OBSERVATIONS ON LUMINOUS BACTERIA

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Inoculations of luminous bacteria (*Bacillus Fischeri*, Beijerinck, Migula) (Migula, 1900) on the surface of agar slants of phosphate buffered medium were made by one of us (Shoup, 1928) and sealed in pure hydrogen. These cultures were found to grow and regain luminescence on re-exposure to the air after two months. Preparations made in the same manner have now been found to regain growth and luminescence after fourteen months in the absence of oxygen. It has also been found that no growth or luminescence occurs in the presence of pure nitrogen, but that luminous bacteria sealed in pure nitrogen regain growth and luminescence after twelve months.

Inoculations were also made on agar slants of the calcium carbonate buffered medium previously described (Hill, 1928) and sealed without displacing the air. These were kept at 3.5°. Faint luminescence and slow growth continued for six days until all of the oxygen was consumed. In those tubes opened within five months luminescence reappeared at once and the transfers appeared normal. After twelve months most of the bacteria were dead as shown by the reappearance of luminescence only after the tube had been open to the air for about twenty-four hours, and by the development of only scattered colonies on the transfers. On the other hand, transfers from inoculations on the phosphate buffered medium appeared quite normal after twelve months, even when these had been sealed without displacing the air.

It has been stated (Hill, 1928) that luminous bacteria were soon killed in borate buffers above pH 9.0, the assumption being made that the buffers were non-toxic, but it was later found that

these bacteria were unharmed in NaOH solutions at pH 9.0 (Hill, 1929), and that they survive for about thirty minutes at pH 10.0. Luminous bacteria grow and luminesce best on alkaline media. In the earlier paper, the poor luminescence and slow growth on the barium carbonate buffered medium (pH 9.2) was ascribed to the too-great alkalinity of the medium. It now appears probable that a specific toxic action of the barium ion was also concerned. Bacteria have been grown on a medium buffered with magnesium carbonate and magnesium hydroxide, both of which are strongly alkaline (in the biological range), the latter slightly more alkaline than the barium carbonate medium. On both the magnesium carbonate and magnesium hydroxide media the light was very brilliant and growth was good, continuing with full brilliance for twenty days, and lasting with lesser brilliance for twelve days longer. While the magnesium hydroxide medium was initially the more alkaline, both reached the same pH after a few days, as judged by thymol blue in the medium.

If bacteria are suspended in sea water and shaken with the solid buffers employed, the light is fully brilliant after one hour in calcium and magnesium carbonates, is faint in barium carbonate, and is extinct in magnesium hydroxide. After three hours it is extinct in barium carbonate also. In other words, luminous bacteria live and grow vigorously on the surface of medium having a reaction which is fatal if they are immersed in it. It is suggested that this is because of the constant formation of acid by the bacteria and its diffusion into the solid medium, protecting them from direct contact with the excess alkalinity, while in suspension this protection is lost through rapid mixing of the solutions, due doubtless in part to the motility of the organisms, (in spite of their motility, spreading on the surface of medium if the proper salinity does not occur). If this hypothesis is correct, a simple explanation is offered for the death of the bacteria in the course of months without growth on the surface of a medium initially favorable (calcium carbonate buffered), and their continued viability on the surface of a medium slightly less favorable for growth, but of lower reaction.

In table 1 are given the pH values for sea water shaken with the indicated solids, then the pH after boiling, then, after three hours aeration. The drop in pH of sea water shaken with calcium carbonate is explained by McClendon (1917, 1918) as due to the precipitation of the excess calcium carbonate from the supersaturated solution normal to sea water. Atkins (1922) in a discussion of the substances responsible for the reaction of sea water, gives some figures for the reaction produced by boiling MgO with water (pH 10.0), for $MgCO_3$ boiled with $MgCl_2$(pH 9.2), and for $CaCO_3$ boiled with $CaSO_4$ (pH 8.0); and these are

TABLE 1

	1	2	3	4
Sea water	8.4	8.48	8.02	
Sea water + $CaCO_3$	7.67	8.55	8.14	7.6–8.0
Sea water + $BaCO_3$	8.94	9.06	9.21	9.2
Sea water + $MgCO_3$	9.13	8.93	9.02	9.0
Sea water + $Mg(OH)_2$	9.92	9.41	9.31	9.4

1. pH (with glass electrode) of sea water, shaken with substances indicated.
2. Same, after boiling, cooling under tap, and measuring at once.
3. Same, after three hours aeration.
4. pH (colorimetric) of culture media (1 per cent glycerol, 1.5 per cent peptone, 1.5 per cent agar, 1 per cent beef extract, added to sea water-buffer mixtures, and autoclaved fifteen minutes at 15 pounds).

probably as close as could be expected to the reactions of the same substances boiled with sea water.

The precipitation of Ca and Mg from sea water has been discussed by Irving (1926), by Haas (1922), and by Kapp (1928).

From the figures of Irving and of Haas it seems likely that practically no magnesium was precipitated by calcium and barium carbonates even when boiled with sea water, and that very little calcium was precipitated by either barium or magnesium carbonates. There may have been some precipitation of calcium by the magnesium hydroxide, perhaps 20 per cent (Irving). Kapp unfortunately does not give the pH values of her solutions, so it is hard to apply them here. The reaction of sea water boiled with excess solid should depend principally on the partic-

ular solid added, and should approach very closely that of the culture medium buffered with the same substance. This is found to be the case, as may be seen by comparison of the values given in the third and fourth columns of the table.

Luminous bacteria were inoculated into plain broth, broth containing 1 per cent glycerol, and broth containing 1 per cent glucose. Samples were tested at intervals for several days for indications of the presence of indol by Ehrlich's test (Boehme, 1906). No instances of color reaction were noted, indicating that indol was absent. This species of luminous bacteria is in no sense putrefactive.

Stab cultures into nutrient gelatine of pH 8.0 show abundant growth at the surface after twenty-four hours, and liquefaction begins after forty-eight hours, proceeding down the entire length of the inoculation to form a crater of liquefaction, reaching maximum depth in four days. Luminescence occurs at the top of the crater, occurring in the depths only after complete liquefaction of the stab. A flocculent precipitate gradually collects at the bottom of the crater. Stab inoculations in nutrient gelatine with 4 or 5 cm. of gelatine poured in above will show luminescence at the top of the inoculation and finally growth and liquefaction along the stab.

Luminous bacteria inoculated into plain broth made up in sea water and adjusted to pH 8.0 cause no change in the reaction of the medium, but if 1 per cent glycerol is present the pH will fall to 7.0 in twenty-four hours, without gas production. If 1 per cent glucose is present the culture becomes highly acid within twenty-four hours with marked decrease of luminescence.

If they are grown on agar slants without glycerol there is again no evidence of acid production, and growth and luminescence are both very poor.

SUMMARY

Preservation of luminous bacteria (*B. Fischeri*) in presence of pure hydrogen and pure nitrogen is discussed, also their preservation in absence of oxygen on two kinds of media, with an explanation of the difference in result. Reaction of culture

media produced by excess of different solid buffer substances is discussed. Barium carbonate, alone, of the substances used is toxic, while magnesium carbonate is apparently stimulating. These luminous bacteria do not produce indol. They liquefy gelatine. They produce acid from glycerol and glucose, but luminesce very little on glucose.

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