6208

Immunity in Human Beings to the Pneumococcus.

ARTHUR F. COCA.

From the New York Hospital, the New York Postgraduate Hospital, and the Lederle Laboratories.

In the fall of 1930, I began a study of the products of several bacteria in the search for hitherto unknown exotoxins which could be identified as the pathogenic agents of the respective microorganisms. This paper is a summary of the findings with various culture filtrates of the pneumococcus.

In this work I have enjoyed the cooperation of Dr. Henry W. Straus and of Dr. Norman Plummer. We shall make a detailed report of the experiments, some of which are still in progress.

The existence of a pneumococcus toxin had been suspected and A. B. Wadsworth had recommended the injection of whole cultures rather than the collected bacterial bodies in the immunization of horses.

In view of the consistent failures to demonstrate the toxin in animal experiments it was decided to test the filtrates upon young and presumably susceptible children by subcutaneous injection; the criteria of toxic action were to be a local effect and a rise in temperature.

The experiments were begun with a freshly isolated culture of type II pneumococcus obtained from Dr. Plummer.

The first filtrates produced temperatures ranging from 101°F. to 104°F. in subcutaneous doses of 2.0 cc. to 4.0 cc. or of 0.5 cc. to 1.0 cc. of the filtrate concentrated by acetone precipitation according to the method of Wadsworth and Quigley.[1] There was tenderness and swelling at the site of injection.

The later experiments were carried out with an unconcentrated filtrate from a stock type I culture which produced temperatures of 101°F. and upward in a quantity of 0.5 cc.

Immunity to the fever-producing substance was established in some children after a single pyrogenic dose or 2, and in a series of 21 children of about 5 years of age, immunity was established in 14 within 2 weeks after a second injection.

The serum of the immunized children neutralized the pyrogenic substance in mixture or separately injected. For example, 1.0 cc. of the serum neutralized 4 minimum pyrogenic doses.

[1] Wadsworth, A. B., and Quigley, J. J., *J. Immunol.*, 1931, **20**, 459.

The serum of type I and type II pneumonia convalescents neutralized the pyrogenic substance. Normal children's serum in a quantity of 2.0 cc. did not neutralize a single minimum pyrogenic dose of the type I filtrate (0.6 cc.).

Not all toxic filtrates of pneumococcus cultures are antigenic. Of 5 children that received 3 injections of 2 or more minimum pyrogenic doses at weekly intervals of filtrates from ordinary phosphate broth cultures none was found immune upon the fourth injection one week later.

The study of the conditions favorable to the production of the antigenic toxin is continuing and the results will be published in the full report.

The toxin appears to be stable since none of the filtrates have shown any lessening of toxicity, one of them having been tested over a period of 4 months.

Skin Test. The undiluted type I filtrate injected intracutaneously in a quantity of 0.05 cc. produced within 20 hours, an area of erythema with swelling and tenderness of 1½ to 3½ inches in diameter in *all* of 14 children who were given at the same time 1.0 cc. of the filtrate subcutaneously. Twelve of these children developed temperatures ranging from 100.4°F. to 104.6°F. The other 2, both of whom had had pneumonia, showed temperatures between 98°F. and 100°F., which were considered within normal range.

These results seemed to indicate that the undiluted filtrate was too toxic for practical testing purposes since the 2 immune children exhibited no difference in skin reactivity to the filtrate as compared with the 12 susceptible children.

One week later, 11 of these 12 were tested again with the undiluted filtrate and 5 of them responded with greatly diminished reactions (¾ inch or less). One refused to be tested.

A second subcutaneous injection (of toxoid—described below) was given and one week later 6 that showed no diminished reaction were tested with 0.1 cc. of the filtrate diluted 1-10. At this test 4 showed reactions of ⅞ inch to 1¼ inch diameter which were considered positive; the result in the other 2 was negative—slight redness of ¼ inch diameter or less.

The further results with the skin test, which was carried out with the 1-10 dilution of the type I pneumococcus filtrate, are summarized in the table.

The results of this limited number of tests seem to indicate that, as is the case in immunity to diphtheria, the number of immune

TABLE I.
Results of Cutaneous Tests with 1:10 Dilution of Filtrate of Type I Pneumococcus.

	Positive	Negative
Medical interns; laboratory and office staff; nurses	31*	18†
Ward patients	16	31
Pneumonia convalescents	1‡	26§

* One of these had had one attack of pneumonia and one had had 2 attacks.
† Five of these are known to have had pneumonia.
‡ Dr. Plummer regards the diagnosis of lobar pneumonia in this case as doubtful; an "unclassified" pneumococcus was found in the sputum.
§ Of these, 7 cases were of type I, 3 of type II, 2 of type III, 3 of type IV, 3 of type V, 1 of type VIII, 1 of type XX with Friedlander, and 7 of "unknown" strains.

adults is greater among the class of people represented by ward patients than among a professional class.

Pneumococcus toxoid was prepared as usual by adding formalin to the undiluted type I filtrate in a concentration of 0.3% of formaldehyde and incubating for 4 weeks at 37°C.

Ten children received 2 injections of 1.0 cc. at one week interval and 2 weeks later were tested with the 1-10 toxic filtrate. At this test 3 showed positive reactions (⅞ inch in diameter) the others, negative ones (¼ inch or less). The first toxoid injection produced a temperature of 103°F. in one child, 101°F. in a second and no rise in the others.