being in the experimental stage. An added uncertainty concerns itself with the instability of the strains employed in the development of the sera. And we agree with the recent conclusions of Colebrook,[4] whose experience has made him averse to the conventional practise of submitting such sera to clinical trial generally—and as so often has proved, prematurely. But regardless of the ultimate validity of these working hypotheses we believe the clinical results to be sufficiently decisive to justify thorough inquiry into that most fundamental problem of all serious infections, namely, the tendency to invade distant tissues, instead of remaining localized.

Although diversification of duty has of necessity given a sporadic cast to these observations, the appreciable span of years covered by them makes it desirable to record them to this extent. Control-observations with the conventional streptococcal antisera, as well as frankly nonspecific ones, have been, and are being, employed as opportunity offers.

Conclusions: 1. In a series of 118 cases of general hemolytic streptococcal infections a mortality of but 12% has attended the administration of antisera prepared from 2 anomalous dissociants of a cellulitic strain of hemolytic streptococcus. 2. The objective of the treatment has been the *in vivo* transformation of the virulent types to dissociants whose virulence is diminished or absent. Clinical bacteriological evidence of this effect is submitted. 3. Associated with recovery of the patient is a return of the calcium-phosphorus product of the blood to normal. This product is usually lowered during the active stages of the disease.

8666 C

Serologic Studies with Streptococci Isolated in Cases of Myasthenia Gravis.

EDWARD C. ROSENOW AND FORDYCE R. HEILMAN.

From the Division of Experimental Bacteriology, The Mayo Foundation, Rochester, Minnesota.

Green-producing streptococci from the muscles, foci of infection, and nasopharynges of patients with myasthenia gravis were isolated by methods already described.[2] A carbohydrate was ex-

[4] Colebrook, L., *Lancet*, 1935, 1, 1083.

[2] Rosenow, E. C., *Internat. Clin.*, 1930, **2**, 29.

tracted from a number of these freshly-isolated strains by the following method:

The sedimented growth from 3 liters of dextrose-broth was treated by boiling in physiologic salt-solution for one hour and the bacteria were then removed by centrifuging. The supernatant fluid was cooled and proteins were precipitated by adding glacial acetic acid until a cloud appeared. The proteins were then removed by centrifuging and the carbohydrate was precipitated by adding 2 volumes of 95% alcohol and a small amount of sodium acetate. After a good flocculation appeared the mixture was centrifuged. The carbohydrate sediment was then dissolved in water, reprecipitated with alcohol and sodium acetate, and then redissolved in water, this procedure being carried out 5 or 6 times. The final precipitate was dried in a stream of air and dissolved in 10 cc. of physiologic salt-solution; it was then ready for use as antigen. The activity of the carbohydrate was greatest in freshly-made solutions; it decreased about one-fourth when kept at room temperature for 2 weeks.

Precipitation occurred with the carbohydrate from myasthenia gravis strains in 30% of 31 tests made with serums from 15 persons who had myasthenia gravis, in 15% of 20 tests made with serums from 11 persons having disease other than myasthenia gravis, thought to be attributable to green-producing streptococci, and in no instance in 12 tests made with the serums from 7 normal controls. Precipitation occurred with the carbohydrates from strains of green-producing streptococci isolated from persons having disease other than myasthenia gravis in 15% of 32 tests made with the serums from 15 persons who had myasthenia gravis, in 7% of 14 tests made with the serums from 11 persons having disease other than myasthenia gravis, and in no instance in 6 tests made with the serum from 6 normal controls. In these precipitation experiments the age of serums and solutions of carbohydrate were not sufficiently considered, which led to the relatively low incidence of positive reactions. The serums from 30 patients with myasthenia gravis, kept not longer than 4 months, and freshly-made solutions of the carbohydrate of streptococci isolated from patients with myasthenia gravis, gave a positive "ring" test in 60% of cases. Filtered, glycerin-salt-solution extracts of these streptococci against the same serums gave a positive test in 46.6% of cases.

The preparation of this glycerin-extract has been described previously.[3] The serums of patients with severe forms of myas-

[3] Rosenow, E. C., *J. Infect. Dis.*, 1929, **45**, 331.

thenia gravis regularly gave precipitation; the serums of well persons never reacted nor did control serums of patients with uncomplicated diseases of known cause, other than those related to streptococci. The test was carried out in narrow tubes incubated at 45°C. in a water bath and observed frequently over a period of 2 hours. A dark background behind a bright shaded light was used in recording the test.

The serums of patients with myasthenia gravis were tested for a slowing property on streptococci in the electrical field of the Northrop-Kunitz-Mudd cataphoretic assembly. Various sets of 4 matched antigens were used, one antigen from myasthenia gravis and 3 from various other diseases. The antigens were prepared by inoculating dextrose-broth with freshly isolated cultures in dextrose-brain broth. The sediments of 16-hour dextrose-broth cultures were kept in a dense mixture of two-thirds glycerin and one-third 25% sodium chloride solution. Dilute uniform suspensions of these sediments were then made in water containing 0.05% formalin 3 or 4 days before they were to be used. The serums were then diluted 320 times in physiologic salt-solution. One cubic centimeter of the diluted serum was mixed separately with 1 cc. of each of the bacterial suspensions and was incubated for 14 minutes at 37°C. To each tube was then added 6.5 cc. of distilled water and the readings were made at once as unknowns by a technician.

Of 22 serums of patients with myasthenia gravis that were tested, all showed selective slowing action on the strains from myasthenia gravis. Each test was considered positive only when the serum of a patient with another disease against the same 4 antigens specifically slowed the antigen of that disease without slowing the antigen of myasthenia gravis. Adsorption of the serums of patients who had myasthenia gravis with different strains of streptococci showed the myasthenia gravis strains to remove selectively the slowing effect of the myasthenia gravis serums on myasthenia gravis strains.

The serums of 10 rabbits injected for 6 weeks with different formalized strains from patients with myasthenia gravis, and of 6 rabbits immunized to different strains from other diseases, showed by precipitin tests with various streptococcal carbohydrates, by agglutination and by cataphoresis of the streptococcus, the serologic specificity of the strains from patients with myasthenia gravis.

The serums of 4 horses, each immunized to a different type of green-producing streptococcus, and the serum of a normal horse,

were also studied. The serum of the horse immunized against the strains of myasthenia gravis precipitated filtered glycerin-extracts of the strains of myasthenia gravis more markedly and in greater dilution than did the other serums, and more markedly and in higher dilution than extracts of strains from other diseases. The other serums precipitated more strongly with homologous extracts.

Thus, precipitation occurred in 56% of 37 tests with the myasthenia gravis serum and myasthenia gravis extracts, in 15% of 42 tests with this serum and extracts of strains from other diseases, in 20% of 26 tests with the other serums and extracts of the myasthenia gravis strains, and in 48% of 21 tests with the other serums and extracts of strains from other diseases.

The erythematous-edematous reaction of Foshay[1] was used in the study of cases of myasthenia gravis. Several antistreptococcal equine euglobulins were standardized to a 10% solution by volume of centrifuge-packed, wet euglobulin in physiologic salt-solution. Approximately 0.03 cc. of each of these various immune-globulin fractions, along with normal horse-serum and pneumococcal immune-globulin as controls, were injected intracutaneously into patients. Of 7 patients with myasthenia gravis who were tested, all showed a predominant flare in 10 minutes to the euglobulin fractions of the serums of horses immunized to streptococci isolated from cases of myasthenia gravis. The average area of the flares to the different globulins in these cases was as follows:

Encephalitis streptococcal immune-euglobulin	4.61 cm.^{2}
Arthritis " "	4.11 "
Myasthenia gravis " "	10.5 "
Pneumococcal immune-euglobulin	0.85 "
Normal horse-serum	0.37 "

Normal persons gave no such flare and patients with other streptococcal diseases reacted predominantly to the globulin from the animal immunized with the strains from their type of disease. In none of the cases of myasthenia gravis did sensitiveness to normal horse-serum confuse interpretation of the reaction.

It appears that patients with myasthenia gravis harbor a streptococcus of specific serologic type, that they have antibodies in their blood specific for this organism, and that they have in their tissues antigen of this type of streptococcus, as shown by the Foshay reaction.

[1] Foshay, L., *J. Allergy*, 1935, **6**, 360.