

hydrolysis of the collagen, although the organized skin structure inhibited diffusion of the enzyme and greatly decreased the speed of the reaction.

Specimens of collagen tanned with quinone, gallotannic acid, copper sulfate and formaldehyde were all hydrolyzed by trypsin while chrome tanned collagen was not.

## 216 (2176)

### The specific soluble substance of pneumococcus.

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In 1917 Dochez and Avery<sup>1</sup> showed that there was contained in filtrates from pneumococcus cultures and in the body fluids of experimentally infected animals and of patients suffering from pneumonia, a soluble substance which reacts specifically in anti-pneumococcus serum of the homologous type. This substance, which was found to be thermostable, precipitable by alcohol or acetone, non-dialyzable, and not digested by trypsin, is now being subjected to a more intensive chemical study.

Eight-day, autolyzed cultures of Type II Pneumococcus in phosphate broth were concentrated to 1/15 volume and precipitated with 1.2 volumes of alcohol. The precipitate, centrifuged at high speed, yields a compact middle layer containing the specific soluble substance. By repeated fractionation with alcohol or acetone, first in neutral, then in dilute acetic acid solution, followed by repeated fractional precipitation with ammonium sulfate and final dialysis, about 1 gm. of a highly purified preparation was obtained for each 75 liters of culture used.

In its present state of purity the specific soluble substance is amorphous and yields a viscous solution in water. A 1 per cent. solution gives no biuret test, yields no precipitate with phospho-

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<sup>1</sup> Dochez and Avery, *J. Exp. Med.*, 1917, xxvi, 477.

tungstic acid, mercuric chloride, or neutral lead acetate, gives a faint haze with tannic acid, and is precipitated by basic lead acetate. At a dilution of 1:1,500,000 it still gives the Molisch reaction and yields a precipitate with Type II immune serum.  $[\alpha]_D$  is  $+58.7^\circ$ ; N, 1.2 per cent.; P, trace; S, none; C, 46.2 per cent.; H, 6.1 per cent. Hydrolysis yielded 79 per cent. of reducing sugars, of which glucose was identified by the melting point and optical rotation of its phenylosazone. Earlier preparations containing more nitrogen and yielding less reducing sugars on hydrolysis were not specific at as high dilutions.

While it is not excluded that the non-carbohydrate portion of the preparation is actually the carrier of the specific reaction, it is believed that the evidence points to the identity of the specific soluble substance with the polysaccharide portion, thus linking it with the bacterial gums isolated by others from capsular material, but never before connected with specificity.

## 217 (2177)

### Immunological relationships of cell constituents of pneumococcus.

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In the preceding communication, it has been pointed out that the so-called soluble specific substance of pneumococcus is non-protein in nature, and in its present state of purification is either itself a polysaccharide, or intimately associated with the carbohydrate. Although antigenically this substance appears capable of stimulating little or no antibody response, serologically it is highly reactive and exhibits to an extraordinary degree the reactions of type specificity in antibacterial serum of the homologous type of pneumococcus. On the other hand it is possible to recover from the pneumococcus cell another substance which is protein in character and which is distinctive in its serological behavior from the soluble specific substance. From bile solutions of pneumococci dilute acetic acid precipitates a protein fraction. This precipitate is washed in water and redissolved in dilute al-