

was found to be present in "medium" quantities and in 5 "faint." Three of the spinal fluids showed no Pb.

Observations for Pb were made on the 2833.07 Å line since it is one of the most sensitive. The amount of Pb was estimated by comparing the intensity of the 2833.07 Å line in a suspect with that of a synthetic solution to which had been added a known amount of $\text{Pb}(\text{NO}_3)_2$. From this comparison it was estimated that the specimens designated as having a strong line contained about 10^{-8} parts of lead by weight.

An examination was made of 18 samples of spinal fluid for Sn. Of the group 5 showed definite evidence of this metal with one specimen catalogued as very strong, one medium and 3 weak. The remaining members of the series, 13 in number, showed no trace of Sn. The occurrence of the sensitive 2839.99 Å line was used as a criterion in the estimations.

In addition to the elements named above the ubiquitous Na, Ca, Mg, Cu, K, and P were always found in relative abundance. Ba, Sr, and B were in all specimens examined. We were hampered in our studies by the fact that the electrodes used contained Fe, Mo, Rb, and Si. These elements have been reported as being found in tissues and one might reasonably expect them in spinal fluid.

While our series of cases is too small to permit us to indulge in statistical treatment it may be said that, within the limits of the sensitivity of the method used, all samples of spinal fluid can be expected to show evidence of Al, Ba, Sr, B; about half of them Pb and a fourth of them Sn.

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Action of Immune Serum on Meningeal H. Influenzae in vitro and in Experimental Infections.

DOROTHY WILKES-WEISS.* (Introduced by J. V. Cooke.)

From the Department of Pediatrics, Washington University School of Medicine, and the St. Louis Children's Hospital.

Freshly isolated meningeal strains of *H. influenzae* differ from most respiratory strains in their "smooth" colony forms and their virulence for rabbits and mice, and these closely correlated proper-

* Theron Catlin Fellow in Pediatrics, St. Louis Children's Hospital.

ties may be readily lost on subculture.†^{1, 2} In this instance virulence seems to be associated with ability of the organism to survive and multiply within the host's tissues, rather than with an "endotoxic" effect. In this laboratory filtrates and heat-killed cultures were found to be relatively innocuous to the test animals. Hence, there is little reason to look for "anti-endotoxic" properties in immune serums, and if such serums possess any protective or therapeutic value, this will probably be dependent on bactericidal action. There is reason to believe that a so-called "immune serum" may have little activity against a smooth virulent strain if the strain used for immunization is one which has become rough and avirulent.^{1, 2, 3} In the course of my attempts to develop a product for the treatment of influenzal meningitis, tests were made of the bactericidal activity of various antibody preparations, and the results will be briefly presented.

Through the cooperation of Dr. L. T. Clarke of Parke, Davis and Company, several types of serum from immunized horses were prepared and used in these studies: (1) Felton antibody from a horse immunized against 2 meningeal strains of *H. influenzae*. One of these strains was "smooth" when first used in injecting the horses, but later became "rough". The other was an old "rough" strain. (2) Serum from a horse immunized with a strain kept "smooth" by weekly intracisternal injections into rabbits. (3) Antibody from this serum concentrated according to the "Felton" method. (4) Antibody from this serum concentrated by a method said to conserve both globulin fractions, called "Eureka" by Parke, Davis and Company.

The results of bactericidal studies with these sera are shown in Table I. Guinea pig complement was added for the purpose of enhancing the bactericidal action. A series of dilutions up to 1:100,000 was made from a 24-hour culture of a smooth virulent strain, adjusted to the turbidity of tube No. 3 of the McFarland Nephelometer. With the Petroff-Heuser technic,⁴ such cultures were found to contain 1,850,000 organisms per cc. The various immune

† Although the terms "rough" and "smooth" seem unsuitable as descriptions of these variants, they are retained in deference to prevailing usage. Strains which exhibit a mucoid growth on suitable media are called "smooth," while all other strains are designated as "rough," although in most instances the colonies are quite smooth.

¹ Pittmann, M., *J. Exp. Med.*, 1931, **53**, 471.

² Pittmann, M., *ibid.*, 1933, **58**, 685.

³ Ward, Hugh K., and Wright, Joyce, *J. Exp. Med.*, 1932, **55**, 223.

⁴ Baldwin, Petroff and Gardner, *Tuberculosis; Bacteriology, Pathology and Laboratory Diagnosis*, Philadelphia, Lea and Febiger, 1927.

serums were likewise diluted with normal salt solution. Fresh guinea pig serum was added in a dilution of 1:2. Into a given sterile test tube the following amounts were pipetted: 0.1 cc. of the specified dilution of culture, 0.2 cc. of complement, and 0.2 cc. of the specified dilution of serum. The tubes were incubated for 3 hours at 37°, being vigorously shaken every 5 to 10 minutes. Three drops from a standard capillary pipette were then streaked over the surface of chocolate agar slants, the slants were incubated at 37°C., and the final readings were made after 48 hours.

The immune serums showed definite bactericidal activity. Those made by means of a "smooth" strain were definitely more bactericidal than that made with a "rough" strain. The Neisser-Wechsberg phenomena was observed in tests with the "smooth" serums, while the slight bactericidal activity of normal horse serum, which was apparently due to the added preservative, occurred only when the serum was undiluted.

An attempt was made to study the efficacy of these serums in experimental infections, but the technical difficulties were enormous, and the results can only be considered suggestive. Cisternal puncture in rabbits proved quite difficult, and it was very hard to give an accurately measured dose of organisms by this route, since the inoculum had to be small and concentrated. Moreover, rabbits seemed to vary greatly in their susceptibility to the test organism. However, it may be noted that of 13 rabbits receiving mixtures of "smooth" serum, culture, and complement, only 3 died, while all of the 6 controls died. The results of administration of serum at varying intervals after the infection are not clear enough to warrant detailed presentation. A few of these animals recovered, and it is perhaps noteworthy that the blood cultures of serum treated animals that died were usually negative, even in those treated with "rough" serum, whereas in the controls the cultures were uniformly positive.