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Action of Microorganisms from Soil on Type-Specific and Nontype-Specific Pneumococcus Type-I Carbohydrates.

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The report of Avery and Dubos[1] that the specific carbohydrate of pneumococcus type III was split by an enzyme from a soil microorganism appeared to offer a new approach to the study of relationships and differences between chemically and immunologically different preparations of the specific carbohydrates.

Organisms which decompose the specific carbohydrates of pneumococcus types II and III were described in a previous report.[2] We now have in pure culture a microorganism which utilizes to some extent the specific carbohydrate of pneumococcus type I. As in the study of the bacteria that decomposed the carbohydrates of pneumococcus types II and III, the precipitation test with antipneumococcus serum was used as an indication of the presence or absence of the carbohydrate, but, unlike the results obtained with these microorganisms, the precipitation reaction never entirely disappeared. It has not yet been ascertained whether this residual reaction is due to an unused portion of the original carbohydrate or to products of decomposition, which might either be present in the original sample or be formed as a result of the action of the microorganism from the soil.

In mixed culture, as it was first obtained from soil, this microorganism could not be cultivated on the purified specific carbohydrate, and, until it was isolated in pure culture, it had to be maintained on a mineral medium to which specific carbohydrate only partly purified had been added. In pure culture, the microorganism utilized the soluble specific substance obtained from broth culture and the specific carbohydrate isolated from pneumococcus type-I cells as well.

In the type-I-carbohydrate, mineral-base medium in which routine transfers are maintained, the organisms are small Gram-negative rods tapering to a point at the end. In older cultures in liquid or semisolid, saccharose, mineral medium, rods with a similar appearance are seen, together with many pleomorphic forms. No growth

[1] Avery, O. T., and Dubos, Rene, *Science*, 1930, **72**, 151.

[2] Sickles, G. M., and Shaw, Myrtle, *J. Infect. Dis.*, 1933, **53**, 38.

develops on ordinary, beef-extract, peptone agar, but small yellow colonies are obtained on mineral-base agar containing saccharose. Saccharose, 1%, and dextrose, 0.1%, in a mineral base, are utilized by the organism. Larger amounts of dextrose have a somewhat inhibitory effect on growth. Growth in a mineral base containing 1% of saccharose is vigorous.

No soluble enzyme has been obtained from this microorganism.

It is well known that the pneumococcus may become attenuated and lose, in varying degrees, its virulence, capsule formation, insusceptibility to phagocytosis, and type specificity. The antigenic activity as an immunizing agent and the production of specific carbohydrate are also altered. A nontype-specific carbohydrate has been obtained in small amounts by Wadsworth and Brown[3] from the frozen and thawed cells of such an attenuated strain, which was isolated in 1925 from the blood of a horse undergoing immunization with a typical, type-I strain and which has never regained its virulence.

Several different species of microorganisms from soil have been found to decompose this nontype-specific carbohydrate when precipitation with antipneumococcus serum is used to indicate the presence or absence of the carbohydrate. Two of the species have been investigated in more detail. One is the aerobic, spore-forming rod, described in a previous report,[2] which utilizes the specific carbohydrate of pneumococcus type III and agar. The other acts on the nontype-specific carbohydrate only and is a Gram-negative, aerobic, nonmotile, nonspore-forming rod which grows on standard media. The colonies on blood agar are small and rust-colored; on potato, the growth is a bright orange. Gelatin is liquefied, and acid is produced in dextrose, lactose, saccharose, maltose, inulin, and dextrin serum-water medium.

A soluble enzyme that decomposes the nontype-specific carbohydrate in the absence of bacterial cells has been obtained from both strains. However, no protection of mice against pneumococci of types I, II, or III has been demonstrated with these preparations.

The carbohydrate obtained from the cells of the attenuated strain, like the specific preparations from the cells of pneumococcus types I, II, and III, induces purpuric lesions when injected into mice. It was previously noted that, when the "cellular carbohydrate" from pneumococcus types II and III had been decomposed by the specific microorganism from the soil, purpura no longer appeared after in-

[3] Wadsworth, Augustus, and Brown, Rachel, *J. Immunol.*, 1933, **24**, 349.

jection into mice. The nontype-specific carbohydrate, after decomposition by either strain, also failed to induce purpura.

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Production of Lens Sensitivity in Rabbits by the Action of Staphylococcus Toxin.

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Extracts of lens[1] derived from any of the vertebrates, with the exception of rabbit lens, produce, when injected into rabbits, precipitins for all lens extracts Although a high precipitin titer can be developed neither an Arthus reaction nor an anaphylactic state can be produced in the rabbit. In a previous report[2] it was shown that Staphylococcus toxin, injected intracutaneously into rabbits, produces not only an antitoxin but also a hypersensitive state to the beef bouillon in which the toxin is produced and to beef serum. This result suggested that if such a poor antigen as beef broth could be made antigenically active, a similar antigenic effect could be developed for lens substance if this material were added to the broth. Accordingly the following experiments were undertaken.

Beef eyes, trimmed of all extraocular tissue, were dropped into boiling water for 30 seconds. With a sterile scissors a transverse corneal incision was made and the lens expressed into a tube containing 20 cc. of hormone bouillon. After a preliminary incubation period of 7 days to eliminate the contaminated tubes, the sterile tubes were inoculated with toxin-forming *Staph. aureus,* strain Ha. After 10 days' incubation at 37°C. the contents of the tubes were pooled, ½% trikresol added and filtered through a Berkefeld V filter. Injected intravenously, 0.3 cc. killed a 3000 gm. rabbit within 24 hours. This lens broth toxin precipitated with lens immune serum in dilutions as high as 1-50,000.

Rabbits were injected intracutaneously with 0.1 cc. amounts of lens broth toxin and lens extract, each in a different skin area at intervals of one week. Two rabbits were injected intravenously with

[1] Woods, A. C., Allergy and Immunity in Ophthalmology, Johns Hopkins Press, Baltimore, 1933. Review of the literature on immunology of the lens.

[2] Burky, E. L., *J. Immunol.,* 1933, **25**, 419.