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Hemolytic Streptococci from the Throat of Normal Monkeys.

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In November 14, 1938, a shipment of 500 Rhesus monkeys arrived in San Juan from India. These animals came, on the average, from 300 miles inland from Calcutta. The period of time they remained in this city awaiting shipment varied with different animals from a few days to 3 weeks. The shipment left Calcutta, September 30, 1938. The ports touched enroute were: Colombo, Ceylon, one day; Boston Mass., 2 days; and New York City, 2 days.

We studied the throat flora of 172 of these monkeys soon after arrival, over a period from November 20 to December 12, 1938, and the beta-hemolytic streptococci recovered were studied in considerable detail. The materials and methods used were the same as those employed in a previous investigation.¹

During the second week of December, 1938, the monkeys were taken to Santiago* Primate Colony, where they were set free. Throat cultures from 309 monkeys which were in apparent good health were taken again over a period from January 16 to February 22, 1940, and the hemolytic streptococci recovered were studied, utilizing media and methods similar to those employed before.

Seegal, Heller and Joblanowitz² studied the normal bacterial flora of 48 monkeys in New York City and recovered beta-hemolytic streptococci in 28 instances. Of these, 19 were Group A, 4 Group C, 5 Group G, and one, a minute streptococcus belonging to Group F.

Of the 172 monkeys examined in 1938, 22 (12.7%) harbored hemolytic streptococci in their throat, of which 10 (45.6%) were Group A, 8 (35.9%) were Group C, and 4 (18.5%) were Group G.

Of the 309 monkeys examined in 1940, 39 (12.5%) harbored hemolytic streptococci in their throat. None of them were Group A. Twenty-six (66.6%) were Group C, and 13 (33.3%) were Group G. It is interesting to note that, though the incidence of beta-hemolytic streptococci is the same in both cases, about one-half of the

¹ Pomales-Lebrón, A., *P. R. J. Pub. Health and Trop. Med.*, 1940, **16**, in press.

* This is a small island (about 35 acres) approximately one mile off the eastern coast of Puerto Rico.

² Seegal, B. C., Heller, G., and Joblanowitz, J., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 812.

streptococci recovered from the throats of the monkeys soon after arrival, belonged to Group A, while no streptococci belonging to this serological group were encountered among the strains obtained after the monkeys had been in Santiago Island for about 14 months.

The average final pH in glucose broth in both series varied from 5.1 to 5.3. None of the Group A strains gave reduction of methylene blue in milk. Of 8 type C strains isolated in 1938, 7 (87%) reduced methylene blue. Of 26 type C strains isolated in 1940, 23 (88%) reduced methylene blue. Of 4 type G strains of the first series, 3 (75%) reduced methylene blue and out of 13 strains of the second series only 3 (23%) reduced the dye.

According to Hollman's classification, 17 strains of those isolated in 1938 were *pyogenes*, 10 of these belonging to Group A and 7 to Group C. Five were *equi*; of these, one belonged to Group C and 4 to Group G. None fell in the *subacidus* group.

In the 1940 series, 4 strains fell in the *pyogenes* group, 2 belonging to Group C and 2 to Group G. Twenty-seven fell in the *equi* group; 17 of these were Group C and 10 Group G. Eight belonged to the *subacidus* group; 7 of these were Group C and one was Group G.

Out of the 22 strains isolated in 1938, 21 were fibrinolytic against human plasma clot, the average dissolution time being 1.9 hours. All these strains were only very slightly fibrinolytic for Rhesus monkey plasma in 24 hours. Out of 39 strains isolated in 1940 31 were fibrinolytic to human plasma clot. Eight strains, belonging to Group C, were not fibrinolytic. All strains were very slightly fibrinolytic for Rhesus monkey plasma.

Seegal, *et al.*,² also noted the relative insusceptibility of monkey plasma to the lytic action of their strains, which, however, were active against human plasma clot.

The virulence for mice was tested by injecting 0.5 cc of a 20-hour glucose (.02%) neopeptone broth culture intraperitoneally. Of the 22 strains isolated in the first series, 11 (50%) killed mice in less than 4 days, and of the 39 strains isolated in the second series, 36 strains (92%) killed mice in less than 4 days.

Hemolysis was tested with the whole culture and with the supernatant after centrifugation and reduction with sodium hydrosulphite. Streptolysin broth³ was used in these experiments. Using the whole culture, all strains of both series showed positive hemolysis. Using the supernatant after centrifugation and reduction, in the first series, out of 10 Group A strains, 4 showed a reducible hemo-

³ Swift, H. F., and Hodge, B. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1022.

lysin and all the 12 strains belonging to Groups C and G showed hemolysis. Out of 39 strains of the second series belonging to Groups C and G, 32 showed a reducible hemolysin. All the Group C and G strains failed to produce an erythrotoxic toxin reactive on the skin of a susceptible white goat, and of the 10 Group A strains, 4 produced an erythrotoxic skin toxin, 3 gave a doubtful reaction and 3 were negative. None of the strains studied hydrolyzed sodium hippurate. All except one fermented trehalose and did not ferment sorbitol. The exception was a Group C strain (sorbitol positive; trehalose negative), which produced a good beta-hemolysis in blood agar but gave a negative tube hemolysis both in the whole culture and with the supernatant after reduction with sodium hydrosulphide. It was non-fibrinolytic when tested against human or monkey plasma, and was classified as *Streptococcus pyogenes* (Hollman).

Discussion. The biological characteristics of the organisms studied seem to prove that the hemolytic streptococci found in the throats of normal monkeys are of human origin.

The incidence of hemolytic streptococci in the normal throats of these monkeys is higher than that in the throats of normal humans in this island.⁸

It is of interest to note that Group A hemolytic streptococci were not found in the throat of monkeys after these had been living in the island for more than a year. This is in accord with previous findings in normal human beings living in the island, which show that the incidence of Group A strains among the beta-hemolytic streptococci isolated from normal throats and from throats in the general population of the island of Puerto Rico is lower than the incidence among strains cultured from similar sources in temperate climates.¹ These findings may help to explain the low incidence of certain streptococcal conditions in Puerto Rico.