

BACTERIA AND THE DISINTEGRATION OF CEMENT.

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TO THE SOCIETY.

Occasionally the cement work of water canals and reservoirs disintegrates below the water level, and instead of showing a smooth and apparently hard face, the surface is seen to be more or less eroded. When struck with a pick, the cement easily comes away, and a porous internal structure is revealed. The cement matrix has disappeared, and the sand, grit and stones are practically all that remain. Above the water line the cement remains quite hard, and shows no sign of disintegration.

Stutzer and Hartleb* investigated such a case, and as a result of their work, they considered that the nitrous organisms—that is, bacteria which convert ammonia into nitrous acid—might assist in the decomposition of the cement, through the production of nitrous acid, which dissolves the lime forming the soluble calcium nitrite. They worked upon a sample of brownish coloured mud taken from the bottom of the Bonn water reservoir. Chemically it proved to be disintegrated cement, and bacteriologically it was found capable of causing the nitrification of a solution of ammonium sulphate.

Barth,† in publishing his experience with hydraulic cements, said that a destruction of the cement might take place in so relatively short a time as three years. In the case which came under his notice, the water did not contain an excess of free

* Stutzer and Hartleb, *Zeit. für angew. Chemie*, 1899 (17) 402; Abstract in *Jour. Soc. Chem. Industry*, xviii. 495.

† Barth, *ibid.*, 1899 (21) 489; Abstract, *ibid.*, xviii. 686.

carbonic acid and the decomposition of the cement seemed unaccountable. When the reservoir was faced with a cement containing a percentage of silica higher than the original cement, no further disintegration was observed.

A disintegration of the cement canals used in conveying the Sydney water supply has been observed for some time by the engineers. Acting under instructions from the Council of this Society, and upon the invitation of Mr. Darley, Engineer-in-Chief for Public Works, I visited the faulty canals on September 8th, 1900, under the guidance of Mr. Smail, Engineer to the Metropolitan Water Supply Board, and collected samples for laboratory examination.

The cement above the water line was absolutely hard, while below the water it was soft, and the surface could be easily scraped off with a blunt nickel spatula to a depth varying from $\frac{1}{16}$ to $\frac{1}{8}$ inch. To get samples deeper into the cement a pick was used. The material easily broke away. The samples included the blackish sediment at the bottom of the canal, a scraping from the surface of the side, and the sandy débris at depths of one, four and six inches.

So far as the history of the cement is concerned, the canal was made some 18 years ago, when, I am informed, cement was bought by the brand, whereas now all cements are tested physically by the Board before purchase. This should be borne in mind, because the disintegration may be purely and primarily chemical and not at all the result of chemical action induced by living micro-organisms.

In endeavouring to obtain organisms which might cause the disintegration, it would obviously be useless to separate all the bacteria that are present in the samples. Since the cement is impregnated with water, all the organisms that are in the water would be found. We ought rather to try to exclude bacteria which would not be expected to have an action upon the cement. This means the employment of methods of culture or media as permit the growth only of such organisms. To fix upon these

methods or media certain hypotheses regarding the behaviour of the bacteria must first be formulated. Stutzer and Hartleb would probably have first formed the hypothesis regarding the action of nitrifying organisms, and then have endeavoured to induce nitrification in a solution of ammonium sulphate. The dilute solution of ammonium sulphate employed in testing nitrification is a selective medium, since it favours the growth of the nitrous organisms and hinders the multiplication of others. Since nitrification has been suggested, one of the points of this investigation should be to test the idea, and see to what extent it is corroborated.

Another idea that gives the cue for a selective medium is based upon the disintegration of the cement through loss of lime. Whether it is removed as the hydrate, the bicarbonate, or other salt, *e.g.*, nitrite, we cannot tell. It is probable, however, that the surfaces of the disintegrating cement particles are more or less alkaline, and if such is the case the bacteria that can withstand or grow only in the presence of alkali are more likely to be responsible for the disintegration than those which show an antipathy to alkaline media. The majority of bacteria prefer small amounts of alkali, say a medium containing 0.05 % sodium carbonate, but there are not so many that can grow in a medium as alkaline as lime water, which contains 0.28 % calcium oxide. It may be said in parenthesis that the bacteria which normally inhabit the intestinal tract of mammals can grow in media containing at least 0.75 % sodium carbonate, but as I have indicated in a former paper, the water which supplies Sydney is pure, and therefore the possible presence of these organisms may be dismissed. There is, however, the question of what bacteria in the disintegrated cement can be separated by means of increasing amounts of alkali. Sodium carbonate is the most convenient alkali to use, since it is soluble and does not alter during the processes of sterilising the media and growing the organisms. Lime would be the ideal alkali to employ in this case, but its relative insolubility and its tendency to form the insoluble carbonate precludes its use.

The fluid on the surfaces of the cement particles cannot contain much nitrogenous nutriment, and consequently those bacteria which can grow and therefore be isolated in media which contain minute quantities of nitrogenous material are more likely to be the cause of the cement disintegration (if it be bacterial) than organisms which cannot grow under such circumstances.

There are accordingly three lines of research indicated, and of these I shall begin with the nitrification. Several 100 c.c. bottles were half filled with ammonium sulphate nutrient solution,* plugged with cotton wool and sterilised. Portions of the samples of the disintegrated cement were introduced and the bottles incubated at 22° C. No nitrification had set in when they were tested after 1, 5, 13, and 39 days respectively. On the 65th day, when they were again tested, nitrites were found in quantity in two bottles, one of which had been infected with material taken from the surface of a cemented crack at the bottom of the canal, and the other with mud also taken from the bottom of the canal. On the 81st and on the 101st day the bottles were again tested, and the same fact observed. Two fresh bottles of ammonium sulphate were inoculated with a small loop of the nitrifying solutions, and nitrites were found in these on the fourth day.

It is seen that nitrification was obtained in the solutions of ammonium sulphate that were infected with the surface layer of the cement and with the mud at the bottom of the canal. It was not obtained in the cement at the depths of one, four, and six inches. This is important, because disintegration was in progress at these depths in the cement wall, and if the nitrifying organisms contribute at all to the decomposition of the cement, they should have been found at these places, and not only on the surface where the material had become thoroughly disintegrated. Stutzer and Hartleb found the nitrifying organisms in the mud at the bottom of a cement reservoir. This is exactly what has

* Ammonium sulphate 2 grms., sodium carbonate 1 gm., potassium dihydrogen phosphate 0.1 gm., tap water 1000 c.c. as recommended by Stutzer.

been found in these experiments, but furthermore they were not found in the disintegrating cement below the surface. This points to the nitrifying organisms coming upon the scene at a late period when disintegration is complete and when a considerable amount of organic matter derived from algæ, etc., is present in the brown and black débris.

The next line of investigation was the separation of organisms that could multiply in the presence of an alkali such as sodium carbonate. Taking the disintegrated cement obtained at a depth of six inches into the canal face as being the sample most likely to contain bacteria capable of causing disintegration, portions were introduced into tubes containing 10 c.c. of bouillon and quantities of alkali varying from 0.5 to 1.0 c.c. were added. The tubes became turbid and orange-coloured colonies of one type developed on plates inoculated with the growth of the tube containing the highest amount of alkali. Subcultures of the organism showed it to be *Bact. croceum*,* a bacterium which had previously been isolated from the Sydney water. It grows well at 37° C., and this temperature was employed in subsequent trials with further increasing amounts of alkali. These trials showed that a turbidity, the evidence of growth, took place in the presence of equal volumes of 10 % sodium carbonate solution and bouillon, that is, the bacterium developed in the presence of 5 % by weight of sodium carbonate. A percentage greater than 5 prevented the growth entirely. Plate cultures from the 5 % bouillon culture showed the turbidity to have been caused by the growth of the organism, while plates infected from the clear tubes containing over 5 % remained sterile. It is needless to say that the bacterium grew in bouillon containing less than 5 % sodium carbonate (Na_2CO_3). With the exception of *Bact. croceum*, all the bacteria in the deep cement were inhibited by 0.5 % sodium carbonate at a temperature of 37° C.

This organism is remarkable in being able to withstand so much alkali. The records as to the limiting amount of sodium

* These Proceedings, 1900, Part iii., p. 456.

carbonate capable of permitting the growth of bacteria are few in number. Reinsch† in experimenting with the Elbe water below Hamburg found that 2 % permitted the growth of some unidentified water bacteria, while 3 % did not. Fermi‡ has recorded the action of potassium hydrate upon many micro-organisms. Excluding the micrococci, which appear to be less influenced by the presence of this alkali in culture-media than rod-shaped bacteria, the organism that can withstand most potassium hydrate is *Bact. luteum*, a yellow bacterium which has some affinities with *Bact. croceum*. The growth of *Bact. luteum* is inhibited when 12 drops (= 0.6 c.c.) of normal potassium hydrate have been added to 5 c.c. of nutrient agar. This is equal to 0.67 % of potassium hydrate. Experiments with *Bact. croceum* showed that growth occurred in the presence of 1.6 % potassium hydrate, but no growth took place when double that amount was used.

The presence in the cement of an organism capable of growing with so much alkali is noteworthy. It is also significant that the same organism was found at depths of four and six inches in one place, but also at a depth of four inches into the disintegrating cement face in another portion of the canal below Prospect Reservoir, some miles distant from Kenny Hill, from where the first samples were obtained. The indifference of the organism to the alkali does not warrant the assumption that it is the cause of the disintegration, but there is the possibility that it may have something to do with it. To obtain some knowledge concerning its action, an experiment was made with cement blocks. These consisted of equal volumes of sand and old cement, and also of two volumes of sand to one of new cement. When they had hardened and had been sterilised, a culture of *Bact. croceum* was painted on the surface of the blocks with a platinum loop and the infected blocks were placed on a glass shelf in a desiccator which was filled up to the level of the blocks with boiled and cooled tap water. The whole vessel had been sterilised, and

† Reinsch, Centralblatt für Bakt. i. Abt., x. 415.

‡ Fermi, *ibid.* xxiii, 208.

the water, which was renewed weekly by way of a side tube, was boiled and cooled before being filled into the vessel. The desiccator was kept at 28° C. for five months. At the end of this time the surfaces of the blocks were scratched with a spatula, but no erosion or softening could be detected in the places where the culture had been placed. It is possible that the time was not long enough, and that years are necessary instead of months, while on the other hand the cements were not the same as that with which the canal was made. However, the experiment, such as it was, gave only negative information regarding the action of the bacterium.

Another line of investigation was to discover any organism capable of growing in a poor medium and likely to cause cement disintegration. To isolate such, a solution of asparagin (0.1%), cement (1%) and tap water was prepared, and bottles containing this medium were infected with portions of the samples and incubated at 22° C. A mixed growth appeared in all the bottles, especially those infected with the cement from depths of four and six inches. From these other bottles were infected, and after 10 days agar plates were prepared. The agar medium consisted of washed agar with cement and asparagin, the percentages being the same as those mentioned above. A third transference into asparagin-cement solution appeared to exhaust the bacteria, as no growth was microscopically visible after 30 days. The agar plates were infected with the cultures from the cements taken at a depth of four and of six inches. The former produced two kinds of colony and the latter one kind. Of the three, two were identical and sub-cultures which were made showed it to be *Vibrio denitrificans*, Sewerin. The other organism was a coccus of variable size, and when stained of irregular appearance. Sub-cultures showed it to be *Mic. radiatus*. When these two organisms were grown upon cement blocks, as was done with *Bact. croceum*, no disintegration of the cement could be observed on the places where the culture had been placed. From this we must assume that although probably capable of growing in the fluid on the surfaces of the cement particles, these two organisms have no disintegrating action upon cement.

The presence of a denitrifying organism deep in the cement is neither an argument for nor against the action of the nitrite organisms upon the cement. *Vibrio denitrificans* reduces nitrate, but not nitrite, and in order to form the nitrate the lime of the cement must first be converted into calcium nitrate. The presence of *Vibrio denitrificans*, however, does not necessitate the presence of nitrates. They are not essential to its existence. It can grow freely in media devoid of them, so that the reduction is purely an accessory phenomenon. Moreover no nitrification was obtained on seeding solutions of ammonium sulphate with portions of the deep cement where disintegration was in active progress, and where the denitrifying organism was found.

There are doubtless many bacteria in the decomposing cement that can form acid from carbohydrates, but in the water and the cement there are no carbohydrates, or if there are they are present in such minute traces that they may be ignored. There is a possibility that acid might be formed by bacteria in the absence of carbohydrates, and to test this point neutralised asparagin-cement-water was coloured with litmus and infected with the samples. No change of colour became evident after two months' culture, so that the possibility of acid formation by bacteria in the interstices of the cement may be dismissed.

On the whole there is considerable room for doubt regarding the action of micro-organisms upon cement. There is more reason to believe that the action is purely chemical, and brought about by the decomposing and solvent action of the water alone upon the cement which probably has not been adapted for resisting the action. The débris had an alkaline reaction to litmus, and I noted that in the bottles in which I endeavoured to obtain nitrification of an ammonium sulphate solution there had formed upon the glass a considerable incrustation of crystals of calcium carbonate. It seemed peculiar that this should occur with a sample which, to all appearance, was reddish-yellow sand and grit. Curious to know the amount of free lime in the sample, I added 5 grms. of saccharose to 500 c c. of boiled and cooled distilled water, and after adding a few drops of phenolphthalein

neutralised the solution with decinormal alkali. Two grms. of the unsifted débris (taken from a depth of four inches) were introduced into the flask containing the solution, and after being shaken at intervals during a day, the solution was filtered and titrated with decinormal acid. The determination showed that there was 1.4% of free lime (CaO) in the débris.

With regard to the composition of hardened cement there are several theories. It is not a substance of constant composition, and as the percentages of the constituents differ in every brand only those that actively play a part in the hardening need be considered. According to Le Chatelier, hardened cement consists of hexagonal plates of crystallised calcium hydrate imbedded in a white mass of interlacing needle-shaped crystals of hydrated calcium monosilicate. Michaeli considers that the hardening is caused by the formation of a hydrated basic calcium silicate through the combination of free hydrated silicic acid with free calcium hydroxide. Of more recent date are the Newberrys' researches upon the essential constituents of Portland cement which they find to be such that form on the addition of water, tricalcium silicate and varying proportions of dicalcium aluminate.

It is evident that lime in the hardened cement, capable of being dissolved, is a source of weakness, and it will sooner or later be dissolved by the water. It can, therefore, hardly be doubted that a cement which, after hardening, has become disintegrated and porous after exposure to water for a number of years, and which still contains 1.4% of free lime capable of being dissolved, was not of a nature likely to withstand the action of water.

In a paper published three years before that already quoted, Stutzer* ascribed the disintegration of the cement to the solvent action of carbon dioxide dissolved in the water. Basing his opinions upon the observations of Michaeli, he considered that the addition of an active form of silica such as trass to the

* Stutzer, *Zeit. für angew. Chemie*, 1896 (11) 317; Abstract in *Jour. Soc. Chem. Industry*, xv. 595.

cement would, on setting, bring about the formation of calcium silicate instead of free lime. Barth, by facing the disintegrating canals with a cement containing a higher percentage of silica, showed the importance of minimising the free lime in hardened cement subjected to the action of water. Schiffner† concluded from the results of experiments conducted upon the Bonn reservoir (the disintegrated cement of which Stutzer investigated) that no calcareous material is capable of permanently resisting the action of running water. "As regards protective coatings for cement-lined reservoirs, an experience of 33 months teaches that the fluorine preparations sold for this purpose give satisfactory results; and oxalic acid and ammonium oxalate (the latter being best) also considerably retard the corrosion. Similarly asphaltum varnish preserves the cement."

POSTSCRIPT (*added May 16th, 1901*).—In the discussion that followed the reading of this paper, it was suggested that the lime had in the experiment been dissolved as a silicate, because it seemed incredible that there could be free lime in the exhibited sample of disintegrated cement. I was led to the conclusion that it was there as free lime by the relatively abundant formation of calcium carbonate crystals on the walls of the culture flasks. To test the matter thoroughly, however, I repeated the experiment, using 5 grms. of sample and 1 litre of boiled and cooled neutral distilled water. After three days the solution was filtered, neutralised with standard acid and evaporated to dryness. The silica was coagulated by repeated evaporation with concentrated hydrochloric acid, followed by heating at 130° C. The results showed that there had been dissolved in the water 39 mgrm. calcium oxide and 1 mgrm. silica, a ratio of $\text{CaO} : \text{SiO}_2 :: 42 : 1$. This is enough to confirm the supposition that the lime is dissolved in the free state and not as silicate. On calculating the calcium oxide to percentage in the sample, 0.78% is obtained. I

† Schiffner, Thonind. Zeit. 24 (100, Suppl.) 1320-1321; Abstract in Jour. Soc. Chem. Industry, xix. 1114.

attribute the difference between this result and the former one chiefly to the lime having become carbonated during the six months' storage in the sample bottle.

The fluid in one of the culture flasks was filtered, and the dissolved silica found to be 1.5 mgrm. The sand in the bottle was washed away by a stream of water, and the grains adhering to the bottom scraped off. After everything excepting the incrustation of carbonate on the wall had been removed, standard acid was run in and allowed to decompose the carbonate. The carbon dioxide was removed and the residual acidity determined. The difference was found to be equal to 22 mgrms. calcium oxide. The small amount of silica in solution in the culture flasks corroborates the conclusion that was arrived at from the observation of the incrustation.



Greig-Smith, Robert. 1901. "Bacteria and the disintegration of cement."
Proceedings of the Linnean Society of New South Wales 26, 107–117.

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