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IV. Negative Effect of Colloidal Carriers on Enhancement of Antigenic and Sensitizing Properties of Polysaccharides.

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The rôle played by inert colloidal substances on the enhancement of antigenic properties of bacterial polysaccharides is still a debatable one. Although positive evidence of such an increase in immunological activity has been reported by Zozaya[1] and by Hoffstadt and Clark,[2] the general opinion[3, 4, 5] is that such an effect occurs with polysaccharides that are weakly antigenic themselves. In view of these discrepancies it appears to be of interest to determine whether the sensitizing and antigenic properties of the polysaccharides of *Bacillus rhinoscleromatis* can be improved by colloidal carriers. The polysaccharides of this organism were thought to be especially suitable for this study because of 2 serologically very similar preparations, one is weakly antigenic in rabbits and weakly sensitizes guinea pigs, while the antigenic properties of the other are insignificant.[6]

The polysaccharides used for this experiment include a fraction prepared by hydrolysis with 1% acetic acid and a fraction prepared by hydrolysis with 0.5% potassium hydroxide of the organism remaining after acid-hydrolysis. The details of the method of preparation and the chemical and immunological characteristics of the 2 fractions have been previously reported.[6] The colloidal substances included collodion particles, aluminium hydroxide, charcoal, and hog serum. The collodion particles were prepared according to the methods of Zozaya[1] and the aluminium hydroxide according to the method of Hektoen and Welker.[7] Adsorption was carried out by mixing varying concentrations of polysaccharide with the colloidal substance, incubating at 56°C for 2 hours and leaving in the icebox overnight. In the case of hog serum equal volumes of a 1:250

1 Zozaya, J., *J. Exp. Med.*, 1932, **55**, 324; *Ibid.*, 1933, **57**, 21.

2 Hoffstadt, R. E., and Clark, W. M., *J. Inf. Dis.*, 1935, **56**, 250.

3 Lewis, J. J., *Ibid.*, 1935, **57**, 94.

4 Landsteiner, K., and Jacobs, J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1055.

5 Jacobs, J., *J. Exp. Med.*, 1934, **59**, 479.

6 Wong, S. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 107.

7 Hektoen, K., and Welker, W., *J. Inf. Dis.*, 1933, **53**, 309.

concentration of polysaccharide and serum were mixed and treated in the same manner. Twenty-eight normal rabbits were divided equally into 2 groups: Group I receiving the acid-prepared polysaccharide mixed with colloidal substances and Group II receiving the alkali-prepared polysaccharide. Three rabbits were used for each type of colloidal carrier in each group and 4 rabbits received only the polysaccharides. Immunization of rabbits was carried out by giving 1 cc of the colloidal suspension on 3 successive days, resting 4 days, followed by 2 more series of injections, making a total of 9 injections representing 18 mg of polysaccharides.

For sensitization of guinea pigs only aluminium hydroxide containing 9 mg per cc of the acid-prepared polysaccharide was used. Two series of 8 guinea pigs each, weighing between 300 to 350 g, were sensitized by one subcutaneous injection followed by 2 intra-abdominal injections of the acid-polysaccharide colloidal mixture and of the acid-polysaccharide alone, each animal receiving a total of 23 mg. The injections were made at 5-day intervals and the test for supersensitivity was done 15 days after the last injection. It was found that the injection of 2 to 5 mg of the acid-polysaccharide either intravenously or intracardially into the guinea pigs of both groups elicited the same type of chronic non-fatal anaphylactic response, not a single fatal outcome having been observed.

Sera obtained from rabbits immunized with the preparations adsorbed on various colloidal carriers gave the same results as sera from control animals immunized with the polysaccharides alone. There were no demonstrable antibodies in any sera of rabbits immunized with the alkali-polysaccharides; there was no increase in the complement-fixation titer beyond 1:50,000, and there was no precipitation of a 1:1000 dilution of either polysaccharide by any serum from the animals immunized with the acid-polysaccharide.

It may be concluded that colloidal carriers such as aluminium hydroxide, charcoal, collodion particles, and hog serum, play no rôle in increasing either the antigenic or sensitizing property of the polysaccharides derived from *B. rhinoscleromatis.*