A NOTE ON THE CAUSE OF CERTAIN RED COLORATIONS ON SALTED HIDES AND A COMPARISON OF THE GROWTH AND SURVIVAL OF HALOPHILIC OR SALT-LOVING ORGANISMS AND SOME ORDINARY ORGANISMS OF DIRT AND PUTREFACTION ON MEDIA OF VARYING SALT CONCENTRATIONS.

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(With 12 Text-figures.)

Hides and calf skins cured with marine salts, especially if kept under conditions of warmth and moisture, tend to develop on the flesh side large areas of brick-red coloration. Similar red colorations have, for many years, been noted as occurring in similar conditions on salted fish, and a number of investigations have been made as to their cause, the first recorded being that of Farlow (1878). Following him were Mauriac (1886), Ewart (1887), Layet (1887), Le Dantec (1891) and, in recent years, Harrison and Kennedy (1922), Browne (1921–22) and Cloake (1923). All these workers have agreed in attributing the red coloration on salted fish to the growth of one or several species of red-coloured organisms, and the more recent workers (Harrison and Kennedy, Browne, Cloake) trace these organisms to the sea salt used in curing the fish, and find them to be chromogenic bacteria only capable of growth in a high concentration of salt. Clayton and Gibbs (1927) have examined many solar brines and salts, and have found that "they almost invariably contain micro-organisms which only exist and develop in the presence of abundance of common salt." Sturges and Heidemann (1923, 1924) have isolated many salt-loving organisms from meat-curing solutions, and Le Fèvre and Round (1919) from the brine of pickled cucumber.

Clayton and Gibbs found that stains on hides might be produced by salting with solar salts, and it was as part of some work undertaken with the object of finding whether certain extensive brick-red colorations on salted hides sent for examination were produced by true halophilic organisms coming from the curing salt or by the ordinary organisms of dust and dirt, that the experiments to be described were done.

A number of curing salts were examined for the presence of halophilic organisms, a solid fish-broth-rice-flour preparation of salt concentration of 25–30 per cent., modified from a fish-broth and rice formula suggested by

Clayton and Gibbs, being used as culture medium. The composition and preparation of the medium were as follows:

Fish-broth. 1 lb. of minced fresh cod was allowed to stand overnight in a litre of water. The resulting fluid was then strained through muslin and to it were added 30 per cent. of sodium chloride (British Drug Houses) and 0·01 per cent. of peptone. When the salt and peptone had dissolved the broth was again made up to 1 litre by the addition of tap water. The *p*H value was then adjusted to 8.

To make the solid medium for Petri plates, 30 gm. of rice flour were added slowly and with careful mixing to 100 c.c. of this broth. The resulting soft paste was poured onto Petri plates and autoclaved at 110° C. for 15 minutes. On this medium halophilic organisms grow freely, and such of the ordinary organisms of dirt and dust as have been examined do not grow. From all salts of marine origin examined, brick-red growths of apparently the same nature as those found on the hides were obtained, and red growths were produced on fresh hide pieces both by inoculation with red cultures obtained from the curing salts and by salting with the curing salts themselves.

The question whether halophilic organisms alone were to be held responsible for the red growths on the hides, or whether ordinary micro-organisms of dirt and dust, such as the coloured sarcinae, might also play a part, suggested an investigation into the capacity for growth and survival at different salt concentrations of the two types of organisms, and the following series of experiments was carried out.

Halophilic organisms giving brick-red growths were cultivated from four varieties of curing salt by sprinkling some of the salt on the surface of a Petri plate of the special medium, incubating in a moist atmosphere at 37° C. for about a week (these organisms grow slowly and grow best in the presence of a good deal of moisture), and sub-culturing, again on to the special medium, from the red colonies which developed. Films made from these red growths and stained with methylene blue showed, for the most part, large cocci, sarcinal in type and arrangement, but also a considerable amount of an amorphous material in which short, pleomorphic organisms, ill-defined in shape, could be vaguely made out. Later work has shown that there are at least two, probably many more, types of these organisms, one giving a dryish, wrinkled, red growth, and consisting of large cocci, which retain their shape when stained in the ordinary way with methylene blue, and another giving a moist colony, consisting of a rather clear, yellowish-pink jelly-like base, streaked and veined with the bright red colour, and breaking up into the amorphous material described above when rubbed up in a drop of water and stained with methylene blue. Some of the halophilic growths used in the experiments apparently contained more than one type of halophil, and no attempt was made to separate those growths into their component parts; they were used in the mixed condition.

Four series of slopes of the rice-flour-fish-broth medium, to which had been

added sodium chloride in concentrations of from 2–33 per cent., were prepared in small specimen tubes and strokes made on these from the halophilic growths obtained as described above from:

(*a*) A salt from the Argentine.
(*b*) A salt from Brazil.
(*c*) A salt from New Zealand.
(*d*) A salt from Bordeaux.

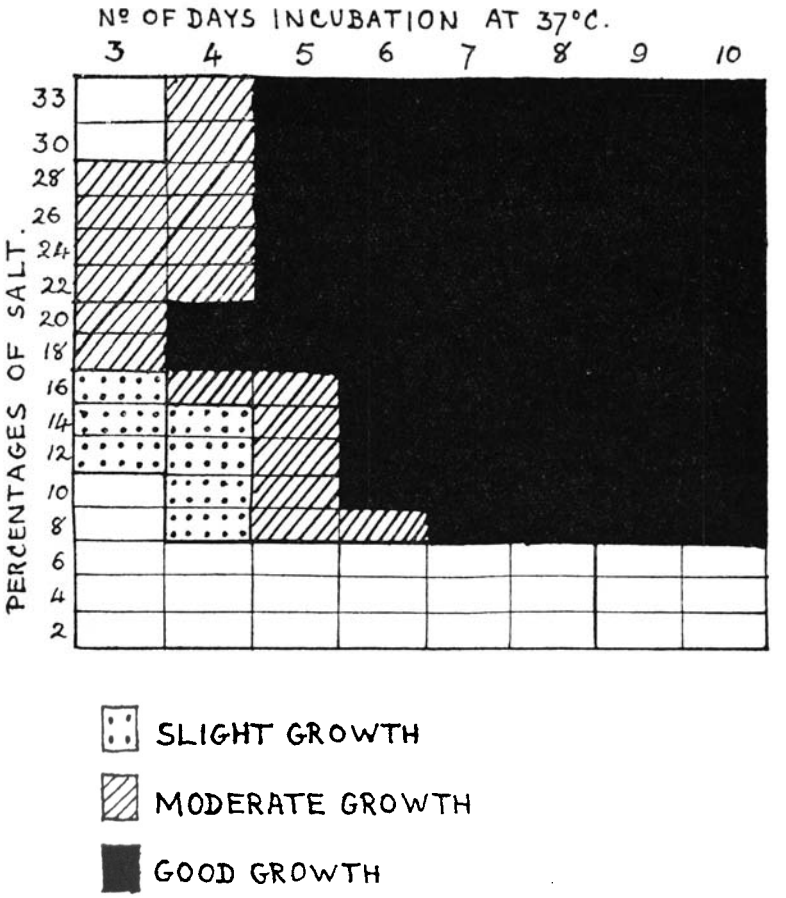

Fig. 1. Red culture from Argentine salt.

The inoculated tubes were kept over water in a covered vessel at a temperature of 37° C. and examined daily for growth. Ordinary laboratory agar slopes (0·5 per cent. sodium chloride) were also inoculated with these halophilic organisms and incubated at 37° C. but none showed any sign of growth. Figs. 1–4 indicate the manner in which growth proceeded and it will be seen that the heaviest growths were obtained at high salt concentrations and that no growth took place at salt concentrations below 6 per cent. (A blank space on the diagrams indicates "no growth.")

The non-halophilic organisms selected for experiment were: (1) *Sarcina lutea*, (2) *S. aurantiaca*, (3) *S. rosea*, (4) *M. tetragenus*, (5) *Actinomyces*, (6) *B. proteus*, (7) *B. pyocyaneus*, (8) *B. fluorescens liquefaciens*. The first six were selected, as they had all been isolated by Stather and Liebscher (1929) from hide pieces showing the brick-red coloration described above. The last two were selected, as they are amongst the organisms giving rise to putrefaction, and the

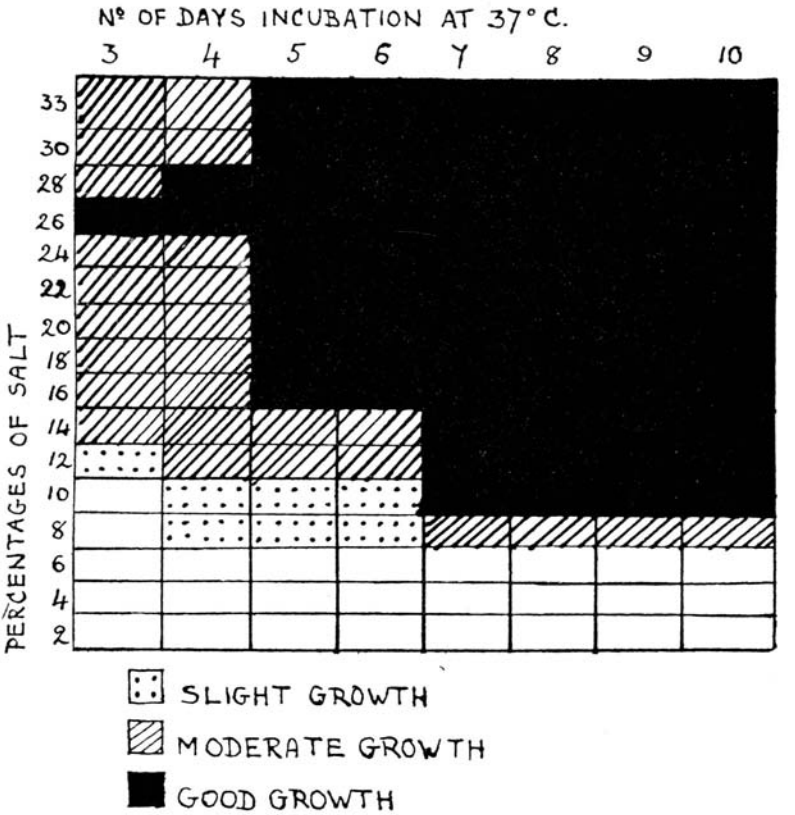

Fig. 2. Red culture from Sal Diamanté, Brazil salt.

question as to how far different degrees of salt concentration were likely to inhibit the growth of putrefactive organisms was also under consideration.

The procedure used with these organisms was rather different from that adopted with the halophils, as it was designed to find out not only whether any growth could take place at high salt concentrations, which could be ascertained by examining the broth tubes for turbidity, but also how long the organisms under investigation could remain alive under such conditions and be capable of development when removed to more favourable conditions. Sets of ordinary laboratory broth tubes were prepared with concentrations of sodium chloride ranging from 0·5–33 per cent. The *p*H value of these tubes in the range be-

tween 0·5–6 per cent. was 7, from 8–20 per cent. between 6·5 and 7, and from 20–33 per cent. approximately 6·5. The tubes were inoculated from 48-hour agar cultures of the organisms to be tested, and were kept at room temperature (approximately 20° C.). Sub-cultures were made daily from the salt broths onto ordinary agar slopes (0·5 per cent. NaCl), and the charts represent the readings obtained from these slopes. The experiment was not originally intended to measure the gradual decrease in numbers of organisms, but only to

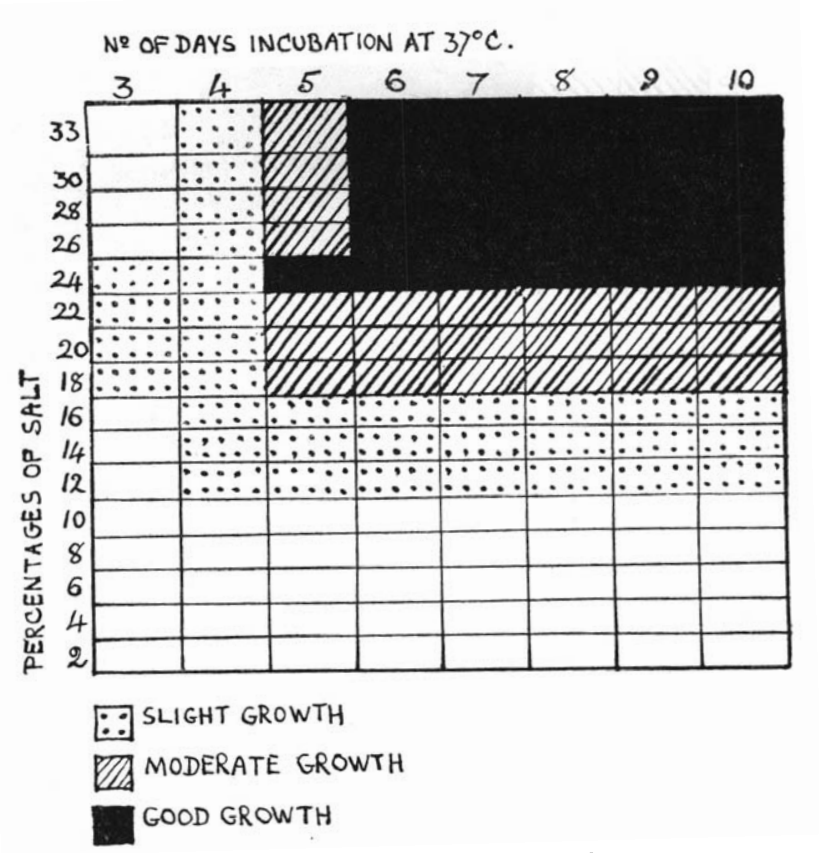

Fig. 3. Red culture from New Zealand salt.

ascertain how long they could survive at the different salt concentrations—hence the technique employed. It was found, however, that the amount of growth on the agar sub-cultures gave some information on this point and, throughout most of the experiment, care was taken by using the same platinum loop, and as nearly as could be judged the same amount of the salt broths in making the sub-cultures, to make the day to day comparisons of the amount of growth on the sub-cultures of some value as a measure of the rate of destruction of the organisms in the broth tubes. The sub-cultures were examined for growth at the end of 24 and 48 hours, and diagrams made from the results of

the 48 hours' observations. Figs. 5–12 show the results of these experiments. (A blank space on the diagrams indicates "no growth.")

It is clear from a comparison of the two sets of diagrams, making all allowances for accidental findings due to slight variations in technique or interpretation, that though there is overlapping of the two groups in the middle of the salt scale, as regards possibility of growth, their optimum growth conditions are widely different. At one end of the salt scale the halophils are incapable of

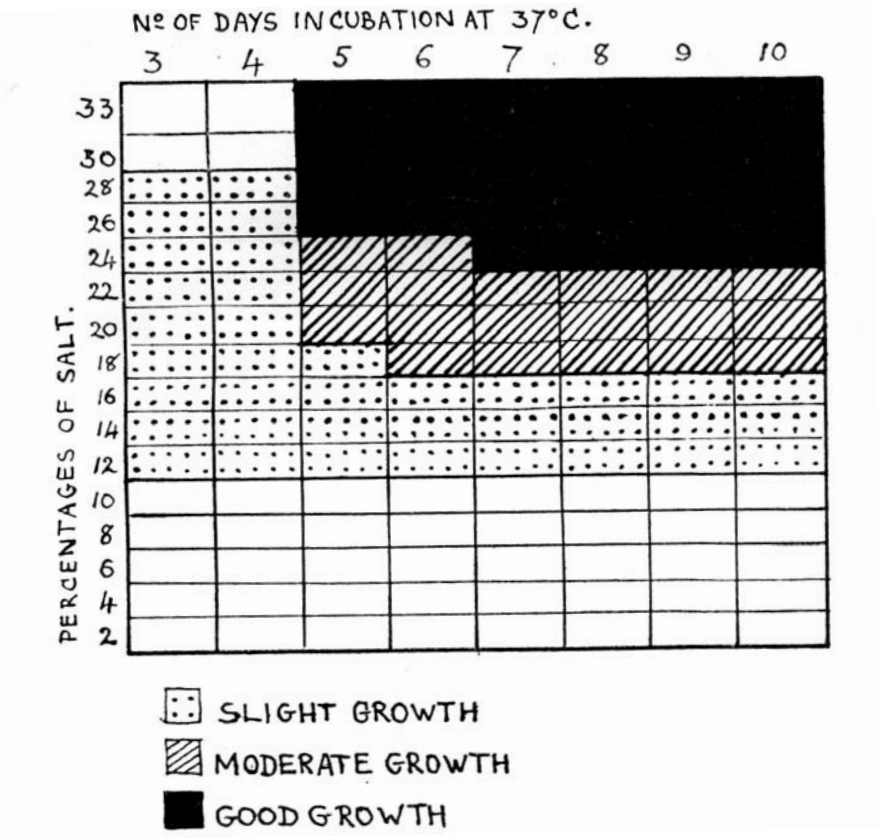

Fig. 4. Red culture from Bordeaux salt.

growth, at the other end only one of the non-halophils, *S. lutea*, is capable of survival for more than 10 days. None of the non-halophilic organisms, as is shown in Table I, gave any sign of growth in broth of a salt concentration of more than 8 per cent.

It was concluded, therefore, that the extensive red patches under examination in salted hides were almost certainly due to the growth of genuine halophilic bacteria, and not to the ordinary organisms of dirt and dust, even though these might be present on the hide and be capable of survival in high concentrations of salt.

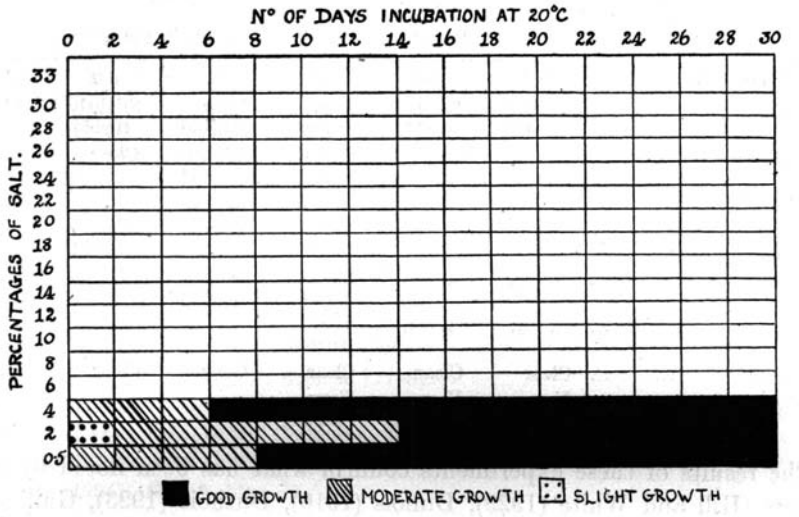

Fig. 5. *S. lutea.*

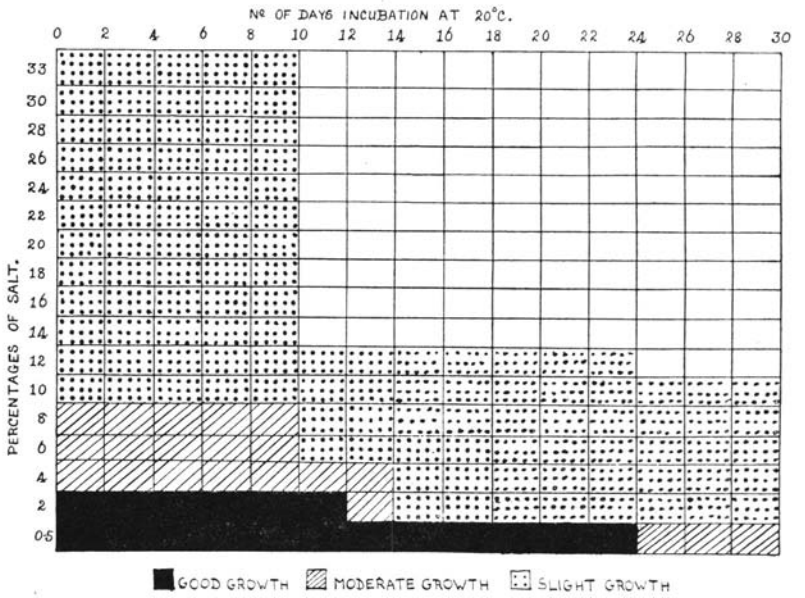

Fig. 6. *S. aurantiaca.*

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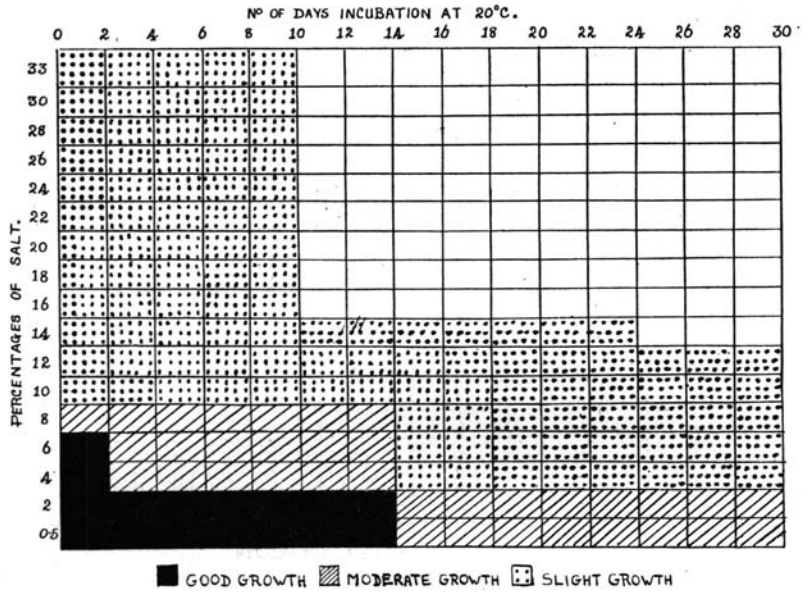

Fig. 7. *S. rosea.*

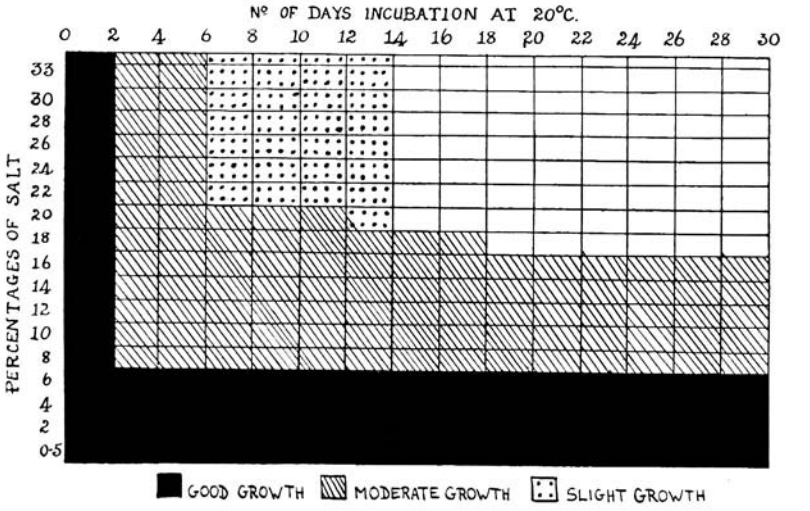

Fig. 8. *M. tetragenus.*

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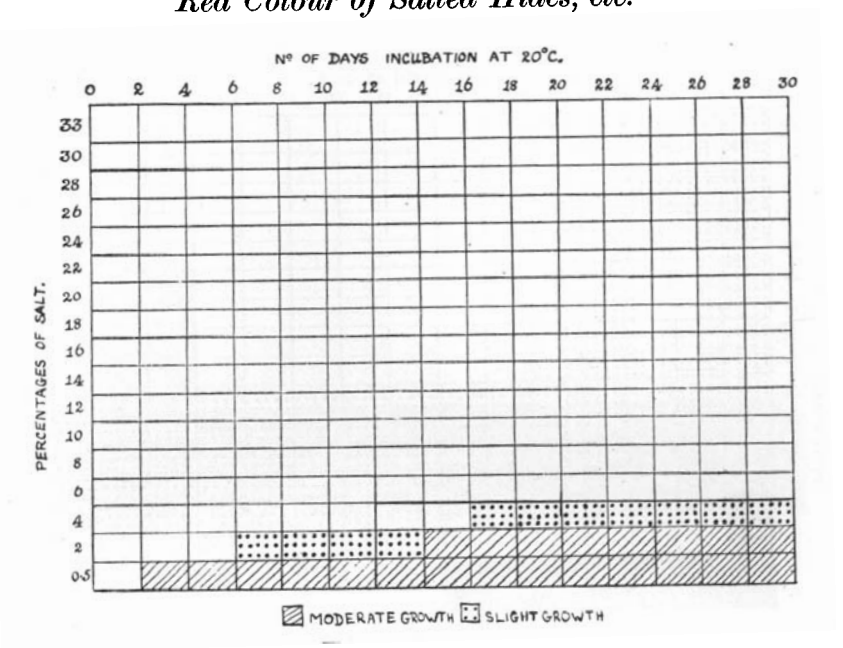

Fig. 9. *Actinomyces.*

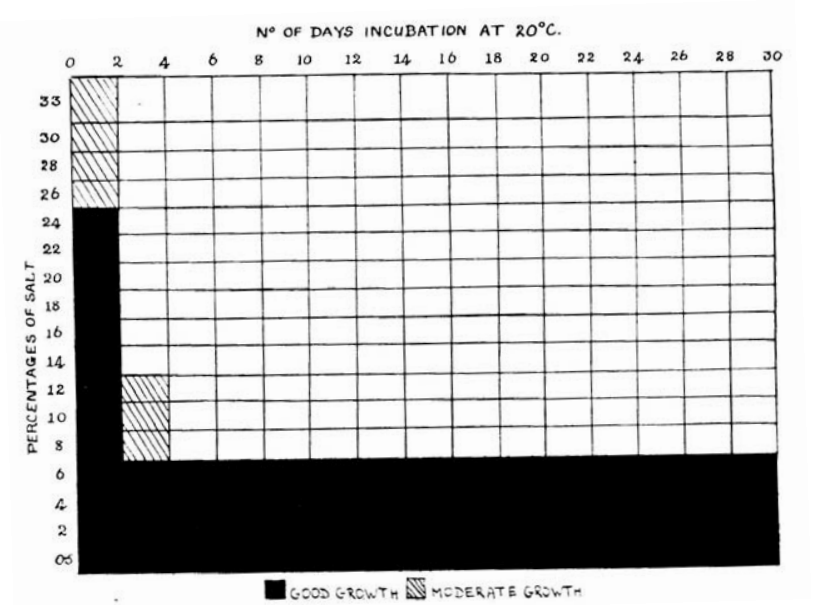

Fig. 10. *B. proteus.*

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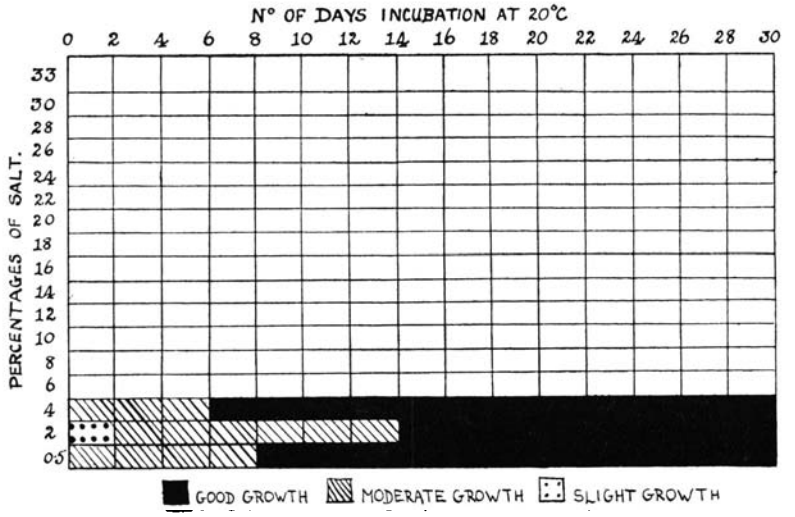

Fig. 11. *B. pyocyaneus.*

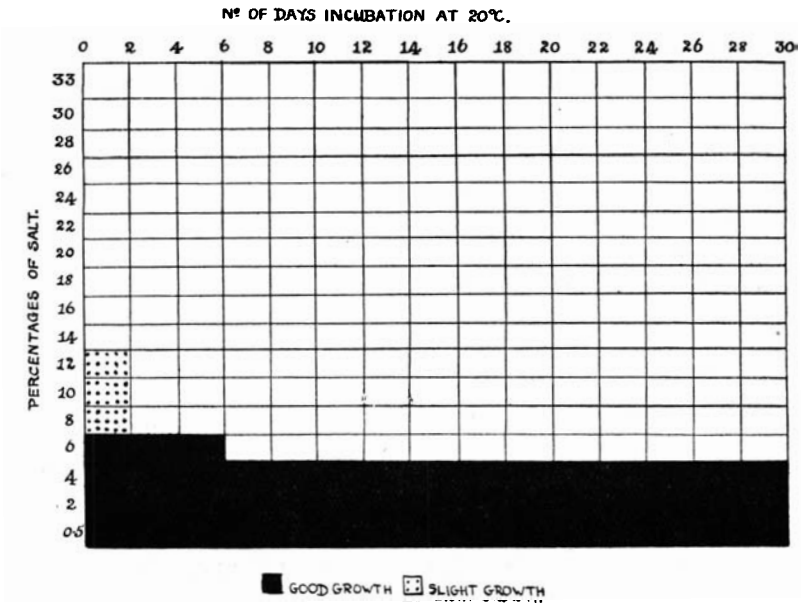

Fig. 12. *B. fluorescens liquefaciens.*

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Table I. *The growth of organisms of dirt and putrefaction as shown by the turbidity of the broth cultures after* 48 *hours.*

Organism	Percentage of sodium chloride in the broth					
	0·5	2·0	4·0	6·0	8·0	10·0–33·0
S. lutea	Very turbid	Very turbid	Very turbid	Very turbid	Slightly turbid	Clear
S. aurantiaca	,, ,,	,, ,,	Slightly turbid	Slightly turbid	Clear	,,
S. rosea	,, ,,	,, ,,	,, ,,	,, ,,	,,	,,
M. tetragenus	,, ,,	,, ,,	Very turbid	Very turbid	Slightly turbid	,,
B. pyocyaneus	,, ,,	,, ,,	Slightly turbid	Slightly turbid	,, ,,	,,
B. fluorescens liquefaciens ...	,, ,,	,, ,,	Very turbid	,, ,,	,, ,,	,,
Actinomyces	Clear	Clear	Clear	Clear	Clear	,,
B. proteus	Very turbid	Very turbid	Very turbid	,,	,,	,,

The results of these experiments confirm what has been noted by other workers (Hill and White (1929), Dubois (1910), Duthoit (1923), Guillemard (1909)) on this subject, *i.e.* that cocci can resist high concentrations of salt much better than bacilli. They indicate also, and most of them were repeated once or twice with the same results, that each type of organism examined reacts in a specific manner to the presence of different salt concentrations in the medium. They thus support the suggestions that have been made by the above-mentioned workers that media of varying salt concentrations might be used as a method of separating organisms in mixed culture and might be of service also in helping in the identification of different kinds of bacteria.

Conclusions.

1. Certain brick-red stains on salted hides are produced by the growth of "halophilic" or salt-loving organisms which come from marine salt used in curing the hide.

2. There are probably many varieties of these organisms, their common characteristic being that they flourish best in high concentrations of salt, and will not grow when the salt concentration falls beneath 6 per cent.

3. The organisms of dirt and putrefaction examined, though certain of them survived for a considerable time at salt concentrations as high as 30 per cent., did not multiply when the salt concentration of the medium was above 8 per cent.

4. The non-halophilic cocci examined survived at high salt concentrations much better than the non-halophilic bacilli.

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