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A chemical study of tuberculin.

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Some time ago in the course of a study of the tuberculin and allied reactions, Zinsser[1] showed that extracts of powdered tubercle bacilli, rendered as free as possible from protein, by acid precipitation and boiling, contained substances precipitable by alcohol, which substance produced skin reactions of the delayed type, in tuberculous guinea pigs. The acetic acid precipitate gave a similar reaction, which was not lost on repeated solution in alkali and reprecipitation with acid. Several possibilities of the relationship between the protein precipitate and the so-called "residue antigen," from which the protein had been removed, were discussed and the question was left open for further work.

The investigation of the residue was continued by Zinsser and Parker,[2] who extended the observations to other bacteria, and found that in the case of each one tried, including staphylococci, pneumocci and meningococci, a similar preparation could be obtained. In the case of these organisms, however, the products were tested by the precipitin reactions, since chronic infections of a type suitable to produce skin hypersensitiveness could not readily be produced in guinea pigs. In all cases these bacterial residues reacted specifically with immune sera by the precipitin test.

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[1] Zinsser, H., *J. Exp. Med.*, 1921, xxxiv, 495.

[2] Zinsser and Parker, *J. Exp. Med.*, 1923, xxxvii, 275.

It was shown that these preparations were made up largely of non-nitrogenous material, which gave the Molisch test for carbohydrates. Heidelberger and Avery[3] have shown that substances prepared from Pneumococcus II and III broth cultures, and which are undoubtedly identical with the active material of the residue antigen, are complex carbohydrates or gums. The writer has come to the same conclusion in the study of a specifically precipitable material prepared from bread yeast. The similarity of results from such unrelated types of microörganisms indicates that the production by bacteria of specific gums, reacting with immune sera by the precipitin test, may be a general phenomenon. It should be noted that these substances, while reacting with immune sera, are apparently incapable of themselves inducing antibody formation, and hence are not antigens in the strict sense.

It has been the purpose of the present investigation to determine whether the tubercle bacillus also produces a carbohydrate residue antigen, and if so, whether it is the substance calling forth allergic reactions in tuberculous animals.

Because of the success of Heidelberger and Avery in purifying their pneumococcus material from broth cultures, the material used in the work here reported has for the most part been old tuberculin. Prepared from the usual glycerine infusion broth, this will usually give a precipitin reaction, by the ring test with good immune sera in a dilution of about 1-1000, and a skin test in tuberculous guinea pigs in a dilution of 1-50 to 1-100. In comparing the two types of reaction after various methods of attempted purification, serious discrepancies began to appear. It has finally been possible to show definitely that they are dependent upon entirely different substances in the tuberculin. For example, after several precipitations with alcohol, dilute acetic acid precipitated a substance which gave a strong biuret test, a powerful skin reaction in high dilution, but gave precipitin tests only in dilutions up to 1-1000 of the dry material. On the other hand from filtrate which gave both reactions, due to incomplete removal of the skin reactive material, of the tannic acid precipitation, followed by removal of the excess of the reagent from the filtrate by barium hydroxide, lead hydroxide, etc., a preparation was obtained containing less than 1 percent of nitro-

[3] Heidelberger and Avery, *J. Exp. Med.*, 1924, xl, 301.

gen. This gave no skin reaction in a dilution equivalent to the original concentrated tuberculin, but gave a precipitin reaction up to a dilution of 1-40,000.

The tannic acid method, in the form so far used, has served to establish that the skin test and the precipitin test are manifestations of separate and distinct substances in the tuberculin. It, however, causes too great a loss of these substances to be used in quantity production. It was found, that by the cultivation of the organisms on synthetic media, a broth filtrate could be obtained containing both compounds in quantity not markedly inferior to meat broth cultures. From such filtrates it has proved an easy matter, by a single fractional alcohol precipitation, followed by dialysis, to prepare a substance giving the precipitin reaction in a dilution of 1-1,000,000. This material in a dilution of 1-100 gives a doubtful or negative skin test, and is now being collected for further chemical study.

The nature of the component which produces the skin reaction is also under investigation, but beyond the fact that the fraction is largely protein, no definite statement can yet be made.

102 (2625)

Studies on the purification of antibodies. III. Certain methods for the precipitation of pneumococcus protective antibody.

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We have tried to apply the principles developed in our work on typhoid agglutinin to the purification of pneumococcus protective antibodies. The task has turned out to be particularly difficult. The great solubility of the pneumococcus makes it impossible to get the primary extracts of sensitized bacteria as free from bacterial substances as in the case of typhoid. The variability in the resistance of mice to pneumococcus infection, and the variable virulence of pneumococcus under culture, make