that deviations may be even further reduced. Studies are now in progress to test the reliability of the method and to determine the effect of various factors, such as food, visible perspiration, activity, body and environmental temperature and humidity on the insensible perspiration of infants.

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Rate of Development of Pneumococcus Immunity.

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Inasmuch as pneumonia is a self-limited disease ending by crisis or lysis generally between the 7th and 10th days, 3 to 5 days are frequently afforded in which an attempt may be made to produce active immunity before the natural termination of the disease takes place. At the suggestion of Dr. A. R. Dochez a study was therefore undertaken of the onset and rate of development of pneumococcus immunity.

The development of pneumococcus immunity after the introduction of pneumococcus vaccine has been studied from many points of view. The onset of immunity has been noted from the 5th to the 14th day by different workers,[1] in a few observations as early as the 3rd day. Many preparations of the antigens have been employed. Some workers[2] have believed the intact cell necessary for the production of well-marked type-specific immunity, whereas others have found that extracts or solutions of the cells were equally effective.[3] As we were interested in the preparation of a vaccine that would be especially effective in initiating early immunity, we compared in this regard a vaccine made from the intact cell with that obtained from a watery extract of the cell.

Three antigens were employed (1) a vaccine made from the intact cell, (2) a Berkefeldt filtrate of shaken bacteria, (3) a Berkefeldt filtrate of the broth culture. The method of preparation was as follows:

A pneumococcus type 11 organism of high virulence was grown for 6 hours in 2.5% human serum beef-infusion broth. This was used to inoculate 250 cc. flasks of similarly prepared broth in the

* With the technical assistance of Mr. Max Soroka.

proportion of 0.1 cc. inoculum to 5 cc. broth. At the end of 11 hours' incubation, part of the broth culture was centrifuged and the supernatant fluid passed through a Berkefeldt filter, the so-called broth filtrate. A second portion of the broth culture was centrifuged, and the supernatant fluid discarded. The sedimented bacteria were kept intact by rinsing the test-tube carefully with distilled water, removing traces of broth. The bacteria were then taken up in distilled water, to make a concentration of 1 billion organisms to 1.0 cc. The suspension was divided into 2 parts. One was heated to 60° for 1 hour, allowed to cool and tricresol added (final solution 0.3%)—the serum-vaccine. The second part was shaken by hand for 5 minutes, centrifuged, and the supernatant fluid passed through a Berkefeldt filter. It consisted of a clear watery solution, free from formed elements, sterile on culture, and was called the filtrate of the shaken bacteria or the bacterial filtrate.

Active immunity experiments were conducted by injecting a series of mice intraperitoneally with the 3 antigens and testing their resistance to a virulent culture from 1 to 7 days after inoculation. The dose of the vaccine was 200 million organisms, of the filtrates that amount of solution which represented previous contact with 400 million organisms. The test culture employed was fatal to a mouse in dilution of 10^{-6} cc.

The onset of definite immunity occurred on the 3rd day after inoculation of the vaccine, on the 4th day after the broth filtrate and the bacterial filtrate. In all instances the immunity increased markedly to the 5th day, and remained approximately stationary to the 7th day. On the 5th day the mice withstood an injection of 0.001 cc. of culture. In some animals this degree of resistance was evident on the 3rd day after inoculation. The degree of active immunity which the mice developed on the 5th day protected against 10,000 minimal lethal doses of virulent pneumococci, in some instances, particularly when Type 1 was employed as an antigen, 100,000 minimal lethal doses. In order to prove that the immunity produced was type-specific, the mice who survived were given an equivalent dose of Type I test-culture of the same virulence. None survived. In addition, a series of mice were immunized by the above 3 antigens derived from Type II and given a test-culture of Type I on the 3rd, 4th, and 5th days after inoculation. None survived.

Passive immunity experiments were conducted by injecting rabbits with the above antigens. Their sera protected mice in the same degree as that obtained in the active immunity experiments with the exception that individual rabbits varied in their capacity to produce protective substance. Tests were made to determine whether sera

which protected against the homologous organism had any protective power against the heterologous organism (Type I) with completely negative results.

Two conclusions may be drawn from these experiments: (1) Active and passive immunity of considerable degree may be produced 3 days after injection of a suitable vaccine derived from the intact organism, Type I or Type II. The immunity increases markedly to the 5th day and remains approximately stationary to the 7th. In the case of the filtrates either from the broth culture or the shaken bacteria, the immunity begins on the 4th day, increases on the 5th day and remains stationary to the 7th day. (2) The immunity produced during this period was not due to the elaboration of the common protein antibody but to the development of type-specific protective substances. This was equally true after the injection of an extract of the pneumococcus obtained by simple Berkefeldt filtration as well as after injection of the intact cell.

The study was undertaken primarily with the object of determining whether an active immunity to the pneumococcus could be established in a sufficiently short space of time as to make the injection of vaccine a therapeutic possibility in lobar pneumonia. As far as the time interval is concerned our results support this hypothesis. Whether the patient with lobar pneumonia would react by an earlier initiation of his immunity mechanism is not within the scope of this paper.

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A New Phenomenon of Local Skin Reactivity to B. Typhosus Culture Filtrate.

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A phenomenon of local reactivity to *B. typhosus* culture filtrate is described. The work was done on rabbits. The reactivity was induced by skin injections of the filtrate 24 hours prior to the intravenous injection of the same filtrate. The local reactions appeared at the site of previous skin injections; they were fully developed in 4 to 5 hours after the intravenous injection; they were extremely severe and microscopically showed very pronounced necrosis of tissue with rupture of blood vessels and extensive local hemorrhage. The reactions in different rabbits varied in size from 1x1 cm. to 4x4 cm.