On the Nature of the Toxic Action of Electric Discharge upon Bacillus coli communis.

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Introduction.

In a recent paper, Thornton* has drawn attention to some results he had obtained in experiments upon the bactericidal action of electric discharge. Plates of agar were infected with bacteria of various species, and subjected, under different conditions, to the discharge from an electrified point. The plates of agar were subsequently incubated and observations taken of the development of colonies from the surviving bacteria. From experiments upon these lines he concluded that the ionised air, *i.e.* the small current (the whole of the current passing from the point was about 4 micro-ampères) produced by his discharge methods, proved fatal after longer or shorter periods to all the species of bacteria subjected to it.

This conclusion is of considerable interest, suggesting, as it does, the possibility of electrical treatment of tissue attacked by pathological bacteria, with a view to retarding bacterial action. Our attention was attracted to this paper by the fact that its conclusions seem at variance with some conclusions previously arrived at by one of us in conjunction with Miss E. M. Lee, in an investigation carried out at the University of Bristol, of which only a brief preliminary note has so far been published,† pending the further experiments which Miss Lee hopes to be able to carry out.

In this research cultures of the sour-milk bacillus, *B. Bulgaricus*, were subjected to small electric currents, and observations were made to determine the effect of such treatment upon their vitality. Contrary to Thornton's experience it was found that current densities below about 58 micro-ampères per square centimetre served to increase both the fermentation power of the bacteria as determined by electrical conductivity, and also the rate of growth as determined by countings. The fact that the current density required to produce any inhibitory effects in these experiments had to be greater than about 60 micro-ampères per square centimetre may have been due to the fact that in these cases the electric current was derived from

* "Influence of Ionised Air on Bacteria," 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 280.

† "The Influence of Electricity on Micro-organisms," J. H. Priestley and E. M. Lee, 'Brit. Assoc. Report,' 1911, p. 603.

a source of comparatively low voltage, and transmitted to the nutrient medium through the ordinary form of Kohlrausch platinum electrode which was immersed in it. But this suggestion immediately raises the question as to whether the effect detected by Thornton bore any relation to the direct action of the current, or was connected with the chemical changes produced in the atmosphere surrounding the discharge point. Thornton considers that the fatal result of the discharge may be wholly attributed to "the direct influence of, and contact with, ions in the electric wind." It is hardly conceivable, however, that mere ionic bombardment could be responsible for such deep-seated action as was observed, especially in consideration of the fact that ions have practically no penetrating power in the presence of water, a film of which must have always intervened between the organism and the discharge.

Foulerton and Kellas,* as the result of experiments carried out along lines similar to those described by Thornton, employing in many cases the same species of bacteria, had previously arrived at the conclusion that electric discharge itself was not deleterious to the organisms. They found that "emulsions" of bacteria in water became sterile after subjection to the discharge in air and in various artificial atmospheres, but considered that the fatal effect was due, not to the current, but to the products of the discharge, viz., nitric and nitrous acids in air and hydrogen peroxide in hydrogen. Qualitative and quantitative tests of distilled water, after subjection to the discharge, revealed the fact that these substances were indeed present in measurable quantities, and subsequent trials showed that such concentrations of them were fatal to bacteria, independent of the discharge. It is possible that the results obtained by Foulerton and Kellas cannot be directly applied to explain Thornton's experiments, because of the different electrical conditions. In their experiments the bacteria were contained in water in a test-tube and the current was discharged from the points of a platinum brush suspended over the surface, earth connection being made through a platinum wire sealed into the bottom of the tube. In all cases the high-tension discharge from the brush of platinum points was oscillatory in character, and it might therefore be expected that any effects produced by the action of the discharge upon the atmosphere would be enhanced, while effects due to direct action of an electric current should be far less apparent.

The results obtained by Thornton with the apparatus depicted in his fig. 2 suggest that the products of discharge, and not the ions, were the active factor. In these experiments the current passed, not through the bacteria-

* "Action on Bacteria of Electrical Discharges," 'Roy. Soc. Proc.,' 1906, B, vol. 78, p. 60.

infected plate to earth, but through the air between two metal points above the culture, and in this case, where the current through the bacteria was a minimum, "the (sterilising) action was much stronger than in the first arrangement," in which one point discharged directly on to the culture. The investigation, of which an account is presented below, was therefore commenced with the intention of attempting to ascertain whether current densities of the order used by Thornton, obtained from a high-tension source, could still prove toxic when the influence of all toxic substances produced by the chemical action of the discharge had been eliminated.

Experimental.

Bacillus coli communis, being found by Thornton to be one of the least sensitive organisms he employed, was chosen for the experiment. The high-tension discharge was obtained from the ordinary 100-volt direct-current circuit by leading this current after interruption by a mercury break through the primary of a large induction coil. The alternating discharge from the secondary was then obtained as a continuous positive and negative charge at either side of a spark gap by leading the alternating discharge through five Lodge valves arranged in series; these valves act as rectifiers, only permitting the current to pass in one direction owing to the structure of the electrodes. This apparatus, which was purchased from a special research grant obtained from the Board of Agriculture and Fisheries, was available during the intervals when not required for other experiments in progress in the Department. By this method it was then easy to maintain as long as required a difference of potential of some 70,000 volts between the poles of the spark gap, one pole was then connected to earth and the other to the discharging point. The current passing from the discharge point was measured by placing a plate of tinfoil of definite area beneath the discharge point, and connecting this by a carefully insulated wire, shielded by an outer metal tube connected to earth, to a Paul micro-ammeter, which was carefully screened by an earthed metal cover.

In this way it was ascertained that the current density of the discharge to which the bacteria were subjected was of the order of from 10^{-6} to 10^{-5} ampères per square centimetre.

The method of treatment of the bacteria was almost identical with that employed by Thornton, viz., a Petri dish containing a sterilised agar medium infected with the bacillus was supported on a small metal tripod, which itself stood on an earth-connected metal plate. Dish and tripod were then covered with a small bell-jar fitted with a rubber stopper, through which passed a glass tube, open at the upper end, and with a platinum wire sealed into the

lower. By means of mercury, connection was made between this wire and the cable from the discharge set, and so the platinum point discharged downwards towards the Petri dish and metal plate.

1. *Discharge in Air.*—A repetition of the original experiments seemed first desirable, and accordingly cultures were exposed as described above. In 30 minutes the plates were almost cleared, subsequent incubation producing only a few colonies around the edge. In one case one side of the bell-jar was inadvertently wet, and instead of a continuous discharge, intermittent sparking down that side to the metal plate took place. The Petri dish afterwards showed sterilisation over about one-third of its area, and that on one side only. Exposure of 40 minutes or more always resulted in complete sterilisation of heavily infected dishes.

Since, then, the discharge in air was definitely deleterious to the organisms, and as it did not seem likely that ionisation effects could be the cause, the *rôle* played by the products of discharge needed investigation. These would be chiefly ozone and nitrous and nitric acids, which would be carried well on to the infected surface by the electric wind, thus providing every facility for their absorption. To test the action of these products, apart from the direct action of the discharge, a Petri dish of distilled water was exposed to the discharge under conditions identical to those obtaining in the original experiments with infected agar. The liquid was then removed and heavily infected, plate subcultures being made from it after an hour. In no case was there any development in these subcultures upon incubation, even if the water, previous to infection, were exposed to the discharge for only 20 minutes, thus confirming the idea that the products of discharge alone proved fatal.

Particular investigation of these products was now carried out by means of qualitative and quantitative tests of the distilled water after exposure. Abundance of NO_3 radical was present, as shown by the diphenylamine test, whilst addition of starch and potassium iodide solutions produced a deep blue coloration, due to nitrite or ozone, or both, also the liquid gave a distinctly acid reaction, the acidity being measured in a few cases by titration :—

No. of experiment.	Acidity as grammes of nitric acid per c.c. per hour.
1	0·00034
2	0·00072
3	0·00070
4	0·00053
5	0·00067
6	0·00060
7	0·00054

The following experiments show that solutions of about this strength of acidity, and containing nothing but nitric and nitrous acids, are capable of destroying the bacteria. A solution containing approximately 0·002 grm. of nitric acid and 0·003 grm. potassium nitrate per cubic centimetre was made up and its acidity determined. From it were made solutions corresponding respectively to

No.	Grammes of nitric acid per c.c.
1	0·0007
2	0·0003
3	0·0005
4	0·0003

Each was then infected with the bacillus, well shaken, and after an hour subcultures were made. All the plates proved sterile upon incubation, indicating the failure of the organisms to exist in such solutions. Controls with untreated distilled water were carried out simultaneously with the above experiments, and plates infected from these showed luxurious growth.

Thornton, in his paper, intimates that the criticism had been made that hydrogen peroxide might be responsible for the sterilising action of the discharge, but on discharging on to a test solution of potassium titanium sulphate, which detects minute quantities of hydrogen peroxide by the formation of yellow titanium peroxide, we found that no measurable amount of that compound was formed. Indeed, it seems unlikely that hydrogen peroxide would exist in the presence of excess of ozone, the two tending to interact with mutual reduction:—

$$H_2O_2 + O_3 \rightarrow H_2O + 2O_2.$$

2. *Discharge in Hydrogen.*—It is chiefly upon his experiments in hydrogen that Thornton bases his conclusions as to the direct instrumentality of the current in the bactericidal action. These experiments have therefore been carefully repeated, using the same form of electrode by means of which a continuous stream of hydrogen was caused to enter the discharge vessel by sweeping past the discharging point. Pains were taken to obtain the hydrogen in a comparatively pure state, since discharge in the unpurified gas resulted in the formation of a film of metallic appearance, possibly arsenic, on the object discharged upon. Therefore all hydrogen, after leaving the cylinder containing it under pressure and before being used, was passed slowly through three **U**-tubes containing respectively soda-lime, silver nitrate crystals and lumps of a mixture of lime and mercuric chloride, and, finally, through wash-bottles of strong sulphuric acid and potassium pyrogallate solution. In the latter, solutions of caustic potash and pyrogallic

acid were mixed, after all air had been replaced by hydrogen, by means of repeated exhaustings with a Geryk pump and refilling. When the potash and pyrogallol were allowed to mix they constituted both a test and an absorptive agent for oxygen, very little of which was present, judging from the very faint coloration of the solution.

After this treatment the hydrogen was led to the bell-jar, which for these trials was fitted with an exit delivery tube. This exit tube was connected through two more wash-bottles, the first being another test of pyrogallate, and the second merely to prevent diffusion of air back into the first. The bell-jar was rendered air-tight by sealing it to the metal plate with a stiff wax. The apparatus was then exhausted by means of a Geryk pump and slowly re-filled with purified hydrogen, this being done three times, after which the bell-jar was found to be free from oxygen. The discharge was now switched on, a stream of hydrogen being kept continually passing through the apparatus during any exposure.

Continuous discharge upon infected agar, for periods varying from 30 minutes to 2 hours, failed to produce any toxic effect, the colonies developing as quickly and in as great a number after exposure as normally. This result is the reverse of that obtained by previous investigators, Thornton, and also Foulerton and Kellas, having stated that the discharge proved fatal in hydrogen as well as in air, though the latter give no indication that any attempt was made to exclude oxygen completely. They attribute their result to the formation of hydrogen peroxide, which, by quantitative tests and subsequent trials with definite concentrations, they show to be produced in quantity sufficient to destroy the bacteria.

Thornton, on the other hand, assumes at the outset that no hydrogen peroxide was formed in his experiments, but makes no statement as to any test employed to detect it. It may have been that the compound was indeed formed, and that it was responsible for the sterilisation. Such a state of affairs is probable if the hydrogen atmosphere contain small quantities of oxygen, as was shown by some experiments of ours with such mixed atmospheres. Infected plates exposed to the discharge for 40 minutes, under such conditions, show after incubation a small clear space immediately beneath the discharging point, but the effect never approaches that obtained in air. Quantitative determinations, made by a series of comparative colour tests with the above-mentioned titanium solution, disclosed the fact that the presence of oxygen induced the formation of hydrogen peroxide in varying quantities.

No.	Total amount of H_2O_2 per hour.	No.	Total amount of H_2O_2 per hour.
1	0·000197 grm.	8	0·000099 grm.
2	0·000085 „	9	0·000114 „
3	0·000051 „	10	0·000029 „
4	0·000019 „	11	0·000174 „
5	0·000046 „	12	0·000677 „
6	0·000112 „	13	0·001459 „
7	0·000195 „		

The variation is probably due to the different amounts of oxygen present, and although quantitative determinations of the relative proportions were not made, the amounts of oxygen present in Nos. 12 and 13 were certainly larger than in the other cases.

Herein, then, may lie the explanation of the discrepancy alluded to above, the unsuspected presence of small quantities of oxygen serving to produce hydrogen peroxide in amount sufficient to cause the death of the bacteria. From the foregoing results we are led to conclude that in the destructive action of the discharge upon bacteria, the current itself plays no part, but that the gaseous products of such a discharge in air are the actively toxic agents, causing the death of the organisms, independently of the current.

Summary.

1. Electric discharge in air is fatal to bacteria exposed to its action.

2. The effect is due to the products of the interaction of the constituents of the air, namely nitric and nitrous acid and ozone.

3. Discharge in air-free hydrogen has no deleterious effect on the organisms, but the presence of small quantities of air allows the formation of a toxic substance, probably hydrogen peroxide, which again exerts a bactericidal action.

4. It, therefore, follows that electric discharges in which the current density does not exceed 10^{-5} ampère per square centimetre do not exert any directly toxic action upon micro-organisms, a result which is contrary to the statements made by some previous investigators.