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"Residue antigen" from a strain of Friedlander bacillus.

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The work of Zinsser and Parker,[1] Heidelberger and Avery[2] and one of us (Mueller)[3] has indicated that most bacteria produce carbohydrate gums possessing the property of reacting specifically with antibodies formed in response to injections of the original bacteria. It is, therefore, of interest to study a number of these gums, called by Zinsser "residue antigens," from different bacterial sources. Only by such study can it be determined to what extent it will be necessary to alter the present conception of the chemical basis of immunological specificity.

Since the Friedlander Bacillus grows readily and abundantly on simple media, and is known to produce considerable quantities of a complex carbohydrate presumably related to the capsular material,[4] * this organism was chosen as being particularly adapted to the work.

An old stock strain has been used, the source of which is unknown. It may be mentioned here that among several strains examined, there are distinct serological differences, as might be expected from the unsatisfactory cultural classification of this group. Work upon other strains is being continued by one of us, (Smith) and it is possible that a classification of the group based on relationship of the residue antigens produced may be developed as an incident to the purely chemical study of these substances.

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

[2] Heidelberger and Avery, *J. Exp. Med.*, 1923, xxxviii, 73, and 1924, xl, 301.

[3] Mueller, PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 209.

[4] Toeniessen, *Centralbl. f. Bakt. I Abt. Orig.*, 1920-1, lxxxv, 225.

* Since this article was written, we have found that by following exactly Toeneissen's technic for the preparation of "capsular material" from cultures grown on ordinary agar in Petri dishes, a substance is obtained which precipitates with immune serum more rapidly at a dilution of 1-1,000,000 than the material obtained from broth cultures. The yield is somewhat greater, and the preparation is much simpler. Not enough of this substance is available for analyses, but it is apparently similar in being largely carbohydrate.

Cultures were made in broth, prepared from casein hydrolyzed for six hours with four times the weight of hydrochloric acid. After removal of the excess acid, and neutralization, there is added an inorganic salt mixture and glucose. One hundred liters of broth were used for the preparation here reported. After a week's growth in the incubator, the flasks were Arnolded for one hour, passed through a Sharples centrifuge to remove the bacteria, concentrated to about one tenth on the water bath and precipitated by adding two volumes of alcohol. Upon centrifuging, a two-layer precipitate forms, the lower layer being a brown syrup, the middle layer a grayish white solid cake. Most of the residue antigen is in the lower layer. This material, after reprecipitating with alcohol, dialyzing and again precipitating with alcohol, yields about 25 grams of material, giving a ring with immune rabbit serum at 1-100,000. It is purified further by dissolving in water, and acidifying with hydrochloric acid until no more precipitate separates. This is removed by centrifugation and proves to be relatively inactive with serum. By the addition of about one and one half volumes of glacial acetic acid to the somewhat turbid supernatant fluid, another bulky precipitate separates and is centrifuged off. This also is mostly impurities. To the supernatant, more glacial acetic acid is added, to make a total of about two volumes. A heavy, curdy yellowish white precipitate separates quite sharply, which, after reprecipitating once in the same way with acetic acid, is washed with alcohol and ether and dried. The yield is about 3.0 grams.

It does not dissolve readily in water but forms a very turbid opalescent suspension, which clears quickly with a small quantity of NaOH solution, giving a yellowish solution. It yields a ring with immune serum at 1-1,000,000. The nitrogen content is 1.3 per cent, and there is an appreciable quantity of phosphorus present, but no sulfur. Reducing sugars are formed in considerable quantity on acid hydrolysis, and from these an osazone has been obtained which has not yet been definitely identified.

A further comparative study of the chemical composition of this substance, and of similar substances isolated from other strains of Friedlander bacilli, is being made.