THE PRODUCTION OF ANTITOXIN BY THE PASSAGE OF ELECTRICITY THROUGH DIPHTHERIA CULTURES.

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Prochownik and Späth, in 1890, made the observation that bacteria adhering to the positive pole of an electrical current are retarded in growth or killed, according to the strength and duration of the current. In these experiments results were obtained only by growing the bacteria in agar on the electrodes themselves, and then dipping these in salt solution and passing the current. Simply passing the current through a liquid culture or suspension of the bacteria had no effect. They attribute the effect to the chlorine gas liberated at the positive pole.

Apostoli and Laquerrière found that passing a current through bouillon cultures had the effect of killing bacteria if the poles are placed close together. The action is independent of the heat that is produced, and takes place only at the positive pole. The action is believed to be due to the decomposition of the nutrient solution into acids and to the liberation of oxygen. The electricity itself is not believed to play any part directly.

Foth found that electrical currents lessen the fermentative power of yeast cells. He concludes that the action is due to the products of electrical decomposition, notably to the development of ozone.

Tolomei found that only strong discharges from a Ruhmkorff apparatus, placed very close over the surface of the liquid, stops acetic-acid fermentation. These discharges do not cause a sterilization of the liquid, and fermentation begins again slowly after the discharge.

Spilker and Gottstein tested the effect of electricity upon cultures

and suspensions of bacteria in various fluids. The bacteria were destroyed in suspensions in water and in " weiss Bier," but not in milk. The suspensions in blood were sterilized by the electrical current, and it was supposed that the iron in the blood had something to do with the action; but suspensions in solutions of various salts of iron were not sterilized by the electrical current. Suspensions in solutions of iron albuminate were retarded in growth, but development of bacteria recommenced after eight days.

Verhoogen found that there was a destruction of bacteria around the positive pole with weak currents, but if the current is made strong enough there is this action also around the negative pole.

Charrin reported some observations of Gautier made on suspensions of bacillus pyocyaneus in solutions of iodide of potassium and in artificial serum. The positive electrode consisted of copper, and was dipped into the suspensions of the bacteria. In both cases the bacillus was deprived of its power of producing any colour when grown on agar after the current was passed for seven minutes; longer action was found to retard subsequent growth and even to cause sterilization.

Krüger found that the constant current is able to suspend growth, but not to kill bacteria, where all chemical action due to the products of decomposition of the nutrient material is excluded. Cultures subjected to a certain strength of current for a certain length of time possess immunizing properties.

Smirnow inoculated a large number of rabbits with 0.5 to 0.7 cubic centimetre of a bouillon culture of the diphtheria bacillus two or three days old, and twenty-four hours afterward, when the animals were very sick, he injected 8 to 10 cubic centimetres of the product from the positive pole of electrolyzed toxin. The animals all recovered. In order to convert 200 cubic centimetres of toxin into antitoxin, he finds it necessary to use a current of 80 milliampères for not less than sixteen to eighteen hours. The action of the current on the toxin is apparent at the positive pole by a bleaching of the bouillon; at the negative pole by a darkening of the bouillon. In applying the current, Smirnow used a U-shaped tube, with a glass stopcock in the middle.

Krüger finds that the calibre of the U-tube used to contain the culture should not be more than 18 millimetres, and that the entire length should not be more than 22 centimetres. The current should be made to pass upward through the liquid. Krüger, moreover, obtained the best results by scraping the diphtheria bacilli off of agar cultures and making a suspension in one-per-cent salt solution. This he finds better than bouillon cultures. He was able to prevent death in rabbits inoculated with virulent cultures by injection of 3 cubic centimetres of cathode product, and he found he could save rabbits with 2 cubic centimetres of the crude product.

Smirnow finds that diphtheria antitoxin, which cures inoculated rabbits and guinea-pigs even in advanced stages of the disease, can be obtained by electrolysis; that the strength of the antitoxin depends largely upon the strength of the toxin that has been subjected to the current; moreover, that it depends upon the degree of acidity of the liquid at the negative pole. This acidity should be such that 0.9 to 1.3 cubic centimetre of normal soda solution neutralizes 1 cubic centimetre of the electrolyzed product. This degree of acidity is the optimal for guinea-pigs, but for rabbits the current must be allowed to act for a longer time and the acidity should be such that 1.5 to 1.7 normal soda solution is required to neutralize it. He finds that any of the electrolyzed product above that which is just sufficient is injurious.

D'Arsonval and Charrin find that continued or intermittent currents of high potentiality attenuate toxins as well at the positive as at the negative pole. They find that toxin treated with a continuous current is simply destroyed by the chemical products of electrolysis, and possesses little or no antitoxic properties. But toxin treated with an intermittent current, where the current was reversed about 200,000 times a second, produced fairly strong antitoxin in a quarter of an hour. They conclude that the production of antitoxin by an electrical current is due to a violent vibration of the molecules, resulting in a rearrangement, and not to any chemical decomposition.

In the following tests we employed a current from the Edison electric light, which usually has a strength of 110 volts; this was re-

duced and controlled by a simple rheostat inserted in the current. The apparatus used to hold the liquid cultures consisted of two test tubes, connected about the middle by a tube with a stopcock. In some cases the stopcock was of glass, in others of hard rubber. The resulting shape of the apparatus is that of the capital letter H, the vertical arms open at the top and closed below with a platinum-wire electrode melted in. In order to expose a large surface, the wire projects inside the tubes several inches, and is coiled up. The cross arm of the H carries the stopcock. Following Krüger's suggestion, the length of the lower half of the vertical arms and the transverse connection together were made to measure 22 centimetres, and the diameter 19 millimetres. The apparatus with these dimensions gave better results than a larger apparatus made after a similar pattern with a hard-rubber stopcock. In all cases the current was made to pass upward through the liquid. The strength of the current was determined with a Weston milliampèremeter.

The action of the current is made evident by the evolution of gas bubbles, which are given off from the electrodes, and rise and form a foam at the top. A precipitate quickly forms in both tubes, but more abundantly over the positive pole, and the liquid in this tube also becomes lighter in colour. After the stopcock is turned off, cutting off the current, the precipitate soon settles to the bottom. The fluid over the negative pole becomes darker in colour. There is a peculiar odour given off, resembling that of chloride of lime; but it was found that the fluid contained only one tenth of one per cent of chlorine. The strength of the current increases gradually as it is allowed to pass, and has to be regulated with the rheostat from time to time.

Tests were made on bouillon cultures of the diphtheria bacillus grown for varying lengths of time in the incubators. Some of them were filtered through several thicknesses of filter paper, and some of them were unfiltered. The current usually employed varied from 44 to 72 milliampères, and was allowed to act, as a rule, for two hours, though some tests were made with cultures that had been subjected to the current for longer and shorter periods than this. In all cases the strength of the cultures was tested on guinea-pigs previous

to the passage of the current. The tests as to whether antitoxic property was developed by action of the electric current were made by Behring's method, i. e., by administering ten times a certainly fatal dose of diphtheria toxin along with varying amounts of the electrolyzed products, either mixed with the toxin or injected at a different place in the animal.

These tests soon showed that there is a production of antitoxin over the positive pole, as the following result of one of several experiments will show:

A guinea-pig, weighing 230 grammes, was inoculated under the skin with a mixture of 1 cubic centimetre of diphtheria toxin and 5 cubic centimetres of the product from the positive pole. A similar guinea-pig was inoculated at the same time with 1 cubic centimetre of the same toxin mixed with 5 cubic centimetres of the product of the negative pole. Before the current was passed the toxin was found to be of such strength that 0.3 cubic centimetre would kill an average-sized guinea-pig in forty-eight hours. The toxin used to mix with it after the passage of the current killed in doses of 0.1 cubic centimetre in forty-eight hours. The guinea-pig inoculated with the mixture containing the product from the positive pole remained alive, and showed no symptoms of disease. The other animal died promptly in forty-eight hours. Two and a half cubic centimetres of the electrolyzed toxin was insufficient to neutralize ten times the minimum fatal dose of toxin. In order to test whether the product of the negative pole was in itself poisonous, a guinea-pig was injected with 5 cubic centimetres of it, and remained perfectly well; so the toxin properties are destroyed at the negative pole, but no antitoxin is developed.

The strongest antitoxin we were able to obtain was such that 2 cubic centimetres neutralized ten times the minimum fatal dose for guinea-pigs. In a single experiment made on rabbits by intravenous injection, neither 2 cubic centimetres of the product of the negative pole nor 3 cubic centimetres of the positive pole counteracted 0.6 cubic centimetre and 0.5 cubic centimetre respectively of toxin.

It occurred to us to try whether the presence of the bacilli them-

selves would influence the result. So unfiltered cultures and the sediment in old bouillon cultures were tried, but the result was the same, viz., 2 cubic centimetres of the product from the positive pole counteracted ten times the minimum fatal dose, while the same amount from the negative pole had no effect.

Tests with a much weaker current for a somewhat longer time seem to indicate that this method offers an advantage. But if the current, even though very weak, be kept up very long—for example, for a week or more—the antitoxin seems to be less potent.

References.

Apostoli and Laquerrière.—*Compt. rend. de l'Acad. de Paris*, 1890, cx, p. 918.

Prochownik and Späth.—Ueber die keimtödtende Wirkung des galvanischen Stromes. *Deutsche med. Wochenschr.*, 1890, No. 26. (Ibid.)

Foth.—Die Conservirung gegohrener Getränke durch Elektricität. *Wochenschr. für Brauereien*, 1890, No. 3.

Tolomei.—Einwirkung von Elektricität auf die Essiggährung. *L'Orosi*, vol. xiii, p. 401. (Abst.: *Centralbl. für Bakt. u. Paras.*, 1891, Bd. ix, No. 16, p. 539. Baumgarten's *Jahresber.*, 1891, p. 470.)

Verhoogen.—Action du courant électrique constant sur les microorganismes pathogénes. *Bulletin de la Société Belge de Microscopie*, 1891, t. xvii, No. 9. (Baumgarten, ibid.)

Spilker and Gottstein.—Ueber die Vernichtung von Mikroorganismen durch die Inductions-elektricität. *Centralbl. für Bakt. und Parasit.*, 1891, Bd. ix, Nos. 3, 4, p. 77. (Baumgarten, ibid.)

Charrin.—Electrotherapie Microbienne. *La Semaine méd.*, 1892, p. 504. (Baumgarten, 1892.)

Krüger.—Ueber den Einfluss des constanten elektrischen Stromes auf Wachsthum und Virulenz der Bacterien. *Zeitschrift für klin. Med.*, 1893, Bd. xxii, p. 191. (Baumgarten, 1893.)

Smirnow.—Ueber die Behandlung der Diphtherie mit Antitoxinen die ohne Vermittelung des thierischen Organismus dastellbar sind. *Berliner klin. Wochensch.*, 1894, No. 30.

Krüger.—Ueber die chemische Wirkung der Elektrolyse auf toxische und immunisirende Bacteriensubstanzen. *Deutsche med. Wochenschr.*, 1895, No. 21.

Smirnow.—Ueber die Behandlung der Diphtherie mit künstlich dargestellten Antitoxinen. *Berliner klin. Wochenschr.*, 1895, Nos. 30 and 31.

D'Arsonval et Charrin.—*Compt. rend. de la Société de Biologie*, 1896, Nos. 3, 4, and 5.