SPECIAL ARTICLES

COMBINATION OF BACTERIAL POLYSACCHARIDES AND COLLODION PARTICLES AS ANTIGENS

PRELIMINARY REPORT

LOEB and his associates[1] have shown that the adsorption of proteins by collodion particles makes possible the use of certain physico-chemical methods that can not be applied to protein in solution. Jones[2] demonstrated that collodion particles coated with various proteins are agglutinated by their specific antisera. Freund[3] studied the adsorption of diphtheria and tetanus toxin and antitoxins on the surface of the collodion particles. Further work done by Dr. Freund, of the Henry Phipps Institute, and ourselves with the adsorption of bacterial polysaccharides on the surface of collodion particles and the agglutination of them by immune sera proved that its homologous polysaccharides were also adsorbed by the collodion particles as were the toxin and antitoxin. Polysaccharides, as such, when injected have not proven to result in antibody formation. Many have assumed that fairly complex nitrogenous substance had to be a part of the polysaccharide combination to make these antigenic. It occurred to us, however, that this might not be necessary and that combinations might be prepared which may owe their antigenic properties to a physical basis or arrangement rather than to an organic chemical combination. (I am aware of Langmuir's idea of the chemical nature of adsorption in colloids.)

The collodion particles are mixed with anthrax polysaccharide in a dilution of 1–100, (this polysaccharide contains 0.03 per cent. N) cooled for five minutes and washed four times with salt solution, centrifuging each time for 10 minutes. This heavy suspension of particles was standardized (by turbidity) to an approximate concentration of 5,000,000,000 per cc. Agglutination tests were made in test-tubes, diluting the serum up to 1–64 and adding an equal amount of the collodion particle suspension. Incubated for 2 hours at 37° C. The reading was checked with a hanging-drop under the microscope. Proper controls were made with non-homologous polysaccharide adsorbed to the collodion particles, as well as using normal horse serum and other heterologous sera for the agglutination test. We obtained specific agglutination in a dilution of 1–32 and sometimes in a dilution of 1–64, with the collodion particles adsorbed with anthrax polysaccharide and its specific antiserum.

[1] J. Loeb, "Proteins and the Theory of Colloidal Behavior," 1922.

[2] F. S. Jones, *Jour. Exper. Med.*, XLVI, 303 (1927): *Ibid.*, XLVIII, 183 (1928).

[3] J. Freund, *Proceed. Soc. for Exper. Biol. & Med.*, 1930, XXVIII, 65.

For our immunization experiments, several combinations of adsorbed substances to the collodion particles were prepared:

(1) Collodion particles adsorbed with 1 per cent. anthrax polysaccharide.

(2) Collodion particles adsorbed with normal horse serum, washed 4 times, then adsorbed with anthrax polysaccharide.

(3) Collodion particles adsorbed with anti-anthrax globulin, washed, and then adsorbed with anthrax polysaccharide.

(4) The washed precipitate of anti-anthrax globulin and anthrax polysaccharide, without collodion particles.

In the first case, we wanted to find out the results of the polysaccharide alone adsorbed to the particles. In the second case, normal horse serum adsorbed first, washed 4 times, and then the polysaccharide, hoping to find out the effect of a foreign protein and a polysaccharide combination, and lastly adsorbing specific antiserum first, then washing, and followed by the specific polysaccharide with the object of increasing the amount adsorbed according to the findings of Freund in working with diphtheria toxin, where he found that collodion particles, treated first with antitoxin and then with toxin, were slightly but definitely more toxic than particles treated with toxin alone. As controls, rabbits were immunized with the suspended washed specific antibody precipitate obtained from immune serum and its specific carbohydrate. It was not thought necessary to try rabbits immunized with polysaccharide solution alone, as other investigators as well as ourselves have failed.

The rabbits were immunized by injecting intravenously 0.5 cc, 1.0 cc and 1.0 cc on consecutive days and rested for 5 days. A second and third series were made increasing the dose to 3 cc. Seven days later they were trial-bled and agglutination and precipitin tests were made. The results are given in Table I.

The variation in titer of the different series is thought to be due to variation in the response of the individual animal, although the animals injected with the antibody precipitate seemed to give higher value. For the present we are not giving special significance to this finding. The third series also seems to be better than the first.

To ascertain if the foreign protein in the second, third and control series had given anti-horse antibodies, specific horse precipitin was tested for and positive reaction found in all the rabbits injected, except those which received collodion particles and polysaccharide alone. The presence of specific anti-horse precipitins demonstrate that the material ad-

TABLE I

AGGLUTINATION TESTS WITH SERUM OF RABBITS IMMUNIZED WITH COLLODION PARTICLES ADSORBED WITH ANTHRAX POLYSACCHARIDE AND OTHER COMBINATIONS

Injected with	C. particles and anthrax SSS			C. particles and antianthrax Gl. and anthrax SSS			C. particles and normal horse serum and anthrax SSS			Anthrax Gl.-anthrax SSS ppte		
Rabbit No.	6704	6705	6706	6707	6708	6709	6713	6714	6715	6710	6711	6712
Final serum dilution 1/4	4	4	3	4	4	3	4	4	4	4	4	4
1/8	4	3	2	4	4	3	2	3	4	4	4	4
1/16	4	2	2	4	4	2	1	3	2	4	2	4
1/32	4	1	1	2	4	1	0	2	1	1	0	3
1/64	2	0	0	2	2	0	0	0	0	0	0	1

Incubated over night in water bath at 37° C.

sorbed on the particles is available for stimulating antibody response.

These results may offer an explanation of why polysaccharides and other substances (of the class of haptenes) are not antigenic when injected alone in solution in the body. The collodion particles provide an enormously increased surface to the polysaccharide (haptene) and make them available in a different physical form on injection. Other factors no doubt come in.

Further work is being done on many of the problems here suggested, especially in finding other colloids which may be employed for the same purpose, as well as the immunization of different animals with various bacterial polysaccharides. Work is in progress with the protection of mice against pneumococci with the specific carbohydrate to collodion particles. At the same time we have adsorbed several polysaccharides in the same collodion particles with the hope of forming a multiple antigen. The result of all this work will be published later.

JOSÉ ZOZAYA

MULFORD BIOLOGICAL LABORATORIES,
SHARP & DOHME,
GLENOLDEN, PA.

LIGNIN AS A NUTRIENT FOR THE CULTIVATED MUSHROOM, AGARICUS CAMPESTRIS

THE cultivated mushroom, *Agaricus campestris,* in common with other fungi, must obtain all the nutrients necessary for its growth and reproduction from the organic matter of the substrata upon which it grows, this organic matter also serving as a source of energy. Horse manure is commonly used as the source of organic matter for the cultivation of the mushroom, although recently attempts have been made to replace this manure by composts of so-called "artificial manure" prepared from straw and other plant residues, from peat, etc. The chief point to be considered in the use of manure or composts of plant residues is that, in order to obtain a fair growth of the mushroom, the manure and residues must first be thoroughly composted; this process lasts, under favorable conditions, from 4 to 12 weeks. The microorganisms active in the composts bring about a number of chemical changes in the various organic complexes; these can be briefly summarized as a reduction of the water-soluble substances, of the hemicelluloses and cellulose, and an increase in the lignin, ash and protein content.

The problem is to determine which of these chemical constituents in the compost form the nutrients for the mushroom, whether all the organic complexes are attacked alike, or whether some are acted upon to the exclusion of others. This problem is both of theoretical interest and has considerable practical application.

It is known, from studies of the activities of wood-destroying fungi, that some organisms attack the cellulose of the wood, but not the lignin, while a few are capable of attacking the lignin as well as the cellulose. With the exception of these wood-destroying fungi, very few organisms are known so far capable of decomposing lignin and using this material as a source of energy and as a nutrient material. Although lignin has been shown to be generally much more resistant to decomposition by microorganisms than celluloses and hemicelluloses, as shown by its relative persistence in composts, peat bogs and soil, it still undergoes some decomposition, especially under aerobic conditions; otherwise the whole surface of the earth would soon be covered with lignin, since all plant residues, with the possible exception of the algae, contain from 5 to 30 per cent. lignin.

In order to illustrate the changes that take place in the composting of stable manure, a typical analysis of a compost of horse manure is given Table I. If one assumes that the concentration of the mineral