New York Meeting.

New York Academy of Medicine, February 21, 1934.

7215 P

A Type-Specific Substance from the Meningococcus.

HENRY W. SCHERP AND GEOFFREY RAKE.

From the Laboratories of The Rockefeller Institute for Medical Research.

In an earlier paper,[1] there was described the separation, from meningococcus autolysates, of fractions exhibiting marked type-specificity toward precipitin-containing sera. It is now possible to report the preparation of the Type I specific substance (SSS) in a much higher state of purification.

Twenty liter lots of 3 week cultures of recently isolated strains of Type I meningococcus in hormone broth are acidified with acetic acid to pH 4.0. The SSS has a marked affinity for the acid-precipitable protein, the so-called "P" fraction, and about one-half of the amount present is carried down with the latter under the above conditions. If the broth is diluted 10-fold with tap water before acidification, almost all the SSS is adsorbed, but there is a loss in selectivity. This point is undergoing further investigation.

The "P" fraction is washed with distilled water, suspended in 1 liter of the same solvent and dissolved at pH 7.0 with the aid of NaOH. The bulk of the protein is then precipitated with acetic acid at pH 4.8 and centrifuged off. The precipitate is washed with distilled water and again submitted to the process of solution at pH 7.0 and precipitation at pH 4.8. The combined supernatant fluids, which contain almost all of the SSS, are concentrated *in vacuo* to the smallest practicable volume and precipitated with 5 volumes of alcohol. After standing overnight, the precipitate is centrifuged off and dried *in vacuo* with concentrated H_2SO_4. It is then dissolved in 50 to 100 cc. of distilled water with the aid of NaOH, and freed of the remainder of the protein by 0.7 saturation with $(NH_4)_2SO_4$. The latter is removed by dialysis, 10% of sodium

[1] Rake, G., and Scherp, H. W., *J. Exp. Med.*, 1933, **58**, 341.

acetate is added and the SSS is precipitated as a yellowish gum by 4 volumes of ethyl alcohol. Further purification is achieved by repeated precipitation by alcohol in the presence of sodium acetate and through precipitation with basic lead acetate or uranyl nitrate. Finally, the SSS is precipitated as a white powder by 10 volumes of absolute alcohol, from aqueous solution containing a small amount of sodium acetate. In favorable cases the yield from 20 liters of broth is 350 mg.

At a dilution of 1:2,000,000, preparations such as described give the precipitin reaction with homologous rabbit serum; at 1:8,000,000, they precipitate homologous horse serum. At a dilution of 1:100, slight precipitation occurs with Type II rabbit serum, but not at 1:1,000. Doses of 1 to 3 mg. administered to rabbits intravenously or intradermally have no apparent toxic action.

In 2% solutions the biuret and trichloracetic acid tests are negative. The Molisch test has been positive at 1:10,000 but not at 1:100,000. The substance does not reduce Fehling's solution, but after acid hydrolysis it gives 45% of reducing sugars calculated as glucose. It gives no orcin test for pentoses, and no color with iodine. It is unaffected by tryptic digestion and by short exposure to heat at neutrality, but is very sensitive to acids, the serological activity being destroyed by heating for one-half hour at 100° C., at pH 4.0. Standing for one hour at room temperature in N HCl has the same effect. At the same time, about 7% of reducing sugar appears calculated as glucose. The specific rotation, $[\alpha]_D^{25°C.}$, is $+45°$. The SSS does not dialyze appreciably through cellophane or parchment but does pass through collodion membranes prepared in the customary manner. It is precipitated by uranyl nitrate, basic lead acetate and safranin but not by copper sulfate, mercuric chloride, strong barium hydroxide solution, saturated ammonium sulfate or saturated calcium chloride.

The substance contains 4.4% N and 6.5% P. The latter is firmly bound since no precipitate is formed with the molybdate reagent until the SSS is subjected to vigorous hydrolysis. On ignition the SSS leaves 29 to 30% ash which present evidence shows to be an alkaline earth phosphate, probably that of calcium. The ash content is unchanged by prolonged dialysis.

Further studies on the chemical and immunological properties of this substance are in progress.