BACTERIA IN COAL

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Received for publication April 5, 1937

Dr. Charles B. Lipman (1931, 1932, 1934, 1935) has reported the occurrence of bacteria in various geological formations, such as ancient rocks, coal, and meteorites, and in old structures erected by man, such as the pre-Inca pyramids in Peru. His explanation of their presence, if correct, has considerable significance. He believes that the bacteria have remained quiescent or dormant in the coal since its formation, i.e., for millions of years and that the bacteria in the meteorites were present before the latter reached the earth's atmosphere.

Coal is believed to have been subjected to various degrees of pressure and heat during its formation. If the bacteria have survived the various stages through which coal passes in its formation, then it follows that either the coal is not subjected to great heat, or, under the conditions of coal formation, bacteria are far more resistant to heat than has been reported under other conditions. Geologists believe, perhaps not unanimously, that some coal formations have been subjected to temperatures as high as 300°C. during formation. This would be for a long period. If bacteria in coal resist such temperatures, then almost the only way to sterilize a piece of coal by heat would be to burn it. It would be a startling phenomenon to find bacteria resisting such high temperatures over indefinitely long periods of time.

To meet this objection, Lipman has reported extreme heat resistance of bacteria under experimental conditions. However, in considering Lipman's theory of longevity of bacteria in coal, we must admit that perhaps coal containing bacteria has not necessarily been subjected to great heat. If Lipman's theory of the longevity of bacteria in coal is proven to be correct this
will probably be used as evidence that coal has not been subjected to great heat.

Considering the importance of Lipman's work, it seems strange that no more critical analysis has been given to it. Perhaps this can be accounted for as due to skepticism. The senior author became interested in the problems raised by Lipman in 1932, and has been conducting experiments dealing with the subject somewhat intermittently since that time. The present paper is for the purpose of reporting the results.

Three explanations have been offered to account for isolation of bacteria from geological formations or material of human construction such as ancient building material: (1) the organisms recovered are contaminants due to some failure in technique; (2) they are the result of comparatively recent penetration; (3) they were in the material as formed and remained alive for indefinite periods of time, either dormant or vegetatively active.

Dr. Lipman is a firm believer in the third explanation and favors the view that the organisms have remained dormant. Some formations have contained little moisture, probably not enough for bacterial metabolism. Since bacteria can multiply in surface and distilled water, we may assume that with water present growth might occur.

Lieske (1932) has investigated the occurrence of bacteria in coal and obtained results comparable to that of Lipman.

Farrell and Turner (1932) and Turner (1932) and Farrell (1933) believe the presence of bacteria in coal represents recent penetration and criticize Lipman's work. They found the same organisms in the surface above the mine as in the coal, and after a personal inspection of the mine concluded there was evidence of water penetration. We consider Farrell and Turner are justified in believing that recent penetration as an explanation must be eliminated before we can accept Lipman's hypothesis.

The similarity of organisms in the coal and at the surface is, however, to be expected. Coal is brought to the surface and distributed over the surface of the earth. If organisms can survive in the coal and grow in our test tubes, we can safely assume they can survive on the earth's surface. The similarity or
dissimilarity between organisms in buried geological formations and those on the surface of the earth has no bearing on the question of longevity.

The first explanation, that of poor technique, can safely be eliminated. It is possible to take fifty pieces of brick, heat them red hot to insure sterility, and apply the technique described below, which is comparable to that used by Lipman, and end up with sterile cultures. Contamination can be eliminated.

Our work has been restricted mainly to an investigation of organisms isolated from coal and the penetration of bacteria into various formations. For our work Dr. Lipman very kindly sent us two organisms—a Gram-positive, pink-pigment-producing coccus and a Gram-negative bacillus. Both were non-spore-bearing in cultures.

Since coal is formed under pressure and may be subjected to considerable heat, it seemed advisable to determine the heat resistance of Lipman's organisms. In broth cultures both organisms were killed by pasteurizing temperature. The organisms kept on a dried agar slant for three years were also easily killed by heat. The agar slant was cut in half and the organism recovered from the unheated half, not from the heated. Filterable forms of the Lipman's coccus obtained by growth on Kendall's K medium and filtration were killed by pasteurization. The organism apparently does not produce heat-resistant forms under the conditions of our experiments. They may do so in coal, but that requires an assumption that needs confirmation. Lipman, in support of his stand, believes that all bacteria form resistant cells and has recorded extreme heat resistance in coal.

Some formations containing bacteria apparently contain little moisture over long periods of time. The organisms present, if they survive, must resist desiccation. The Lipman's coccus survived in a dried state in our test tubes and on coal for four years. A number of common non-spore-bearing organisms, such as Staphylococcus aureus, Sarcina lutea, and Pseudomonas aeruginosa survived the same conditions. This is too short a time to have any significance in relation to survival in the coal, but
it indicates they do not die quickly upon drying. The experiment would have had significance only if the organism had died. Penetration of bacteria into coal, rock, and other formations has a direct bearing on the problem raised by Dr. Lipman. Bacteria pass through filters of diatomaceous earth. There is considerable evidence supporting the belief that they pass through a minute stage in their life cycle. We must consider that as air and water pass through minute pores, bacteria may be carried along.

By connecting a fifteen- to twenty-pound air pressure to glass tubes, cemented in pieces of anthracite and bituminous coal, marine sandstone of miocene age, a recent sedimentary spring deposit, oligocene sandstone, and eocene basalt, and immersing the objects in water, it was possible to demonstrate the passage of air through the objects where no fissures or pores were visible to the unaided eye. Negative results were obtained in some cases, but moving the tube to another point might have produced positive results.

To determine whether bacteria really penetrated coal ten pieces of bituminous and ten pieces of anthracite coal were autoclaved for four hours at fifteen pounds pressure and then placed in cultures of the Lipman coccus and left there for three weeks or longer. The coal was removed, surface sterilization applied, and the coal then placed in flasks of broth. If no growth occurred in three weeks or longer, the pieces were ground up and the powder and small pieces placed in a fresh flask of broth.

The surface sterilization consisted of immersion in 1:500 mercuric chloride or superoxol for ten minutes or longer. This is sufficient for the non-spore-bearing organisms used. Prolonged exposure was avoided. The pieces of coal were immersed for two minutes in two changes of boiling water to remove the bichloride or superoxol. They were then immersed in 95 per cent alcohol and flamed and then placed in broth.

Of the twenty pieces of coal placed in broth after surface sterilization, six produced growth. However, the organism recovered was not the experimental organism but a spore-bearing contaminant. All of these six pieces of coal were treated with
the superoxol which was not as effective as the mercuric chloride for surface sterilization.

The remaining fourteen pieces of coal were ground up and placed in fresh flasks. A pure culture of the experimental organism developed in six of the fourteen flasks. The rest remained sterile.

The results obtained indicate that bacteria may penetrate coal beyond the reach of surface sterilization as applied. Also that they may not grow out to the surface when the coal is placed in broth. The penetration occurred in three weeks. Under natural conditions there is unlimited time to allow for deep penetration.

We do not know that the autoclaving sterilized the coal, but since only the experimental organism was recovered from the interior we believe this to be a justified assumption, although the possibility of its being in the coal and surviving the heating must be considered.

Brick is more porous than coal. Forty pieces were heated red hot for two hours to sterilize, then placed in cultures of four non-spore-bearing organisms—Lipman’s coccus, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. After three weeks they were tested in the same manner as the coal. No cultures were recovered. The mercuric chloride and superoxol apparently penetrated the brick as far as the organisms. The sterilizing agents were removed by the washing, not enough reaching the flasks to inhibit growth.

In nature penetration may be aided by the pressure of a column of water. A sterile brick was exposed at one spot to a three- to six-foot column, one-half inch in diameter, of a broth culture of Lipman’s coccus. After exposure the brick was sawed into ten pieces and the pieces treated as described. Lipman’s coccus was recovered from two of the ten pieces. This was repeated four more times. Only one of the five bricks gave negative results.

The experiment was repeated with a piece of bituminous coal. When cut up Lipman’s coccus was recovered from six of ten pieces of coal. It is evident that recent penetration into what
appear to be impervious surfaces may account for the presence of the organisms recovered.

Water passes for a considerable distance through cement walls, and wind through brick and mortar. Many geological formations are more porous. Pieces of coal placed in water for forty-eight hours showed an increase in weight from 0 to 60 per cent. An exchange of air between the inside and outside of porous bodies or bodies with open fractures takes place with changes in temperature such as occur between day and night and winter and summer.

Eighteen pieces of brick were sterilized by heating red hot for two hours and then buried six inches deep in a garden. Six months later they were dug up and treated as in the other experiments. Six of the pieces contained organisms beyond the reach of surface sterilization. Both cocci and bacilli were recovered.

Two bricks were taken from the interior of the base of a large smoke stack that had stood for forty-five years and was being demolished. These bricks were cut up into smaller pieces and a number of the pieces tested for organisms by the method described. A number of different cocci and bacilli were recovered from the interior of the brick.

We do not know that brick as manufactured is sterile. However, a brick maker informed us that ordinary red bricks are heated for days to between 1200° and 2500°F. It appears to us that this should result in the destruction of bacteria in the bricks. It is significant that in our heated brick experiments we recovered only the experimental organism when using mercuric bichloride for surface sterilization.

Dr. Lipman has presented problems that are of great significance, difficult of solution, and that will probably be unsettled for some time. Unfortunately, we cannot examine coal as it is formed. We are satisfied that contamination can be ruled out as a possible explanation of the apparent recovery of bacteria from various geological formations. Recent penetration as an explanation has not been ruled out.

The burden of proof still lies with Dr. Lipman. To dispel reasonable doubt concerning the validity of his hypothesis he
should eliminate penetration as a possible explanation. Incredible penetration is no more difficult of acceptance than incredible longevity and incredible heat resistance and it requires no more support from assumptions. If penetration is eventually ruled out as the explanation, there still remains the problem of whether the organisms have amazing longevity in a quiescent condition or whether they survive only in those environments suitable for carrying on their physiological activities.

REFERENCES