fixed with absolute methyl alcohol and stained with Giemsa, showed cells mostly round, some oval, from 2 μ - 4 μ in diameter, appearing singly and homogenously stained, from growth on agar containing 10% solar salt. On 15% round and oval forms predominated, though elliptical, cylindrical, and irregular forms were present. Cells varied from 1 μ to 4 μ in diameter, appeared singly, and were homogenously stained. On 20% cells were very irregular, cylindrical forms predominating, some round, oval, pointed, and also truncate. Many cells were unstained in the centre. The size of round or oval forms averaged 2 μ in diameter, but the general appearance of the slide was more the presence of cylindrical cells, which varied from 2 μ to 5 μ in

length, and averaged 1 μ in width. On 25% cylindrical forms predominated, but many irregularities also were noted, such as dumbbell shaped cells, and cells with very pointed ends. The length varied from 2 μ to 5 μ. Many cells showed the presence of vacuoles, and all cells were very lightly stained. On 30% cells were round to oval with few cylindrical or irregular forms, from 1 μ to 5 μ in length and averaging 2 μ. On 35% the general appearance was round to oval forms, with cylindrical and irregular forms also present - pear shaped the commonest irregular form. Cells were vacuolated, and there was more evenness in size, averaging 2.5 μ. Preparations made from fish broth cultures in the same way as the other series showed mostly round or oval forms in 10%. In 15% cells were irregular, both round and cylindrical forms being present. In 20% cells were practically all rod-like forms of varying shapes and sizes, some as long as 10 μ and 1.5 μ wide, but very few round or oval forms. In 25% only rod-like forms of diverse shapes and sizes were present and here unstained vacuoles occured sometimes at the end, sometimes at the centre of cells. In 30% cells were elongated, slender, and shorter than at 25%, few round cells present, smaller than usual, and again vacuoles were observed in a number of cells. The long cells were of all shapes, branched, bent, twisted and swollen at the ends. In 35% the average cells were shorter and stouter than in 30%, with fewer bizarre forms, no round cells, mostly cylindrical, straight or slightly bent, unevenly stained and no vacuoles.

In some ways the most satisfactory preparations were unstained. A thin film spread in 16% solar salt solution, allowed to dry, and before being examined passed once through the flame - warmed rather than heated, clearly showed the red cell contents in the round or oval forms, and also in the cylindrical forms. Sometimes the red mass was intact, and at other times it was broken up in various ways. In hanging drop preparations the rod-like forms, and also the round or oval forms, were actively motile. It was impossible to note any growth or development on a hanging block preparation, due, possibly, to the strictly aerobic character of the organism. Capsules were observed on practically all of the varied forms in both stained and unstained preparations. The division of cells appeared to take place by fission - when there was a clear zone between the contents of the two cells, and also by budding - when occasionally a thin strand of protoplasm was seen connecting the bud and parent cell.

To determine the pathogenicity of the organism, and the toxic effects of reddened codfish, two rabbits were inoculated, one from a suspension of a pure culture of the red organism in weak solar salt solution, and the other rabbit from a suspension of the red directly from the infected fish in a weak solar salt solution. Neither of the rabbits showed any ill effects. Dogs or other animals were not available in carrying out these experiments, and human susceptibility was not investigated. Cooked finnan haddie,

however, was one day sent to the laboratory, after a considerable amount of it had been served as part of a meal. Through the connective tissue marked coloration was observed, though it was a much paler pink than that occurring on the uncooked fish. No cases of poisoning developed, and it is highly probable that the organism in itself it not toxigenic. In this connection, the important work of Mauriac may be again cited. His investigations showed no ill effects from animals feeding on, or human beings eating reddened codfish.

Several investigators have suggested that the reddening of dried codfish was due to an infection in the salt, which was used in curing the fish, and, certainly, experiments carried on in these laboratories have confirmed the idea. Six samples of salt were received - Mediterranean salt, sent by the Maritime Fish Co., of Digby, N.S.; Spanish salt and Turk's Island salt, sent by the firm of Gardiner and Doon, St. Andrews, N.B.; a sample of salt sent by the Fishery Officer at Souris, P.E.I.; and two samples of Malagash salt from the Malagash Salt Mines, Malagash, N.S. - one a sample of their coarse grade, and the other of their medium grade. The first four are probably all solar salts, while Malagash, as the source implies, is a mined salt. Each sample was sprinkled on a plate of beef peptone agar, and also on a plate of fish agar, which had been poured and allowed to harden. Pink color developed on all of the plates made from the solar salts, but there was not the slightest trace of pink made from either sample of the Malagash.

From the pink growth sloped cultures were made, and from these, microscopical examinations, which showed the presence of forms very similar to those isolated from reddened fish. To develop, if possible, pink or red coloration on fresh cod from suspected salt , each sample of solar salt was sprinkled abundantly on the bottom of a petri plate, a piece of fresh cod placed on the salt, and then the fish itself well sprinkled with the same salt. All samples were incubated at 37°C., and as the salt abstracted moisture from the fish, this brine was poured off and more salt placed on the fish. In two weeks' time all samples of fish, and even the salt crystals about the fish, were very pink in color. Microscopical examinations made from fish infected in this way showed the presence of forms identical with those isolated from infected fish samples, and which developed red colonies on fish agars. Mined salt was not used on fresh cod because of the negative results obtained from it on agar plates. These experiments, undoubtedly, indicate solar salt as the possible origin of the red coloration.

The most important point in connection with these experiments is the fact that solar salt carries the red organism, and is the probable primary source of infection. From this source has resulted the contamination of curing houses, and of all articles coming in contact with the fish, especially those made of wood. This material, in course of time, has become thoroughly impregnated with salt and fish extracts, and thus given food for the continued growth of the red organism, and permitted extensive infection of the

fish. Hence, the obvious means of prevention would be, in the first place, thorough disinfection of the curing houses, and the complete sterilization of butts, puncheons, kench racks, carrying boxes, and every object used for the fish, by means of steam, if it can be applied, and where steam is not available, by burning sulphur; and in the next place, the use of mined salt rather than solar salt for curing fish. The recommendations made by Bitting, and others, would be effective, and in this connection deserve mention.

"The fish should be handled from the vessel to the scales without being thrown upon the deck or dock where they may become infected from the boards or be stepped upon by the workmen. All of the docks are infected with the red organisms, and fish coming in contact with them become inoculated.

"The floors, scales, dressing tables, wash tanks, wheelbarrows, and everything with which the fish come in contact, in making them ready for the butts, should be frequently washed with water under considerable pressure. A relatively small stream of water under strong pressure is far more effectual in cleaning than a larger stream of water at low pressure.

"The fish should be washed by sprays of water or by a machine. The sprays should have sufficient force to do the work well. The present method of pitching the fish into a tank or dory and then out again is not sufficient for cleaning, and, furthermore, it tends to disseminate any organisms which may be present.

"The water used upon the fish or upon anything with which the fish come in contact should be of undoubted purity. The use of harbor water for any purpose can not be justified, as it is filled with the germs which come from emptying the butts and washing fish and docks. It is also apt to be polluted with sewage from the city, as was found to be the case in the investigation here reported.

"The butts should be thoroughly cleaned inside and out and steamed for twenty minutes or sprayed with a solution of sulphurous acid.

"Before fish are taken out of the butts water should be turned in to cause the brine to overflow and wash away any reddening which may have occurred on the top.

"The kenching in the storeroom should permit a circulation of air and not cause dead air spaces. The kench racks should be steamed or sprayed after each period of use.

"The walls, posts, and floors should be sprayed often, once a week during the cool season and twice a week during the summer.

"The skinning or cutting tables should not have shelves or boxes beneath to catch bits of skin or fish. They should be well washed each evening. The simple brushing with a hand broom is not sufficient. The floor should be cleaned often.

"All refuse should be removed from the room promptly. Bits of fish in barrels and boxes act only as incubators to perpetuate the infection.

"Concentrated sulphurous acid should be used as a disinfectant when steam is not available. One part of the acid to 50 parts of water is effectual where much reddening has occurred, and 1 part to 200 parts of water will be effective in preventing growth if used often."

A brief description of other organisms isolated from reddened codfish is included in this outline, because of their halophyllic characteristics and because they may take part in the subsequent decomposition of the codfish. Furthermore, biologically they are not without interest.

An organism, which in growing on fish agar containing 16% solar salt, developed as a sulphur yellow colony, was isolated and cultured. Microscopical preparations showed the presence of cocci, slightly more than 1 μ in diameter. They stained well with Loeffler's methylene blue, and were Gram positive. Growth on beef peptone agar, incubated at 37°C. for two days, was abundant, slightly spreading, raised, glistening, smooth, translucent, bright yellow color, and possessed no odor. Growth at 22°C. for two days was more abundant, but in other respects the same as at 37°C. From a gelatine stab inoculation the growth was uniform, filiform, but there was no liquefaction. In a beef broth culture, incubated at 37°C. for two days, a pellicle was formed on the surface of the broth, and a flaky precipitate appeared upon shaking the tube . Growth at 22°C. for two days was practically the same. There was no change in either milk or litmus milk inoculated and incubated at 37°C. and also at 22°C. for two

days - nor even at the end of seven days. On fish agar slopes containing 16% solar salt, incubated at 37°C. for two days, growth was abundant, filiform, raised, glistening, smooth, translucent, and sulphur yellow color - not so vivid as on beef peptone agar. When incubated at 22°C. for two days growth was very much the same. On brine agar, incubated at 37°C. for two days, growth was moderate, filiform, and rather pale yellow color; and, at 22°C. for the same time, it was practically the same. Colonies on agar plates were punctiform. No gas was produced in lactose, maltose, glucose or saccharose. Fish agar containing varying percentages of solar salt were inoculated and incubated at 37°C. for two days with the following results - on agar containing no salt, 5% and 10% salt, growth was abundant; on 15% it was less, but still fairly abundant; on 20% it was only moderate; on 25% and on 30% it was slight; and on 35% very slight. There was also a gradual change in color, from a bright vivid yellow on agar to which no salt had been added, citrinus on 5% and 10%, sulphureus on 15% and 20% to a cream shade on 25%, 30% and 35%. In many respects this organism resembles Micrococcus luteus of Winslow and Winslow,[21] except that in color ours was more sulphureus than luteus.

Many yellow colonies developed on fish agar plates, containing 16% solar salt, in about three days at 37°C. They were round in form, about 1.5 mm. in diameter when full grown, with smooth surface, raised elevation, and entire edge. A microscopical preparation, stained with gentian violet, showed the presence of forms which resembled torulae, rather oval

shaped, frequently appearing in twos, side by side. It averaged 2 μ in diameter, and apparently grew by fission. Time forbade the cultivation of this organism on various media.

Luteus (Saccardo[20]) colored colonies also developed on fish agar plates containing 16% solar salt; and microscopical preparations from such colonies, stained with methylene blue, showed the presence of cocci, slightly more than 1 μ in diameter, which were Gram positive. Growth on beef peptone agar slopes, incubated at 37°C. for two days, was abundant, slightly spreading, raised and glistening, with a definite orange color. Incubated at 22°C. for two days growth was practically the same. From a gelatine stab inoculation there was infundibuli liquefaction. In both milk and litmus milk, incubated at 37°C. for two days, there was an orange ring around the top, and a deposit of the same in the bottom, but otherwise there was no change in these media, even at the end of seven days. In beef broth incubated at 37°C. for two days, there was a ring around the top, and considerable clouding through the medium. On fish agar containing 16% solar salt, and also on brine agar, both incubated at 37°C. for two days, growth was abundant, slightly spreading, raised and glistening. On the former the growth was luteus color (Saccardo[20]), while on the latter it was more ochraceus (Saccardo). On agar plates colonies were punctiform, mostly under the surface. No gas was produced in glucose, lactose, maltose, or saccharose. Fish agar slopes, containing varying percentages of solar salt, were inoculated, and incubated at 37°C. for two days, with the following results -

on agar containing no salt, 5% and 10%, growth was abundant, filiform, and luteus color; on 15% there was less growth, but still fairly abundant and luteus in color; on 20% growth was slight, ochraceus color, on 25%, 30% and 35% growth was very scant, but approached ochraceus in color. With the exception of color production the cultural characteristics of this organism approach those of Micrococcus citreus of Winslow and Winslow.(21)

On some of the fish agar plates Isabellinus colonies developed, which, when transferred, appeared very pale in color, at first, but in older cultures gradually assumed the darker color - Isabellinus - which had first attracted attention. Microscopically, this organism was a medium sized rod, but further knowledge of it is yet to be ascertained.

A creamy black colony on sloped fish agar had the appearance of Høye's Sarcinomyces Islandicus, but cultivation of this organism has not been completed.

A very dark colony (Fuligineus, Saccardo) developed in beadlike form of growth alongside a streak culture of the red organism on fish agar containing 16% solar salt. The microscopical appearance was that of torulae, frequently resembling figure eight in arrangement. It is hoped yet to obtain interesting results from this organism.

A flesh coloured colony frequently was observed on the fish agar plates, which, when examined microscopically, showed the presence of cocci. They stained well with methylene blue, and also were Gram positive. On beef peptone agar sloped

cultures, incubated at 37°C. for two days, growth was moderate, filiform, flesh color. From gelatine stab inoculations growth was filiform, but very scanty and no liquefaction. In beef broth there was slight clouding, flaky precipitate, and no growth at the top. No change developed in either milk or litmus milk. On a sloped culture of fish agar containing 16% solar salt, growth was filiform, more abundant than on beef peptone agar, and flesh color . On brine agar growth resembled that on fish agar, and was, again, more abundant than on beef peptone agar. There was no gas produced in glucose, lactose, maltose or saccharose. On fish agar containing no salt, growth was beadlike, more appearing near the top of the slope. On 5% and 10%, growth was abundant; on 15% it was slightly less than 10%, on 20% still less, on 25%, 30%, 35% there was still growth, but very scant.

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