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Further studies on a specific pneumococcus toxin.

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In a previous preliminary report[1] on a specific pneumococcus toxin and corresponding antitoxin, the cutaneous reactions incited by the intracutaneous injection of such toxin, and the lung pathology produced by its intraperitoneal or intravenous injection, have been pointed out. Larson[2] has also reported his results in applying intracutaneous tests to a small series of human beings and has confirmed our observations on the effect of pneumococcus toxin on mice. This paper will report further preliminary observations we have made.

Dr. E. Vernon Hahn is collaborating with us in a study of the pathology produced in animals by pneumococcus toxin. Detailed reports of histologic studies of its effects upon various organs, particularly lungs, liver, kidneys, and spleen, will be made later.

Since it has been found possible to estimate the strength of various lots of both toxin and antitoxin on the basis of the ability of the toxin to produce lung pathology, and of the antitoxin to prevent it, when the two are injected together, a few brief descriptions will be given:

Pneumococcus toxin given to mice in lethal or slightly sublethal doses, has been found to produce a more or less characteristic gross and histologic picture. Grossly the lungs show edema, engorgement, and frequently apparent massive consolidation, with hemorrhage.

On section intense congestion and interstitial hemorrhage with decreased air content of the alveoli have been noted. There is inter-alveolar extravasation but no extensive filling up of alveoli with exudate or blood, that is, the processes are patchy. An inflammatory or fibrinous exudate may be found in the bronchioles, with or without marked degeneration and vacuolization of the bronchiolar epithelium. Polymorphonuclear leucocytes may be found grouped within and often adherent to the endothelium of

[1] PROC. SOC. EXP. BIOL. AND MED., 1926, xviii, 294.

[2] *Ibid.*

the blood vessels. Varying degrees of interstitial pneumonitis with pigment-laden macrophages scattered throughout the parenchyma have been noted. There may be a more or less notable catarrhal reaction in the bronchioles and alveoli with degeneration of the alveolar epithelium. Varying degrees of edema just beneath the pleura have been noted. This description relates to animals that have received interaperitoneal injections of sterile pneumococcus toxin. Numerous cultures made at autopsy have proven sterile.

In contrast with this, the lungs of animals that have received broth alone and subsequently been killed by chloroform vapor, usually show hyperemia only, with little or no exudate in the alveolar walls, which may sometimes show slight edema.

Approximately the same result as from broth alone is obtained by the injection of heat-destroyed toxin.

Proof that pneumococcus antitoxin prevents lung pathology is subject to statistical interpretation. There may be little or no gross evidence of injury, yet on section the lungs may show some catarrhal reaction, and patchy pneumonitis. They commonly lack, however, the definite polymorphonuclear reaction, the congestion and degeneration met with when toxin alone has been given.

Our experiments indicate that neither the skin reaction in rabbits nor the characteristic lung pathology we have mentioned can be induced by filtrates, in all respects similar to pneumococcus filtrates, excepting that they are prepared from *M. aureus, S. viridans, S. hem. scarlatinae, B. mucosus capsulatus, B. coli or B. typhosus.*

That we are working with an exotoxin and not an autolytic product or endotoxin is indicated by the fact that eight hour culture filtrates are found to possess marked specific toxicity, as measured by rabbit skin test and mouse lung pathology. The toxicity appears to rise sharply for about twenty-four hours, after which it has not ordinarily been found to increase, but rather to remain stationary or to decrease considerably. Five days is the limit of incubation time we have observed. Doubtless the substrate is an important determining factor. We regularly work with twenty-four hour filtrates. Toxin of 300,000 rabbit skin test doses per cc. has been prepared by twenty-four hours' incubation.

Pneumococcus toxin is relatively thermostabile, though perhaps less so than scarlet fever streptococcus toxin which it resem-

bles in this respect. We have completely destroyed the toxicity of filtrates by an hour's boiling. Simple coagulation of toxin-filtrate constituents has not been found to destroy the toxicity of the filtrate.

Heating pneumococcus toxin for short periods of time at relatively low temperatures seemingly heightens its activity, and it can apparently be destroyed by prolonged heating at temperatures under 100° C. Light appears to be detrimental. Unpreserved lots have been found to lose their toxicity with relative rapidity, whereas the addition of preservative, *e. g.*, 0.3 per cent cresol, has apparently prevented loss of potency over a period of several months, whether at ice-box, room, or incubator temperatures. Since 0.3 per cent cresol itself induces reactions in delicate viscera, we are obliged to use fresh filtrates or filtrates containing not more than 0.05 per cent cresol, to avoid confusing results, in some of our work. Dilution seems to favor loss of potency.

Samples of pneumococcus toxin have been tested and found to contain very little hemotoxin. Moreover, when the hemotoxin has been absorbed out, in the cold, and the absorbed filtrates compared with unabsorbed controls by rabbit skin tests, no quantitative decrease in toxicity has been observed. An hour's combined shaking and aeration is likewise found to produce no measurable decrease in toxicity.

Preliminary observations by Mr. E. E. Swanson on a series of dogs, prepared as for ordinary pharmacologic respiration and blood pressure tracings, indicate that toxic pneumococcus filtrates produce a reduction of amplitude of the respiratory wave on a tracing. This is generally associated with cardiac acceleration and irregular respiration. The original culture medium, heat-destroying toxin, or toxin neutralized by corresponding antitoxin have not been found to produce such changes. Moreover, when a dog has been allowed to develop such toxemic manifestations, we seem to be able to relieve completely the respiratory distress, and to a less extent, that of the heart, by the administration of antitoxin.

Preliminary attempts at active immunization of mice by serial injections of pneumococcus toxin have resulted in protection against 1,000 to 10,000 fatal doses of virulent pneumococci as measured on normal controls injected simultaneously. Our experiments indicate that the immunity operates irrespective of

type. Partially immunized animals killed by an overdose of organisms have been found to exhibit intense hyperemia of the peritoneum and abdominal wall, with serosanguinous fluid in the abdominal cavity.

Normal sera from horses, sheep, rabbits and chickens have been titrated against pneumococcus toxin and have ordinarily been found to exhibit very little power, either to prevent cutaneous reactions in rabbits or lung changes in mice. The same is true of regular antipneumococcus sera or antibody solution, and such immune sera as antistreptococcus serum, scarlet fever antitoxin and diphtheria antitoxin.

Further work is in progress. A detailed account of some of our experiments will be published soon.

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On a specific pneumococcus antitoxin.

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Specific pneumococcus antitoxins have been produced in this laboratory by various procedures, including those of Dochez, Larson, and the procedure developed in this laboratory by one of the authors (Olson), using a specific pneumococcus toxin.

The Larson procedure, which consists of injecting whole culture, appropriately attenuated, by means of a highly purified castor oil soap, has been extensively employed by us, using principally rabbits and sheep. Recently somewhat better yields of antitoxin have been obtained by injecting subcutaneously into rabbits, sheep or horses, progressively increasing doses of the sterile pneumococcus toxin.

The same pathological lesions were observed in the larger animals after the injection of successive large doses of toxin, as had previously been noted in smaller experimental animals and reported in a previous paper.

The highest concentration of antitoxin has been secured by starting with a very small amount of toxin and gradually increasing the size of the dose.