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REMARKS UPON THE PNEUMOTOXIN.

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THE pneumococcus is an important agent in the production of disease in the human subject. It is the most frequent cause of acute lobar pneumonia, having been found in about 80 to 90 per cent. of the cases examined. The organism likewise invades the pleura and the blood, and appears in the bile, urine, milk, etc., whilst pneumococcal infections outside the respiratory tract are constantly met with—for example, in meningitis, peritonitis, pericarditis, endocarditis, otitis, conjunctivitis, etc. The organism is also one of the exciting causes in the forms of acute catarrh clinically grouped as "influenza."

The pneumococci, in the variety of morbid conditions they are capable of producing, resemble a kindred group of micro-organisms—the streptococci.

The virulence of the pneumococcus is subject to wide variations. The most virulent forms are met with in acute inflammatory processes, and the virulence may be preserved and increased by passages through susceptible animals. The strains of pronounced virulence lead on injection to a fatal septicaemia in one to three days, those of feeble virulence simply to local lesions. In experimental work upon the pneumococcus it is essential to use cultures of undoubted and tested virulence.

Whilst acute pneumococcal infections are accompanied by symptoms of a severe toxaemia, little of a definite character is known with regard to the specific poison of the pneumococcus.

The attempts that have been made to obtain a soluble poison from cultures of the organism have not led to encouraging results. The toxic properties of filtered cultures are feeble, and large amounts are required to produce any morbid effect. The specific nature of the soluble poisons that have been described still remains a matter of doubt.

G. and F. Klemperer,[1] in the course of a careful experimental inquiry, employed a variety of methods and of material. The toxins obtained were not of a very potent nature, as doses up to 24 c.cm. were necessary to produce a lethal effect in rabbits. These observers regarded such

poisons as specific, and used them for the preparation of an antitoxic serum. The toxic and antitoxic effects were of a feeble character, and large quantities of the filtered broth cultures were necessary to produce an active immunization of animals.

Foà and Scarbone[2] and Scabia[3] conducted experiments on similar lines, but these did not yield any more striking results. Pane's[4] investigations upon a soluble poison were practically negative, and led him to ascribe little importance to the action of a poison in pneumonia.

Mennes,[5] working with highly virulent strains of the pneumococcus, found that the filtered cultures possessed little toxicity. It was only in large doses that filtered or heated cultures produced a lethal effect. Broth cultures, which had been heated to 62° C. for twenty minutes, caused on injection fever, loss in weight, and at times diarrhoea, but the results were inconstant. Rabbits treated with the toxins were in some instances resistant to a fatal dose of the pneumococcus. Mennes also immunized the goat and horse with heated, then unheated, broth cultures and the blood of infected rabbits.

In the case of the horse, its serum protected against the pneumococcus and the broth toxin, which produced fever and loss in weight. Mennes does not appear to have worked with an acutely lethal toxin, and this detracts from the value of his results.

Isaeff[6] also found that sterilized cultures in general possessed feeble toxicity, and were not fatal in doses equivalent to 3½ per cent. of the animal's weight. On the other hand, the blood of infected rabbits yielded a filtrate toxic on intravenous injection, and sometimes lethal in doses equivalent to 1 per cent. of the body weight—that is, about 10 c.cm. per kilogram of rabbit. A dose of 10 c.cm. rendered rabbits immune to the pneumococcus but not to the toxin. Sterilized broth and serum cultures in amounts of 10 to 50 c.cm. produced a rise in temperature and loss in weight. Isaeff concludes that "one cannot admit the existence of an antitoxic property of the blood in animals vaccinated against the pneumococcus." The toxins obtained by Isaeff were, however, not sufficiently powerful to justify the conclusion drawn from his experiments with them.

Carnot and Fournier[7] describe toxic dialysates obtained from cultures of the pneumococcus after concentration by drying *in vacuo*, and by precipitation with phosphate of lime.

The antipneumococcic serums prepared by Pane, Washbourn,[8] and Römer[9] by the injection of various strains of pneumococci protected animals against multiple lethal doses of virulent pneumococci. No antitoxic action has been claimed for these serums.

The efforts to obtain potent soluble poisons from fluid cultures of the pneumococcus have not proved successful, nor have the soluble poisons described shown much value as immunizing agents. None of the experiments in this direction, it may be added, furnishes any conclusive proof

of the existence of a genuine secreted toxin. There can be little doubt that the essential pneumotoxin is of the endocellular type, and that it is this toxin contained within the body of the organism which is to be regarded as the more important factor in the possible production of any antitoxic immunity.

As few direct experiments have been made in this important direction, it may be of interest to put on record the results I have so far obtained.

The object of the experiments was, in the first instance, to extract a toxin from the bodies of the pneumococci by means of the cold grinding method successfully employed in the case of typhoid, cholera, and other organisms.[10] The pneumococci were isolated from the human subject, and their virulence raised by passages through the rabbit. The cultures were made directly from rabbits which had died of a pneumococcic septicaemia. The heart's blood of the animals was spread over the surface of nutrient agar in Roux bottles. This procedure favoured a rapid and good growth of the pneumococcus, the cultures after two to three days' incubation at blood heat furnishing ample material for each experiment. The resultant mass, after trituration of the cocci, was taken up in 1 in 1,000 caustic potash solution, then spun and filtered. If care is taken to use pneumococci of marked virulence for grinding, acutely toxic filtrates of their cell juices are readily obtained. The strains in which a loss of virulence had occurred did not yield cell juices of any marked toxicity; for example, 1 c.cm. on intravenous, and $\frac{1}{5}$ c.cm. on intracerebral injection did not affect rabbits, nor did 1 c.cm., $\frac{1}{2}$ c.cm., and $\frac{1}{5}$ c.cm. kill guinea-pigs or mice on intraperitoneal injection; on the other hand, the filtered cell juices from virulent strains killed acutely in the above doses. The parallelism between virulence and toxicity was unmistakable, and is further illustrated in the figures given below. It was found that by regarding this point one was in a position to obtain not only constantly toxic cell juices from the pneumococcus, but also to increase the toxicity by further exaltation of the virulence of the organism.

The toxicity of the filtered cell juices was tested on rabbits, guinea-pigs, and mice, the majority of the experiments being made on the guinea-pig. The following figures illustrate the results obtained after three to eight passages of the pneumococcus through the rabbit.

A.—Filtered Cell Juices of the Pneumococcus Directly Isolated from the Rabbit.

Rabbit.—1.0 c.cm. intravenous. Acute diarrhœa and death.
,, 0.5 ,, ,, No effect.
,, 0.2 ,, intracerebral. Dead.

Guinea-pig.—2.0 c.cm. intraperitoneal. Dead.
,, 1.0 ,, ,, ,,
,, 0.5 ,, ,, ,,

Mouse.—0.1 c.cm. intraperitoneal. Dead.
,, 0.5 ,, ,, ,,
,, 0.3 ,, ,, ,,

B.—Filtered Cell Juices of the Pneumococcus after Passages through the Rabbit.

Rabbit.—1.0 c.cm. intravenous. Dead.
,, 0.5 ,, ,, Very ill.

Guinea-pig.—1.0 c.cm. intraperitoneal. Dead.
,, 0.5 ,, ,, ,,
,, 0.3 ,, ,, ,,
,, 0.1 ,, ,, ,,

C.—Filtered Cell Juices of the Pneumococcus after Further Passages through the Rabbit.

Guinea-pig.—1.0 c.cm. intraperitoneal. Dead.
,, 0.5 ,, ,, ,,
,, 0.1 ,, ,, ,,
,, 0.05 ,, ,, ,,

The toxic effects were of an acute character, the animals dying in twelve to eighteen hours. The main *post-mortem* appearance consisted in a more or less acute congestion of the lungs. The filtered cell juices contained about 5 mg. of solid matter in each cubic centimetre.

The above experiments will be sufficient to prove that it is possible to extract an acutely lethal toxin from the bodies of virulent pneumococci, and one of much greater potency than the soluble toxins described by previous observers, inasmuch as the ascertained minimum lethal dose was about $\frac{1}{4}$ mg.

The endotoxin of the pneumococcus is sensitive to the action of heat. A filtered cell juice heated to 55° C. for an hour failed to kill in doses of 0.5 and 1 c.cm., whilst 2 c.cm. only caused death at the end of five days. The unheated juice was acutely lethal in doses of $\frac{1}{20}$ c.cm.

The prolonged action of chloroform vapour is also injurious to the toxin. After an exposure of one hour to chloroform vapour, 0.5, 1, and 2 c.cm. did not kill the test animals, the lethal dose of the untreated toxic cell juice being $\frac{1}{20}$ c.cm.

The immunizing experiments with the above toxin of the pneumococcus are at present in progress, and will form the subject of another communication upon the feasibility of producing an antitoxic serum.

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