which bind the primary valence chains laterally. How successfully methods can be devised for spinning fibers which shall have the same thermal and optical properties as normal nerve remains to be determined.

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Experimental Hypersensitiveness to Staphylococcus.*

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The occurrence of type-specific skin reactions to carbohydrates in patients with Staphylococcus infections[1] presented the opportunity for studying experimentally hypersensitiveness to Staphylococcus and its constituents. The recent demonstration that this organism is separable into two distinct immunological types,[2] A and B, dependent upon the presence of chemically and serologically different polysaccharides, has made it possible to consider the influence of type- and species-specificity on the reactions of increased tissue sensitivity. Both monkeys and rabbits were studied to observe evidence of hypersensitiveness to Staphylococcus and its derivatives.

Monkeys *(M. rhesus)* were given injections of Type A or Type B organisms. In one experiment 4 monkeys were given 9 injections intracutaneously of heat-killed bacteria, repeated at weekly intervals. The injections caused only small nodules at the site of inoculation, and the successive reactions following the repeated inoculations were of approximately the same size and severity. In a second experiment, 4 monkeys were inoculated with live bacteria into a subcutaneous agar focus. This was repeated at 3 different times after the effects of the succeeding inoculation had healed completely. The animals in both experiments were skin tested to Type A and B carbohydrates at different periods during the course of observation, but at no time was skin reactivity elicited despite the employment of graded dilutions of carbohydrate. At the termination of the experiment skin tests to carbohydrates were again repeated using 0.2 cc.

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1 Unpublished data.

2 Julianelle, L. A., and Wieghard, C. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 947.

quantities of dilutions of 1:10,000, 1:25,000 and 1:50,000. In one animal, injected preliminarily with live Type A Staphylococcus by subcutaneous agar focus, skin reactions were observed to dilutions of specific carbohydrate of 1:10,000 and 1:25,000 but not to 1:50,000. The reactions appeared within 5 to 10 minutes and they were of the wheal and erythema variety. They reached a maximum in about 30 minutes and regressed completely in less than an hour. A comparable reaction to specific carbohydrate has been observed in patients during convalescence following Staphylococcus infections. In the other animals the tests evoked no response. In addition to the carbohydrate tests, injections were made with Staph. protein (0.2 cc. of a 1:10,000 dilution) and 0.2 cc. of Staphylococcus toxin prepared according to the method described by Burky.[3] These injections caused no reactions either immediate or delayed. Blood serum taken from the animals preceding the skin tests were subsequently tested for precipitins for both the protein and carbohydrate. The sera were completely devoid of precipitating antibodies. Blood was also taken at different intervals during the experiments to determine any changes in total protein, globulin, albumin and non-protein nitrogen. The results of the analyses indicated that these constituents remained within the limits of normal variation.

Rabbits were studied in a manner similar to that described for the monkeys. Injections of heat-killed Staphylococci were made intracutaneously at weekly intervals for 6 weeks. One group of animals received Type A while another received Type B. As previously brought out in the case of similar experiments with pneumococci,[4] the reactions at the site of injection were generally larger and of greater intensity with succeeding injections of a constant quantity of organisms, until the 4th or 5th injection. While more marked with Type A organisms, the same phenomenon was observed with the Type B strain. In one of 6 rabbits, a secondary reaction was observed to Type A, following complete disappearance of the primary reaction to the first injection.

In a second experiment conducted concurrently with the former, other rabbits were injected with live Staphylococci into a subcutaneous agar focus. The reaction to Type A was extremely violent, while that to Type B was relatively mild. This is in accordance with the observation that Type A strains are virulent, while Type B strains are not. A second injection was made in the same manner

[3] Burky, E. L., *J. Immunol.*, 1933, **24**, 93.

[4] Julianelle, L. A., *J. Exp. Med.*, 1930, **51**, 463.

about a month later when the effects of the primary injection were no longer visible. The response to the second injection of live bacteria was much less marked in the case of both types indicating the acquisition of a definite immunity following the first inoculation.

On one occasion during the course of injections the rabbits were skin tested for sensitivity to both carbohydrates but no reactions were observed. Finally, in terminating the experiment, the animals were tested again to carbohydrate in dilutions of 1:10-25-50-100-200 and 500 thousand. There was no reaction either immediate or delayed. The animals were then tested as described in the case of monkeys to Staph. protein and toxin respectively. All the rabbits reacted in varying intensities to these 2 antigens with a delayed inflammatory reaction. The rabbits were further tested for sensitivity by the more delicate method of direct intracorneal injection of carbohydrate. On the same day one eye was tested with a dilution of 1:25,000, and the other eye with 1:50,000. Since no reaction occurred within 48 hours, the eyes were retested to dilutions of 1:10 and 1:100 thousand, and 4 days later to 1:200 and 1:500 thousand. Two rabbits, one Type A, another Type B, gave clouding and vascularization (pannus) to 1:100,000 dilution. From the nature of the reaction, however, it was considered as a toxic, rather than a hypersensitive response. Final bleedings were made prior to skin testing. It was found that none of the sera contained precipitins for the specific carbohydrate, although all the animals showed varying titers of protein precipitins.

Thus, it is seen that under the conditions specified monkeys are sensitized with great difficulty to Staphylococcus and its derivatives. There occurred no increased reactivity of the skin to the whole organism, since the reaction at the site of inoculation of the bacteria remained approximately constant following each injection. In only one animal was sensitivity to type-specific carbohydrate manifested, and in no instance was a similar state observable to the protein or toxin of Staphylococcus. Despite prolonged inoculations, the sera of the monkeys failed to show precipitating antibodies for either carbohydrate or protein.

Under similar experimental conditions rabbits also proved resistant to sensitization to the carbohydrates of Staphylococcus. All the animals acquired an increased reactivity to the whole organisms, but none of the animals studied reacted to any of the dilutions of polysaccharide used for skin testing. On the other hand, all the animals became hypersensitive to the protein and toxin. Similarly, the sera of these animals while lacking precipitins for the carbo-

hydrates, showed varying titers of protein antibody. As has been shown with Pneumococcus[7] and Streptococcus,[8] this is another example of the difficulty of stimulating type-specific antibodies by intracutaneous injections.

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[5] Andrewes, C. H., Derick, C. L., and Swift, H. F., *J. Exp. Med.*, 1926, **44**, 35.

[6] Julianelle, L. A., Morris, M. C., and Harrison, R. W., *J. Immunol.*, 1934, **26**, 267.

[7] Julianelle, L. A., *J. Exp. Med.*, 1930, **51**, 441.

[8] Seegal, D., Heidelberger, M., and Jost, E. L., *J. Immunol.*, 1934, **27**, 211.