2966

On the heterogenetic haptene.

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In a previous communication[1] it was stated that active fractions of the specific part of the heterogenetic antigen, haptene, were obtained by fractional precipitation with alcohol from a solution in chloroform. The material obtained in this manner was extracted with hot alcohol. The residue was dissolved in water. On the addition of an equal volume of alcohol a precipitate formed. This precipitate was further dissolved in pyridine at 60° to 70° C. A smaller insoluble part was removed (although it also was active) and from the remaining solution, on addition of acetone, the material settled out. This product was soluble in water, unlike the known cerebrosides. It was practically insoluble in ether, acetone and cold[2] alcohol. On shaking with absolute alcohol at room temperature a little more than one part in 1000 went into solution. In hot alcohol it was soluble to the extent of about ½ per cent. While at first it did not seem possible to make a filterable chloroform solution, a colloidal solution in chloroform (strong Tyndall phenomenon) could now be obtained from which the substance did not separate on cooling or on concentration to a small volume. From a chloroform solution of the substance very little passed into water on shaking and conversely, chloroform extracted very little of the substance from an aqueous solution.

A 5 per cent solution of the product in water gave a rotation of approximately $[\alpha]_D = +20°$, in pyridine $[\alpha] = +10°$. With orcinol, hydrochloric acid and copper sulfate or ferrous sulfate the preparation gives a purple color.

On heating with ½ normal hydrochloric acid from 5 to 15 minutes a precipitate is formed. At the same time the serological activity is almost entirely destroyed. The soluble part, serologically inactive, reduces Fehling's solution, gives a strong purple

[1] Landsteiner, K., and Levene, P. A., *J. Immunol.*, 1925, x, 731.

[2] In the former communication the word cold was omitted.

color with orcinol, hydrochloric acid and copper sulfate, but it gave very little or no crystallized osazone with phenylhydrazine. Upon further heating of the acid solution, black products are formed, the solution yielding a crystallized osazone.

If the insoluble part resulting from the treatment with hydrochloric acid is heated further with new portions of acid, more reducing sugar is formed. The final solutions gave with orcinol and copper sulfate a green color instead of the purple. These solutions were dextrotatory and gave a crystalline phenylosazone. The insoluble material resulting from complete cleavage contained higher fatty acids and a base resembling sphingosine.

From beef kidneys, not containing heterogenetic antigen, only an insignificant quantity was obtained of a material similar in its solubility to the above. In a second experiment, such a product was not obtained.

Aside from the fraction described others were found which were similarly active, were water soluble, but gave a weak orcinol-copper test.

2967

Further studies on the production of staphylococcus aureus toxin.

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In a previous paper[1] we showed that certain strains of *Staphylococcus aureus* produce an exotoxin which causes necrosis when injected intradermally into rabbits. With the methods used at that time only 8 out of 118 strains from pathogenic sources produced a toxin of sufficient strength to be demonstrable by this method. Recently, however, by two improvements in method, it has been possible to obtain the toxin from 13 out of 14 strains. These changes were: first, the growth of the staphylococcus culture in an atmosphere containing 10 per cent carbon dioxide and,

[1] Parker, Julia T., *J. Exp. Med.*, 1924, xl, 761.