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A precipitating and neutralizing antistreptococcus (scarlatinae) horse serum.

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The immune serum, used in the experiments to be reported, was prepared in the horse (Horse 10) by repeated inoculation of hemolytic streptococci freshly isolated from the acutely inflamed throats of three typical cases of scarlet fever. The injections were made intravenously on three successive days each week from December 19, 1917, to April 20, 1918. The initial injection consisted of the heated (56° for forty-five minutes) bacteria from 100 cc. of glucose broth, suspended in 10 cc. of sodium chloride solution. The heat-killed organisms were injected during the first five weeks, and live bacteria during the remainder of the period of immunization. The dose was gradually increased until the organisms from 600 cc. of broth were given at a single injection. The horse remained well until the latter part of March, when it developed arthritis which gradually increased until May 6, when it was bled to death under anesthesia. The serum used was obtained from this bleeding and from bleedings made April 8 and May 1. After the serum had been proved sterile, 0.2 per cent tricresol was added, and the serum placed in amber bottles securely stoppered and kept continuously in the ice chest.

Another horse (Horse 19), whose serum was used as a control, was immunized in a similar manner but with hemolytic streptococci from septic infection.

The small epidemic of scarlet fever which occurred in Rochester when inoculation of the horse was begun, had disappeared before immunization was considered adequate to give the serum therapeutic value, and hence it was not used in treatment. However, it was found to have marked agglutinating power over the strains inoculated as well as over other strains from scarlet fever, but little or no effect on a few strains of hemolytic streptococci from other sources.

PRECIPITIN EXPERIMENTS.

The precipitin tests consisted of layering various cleared extracts of the streptococci over the respective serums in small glass tubes (3.5 cm. long by 3.5 mm. inside diameter), and noting the presence or absence of a cloud at the junction of extract and serum, after two hours' incubation and after being kept in the ice chest overnight. The readings were made in a darkened room by transmitted light, obtained from below the shade of an electric light bulb, directing the eye into a dark background.

In my work on specificity in the group of green-producing streptococci, it was found that specific agglutinating and precipitating properties were maintained over long periods when freshly isolated strains were kept in dense suspension in glycerin (two parts) and 25 per cent sodium chloride solution (one part). The glycerin-salt solution extracts used in the experiments were made by diluting such dense suspensions of hemolytic streptococci with water, to the density of the original broth culture, and centrifuging until water-clear. The extracts from the old blood-agar slant cultures were made by adding 2 cc. of distilled water, slanting the tubes so that the water covered the slant, and leaving the tubes over night in the ice chest. The extracts from the throats were made by swabbing the nasopharynx, washing the swab in

Precipitin reactions with antigens from various hemolytic streptococci.

Source of hemolytic streptococcus	Source of Antigens	Number of strains tested	Number of positive reactions with serum from		
			Horse 10 (Scarlet fever)	Horse 19 (Septic infection)	Normal horse
Scarlet fever	Glycerin-salt solution extract	18	17	0	0
Scarlet fever	Glucose-broth culture	12	9	0	0
Scarlet fever	Extract in water of old blood agar slants	5	5	1	0
Miscellaneous	Glycerin-salt solution extract	12	2	4	0
Scarlet fever	Sodium chloride solution suspension of nasopharyngeal swabbings	32	17	1	0
Normal controls	Sodium chloride solution suspension of nasopharyngeal swabbings. Persons not exposed to scarlet fever.	76	3	0	0

2 cc. of sodium chloride solution, squeezing out the fluid from the cotton, and centrifuging until water-clear.

The results obtained with extracts of hemolytic streptococci from scarlet fever and other sources, and with extracts of nasopharyngeal swabbings in cases of scarlet fever and in normal controls, are summarized in Table 1. Only negative results were obtained with immune serums used as controls, which were prepared by injecting green-producing streptococci isolated respectively in poliomyelitis, encephalitis, chorea and influenza, and with antipneumococcus serums.

The results of the precipitin reaction are in agreement with those of Tunnicliffe,[1] Bliss,[2] Dochez[3] and others, in that they show the hemolytic streptococcus from scarlet fever to be quite homogenous, and that hemolytic streptococci from other sources are more heterogenous. Nearly all in the former group reacted positively with the immune scarlet fever serum, while only four of the twelve strains from other sources gave a positive test with the immune serums prepared with the hemolytic streptococcus from septic infection. The hemolytic streptococcus was demonstrated in the nasopharyngeal swabbings in all but one of the seventeen cases of scarlet fever that yielded a positive precipitin test, whereas in the fifteen that reacted negatively, it was found in only three. All tests were positive in the acute stage of the disease.

The reaction appears to be specific, and should prove useful in classifying hemolytic streptococci, and in differentiating between scarlatinal and non-scarlatinal throat infections, and hence be of diagnostic value in scarlet fever.

NEUTRALIZATION EXPERIMENTS

The technique of our nuetralization experiments was similar to that developed by the Dicks.[4] The respective organisms were

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[1] Tunnicliff, Ruth: The specific nature of hemolytic streptococcus of scarlet fever, *J. Am. Med. Assn.*, 1920, lxxxii, 1396.

[2] Bliss, W. P.: Abiological study of hemolytic streptococci from throats of patients suffering from scarlet fever, *Bull. Johns Hopkins Hosp.*, 1920, xxxi, 173.

[3] Dochez, A. R.: Studies concerning the significance of streptococcus hemolyticus in scarlet fever. PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 194.

[4] Dick, G. F., and Dick, Gladys H.: A skin test for susceptibility to scarlet fever. *J. Am. Med. Assn.*, 1924, lxxxii, 265.

grown in human or horse blood broth for four to seven days, and the cleared supernatant broth culture was passed through bacterial filters (Mandler type). The neutralizing power of the immune scarlatinal serum and control serums was tested by mixing equal parts of the serum with the filtrate or dilutions thereof, incubating for one hour, and injecting 0.2 cc. into the skin over the forearm of humans. Severe initial reactions, due to the toxicity of the filtrates; delayed urticarial reactions, due to the horse serum; the danger of rendering persons sensitive to horse serum; and the extreme variations in susceptibility of humans, emphasized the importance of finding a suitable test animal to determine the neutralizing power of this and the newly developed therapeutic scarlatinal horse serums. Many animals were tested. The horse, cow, goat, dog, monkey (Macaccus rhesus), rabbit, guinea pig, white rat, mouse and fowl were all found insusceptible to intracutaneous injection. The skin over the abdomen of pigs (Chester white) weighing from 20 to 50 pounds, and the skin in the groin and axilla of the sheep and lamb was found to be susceptible.

The reaction in these animals is sharply defined, but reaches its height in about eight hours, instead of twenty-four hours as in man, and the secondary reaction some days later is uncommon. Pseudo reactions following control injections of uninoculated blood broth, filtrates, normal and control serums rarely occur. The dilution of filtrate should be about one-tenth as great as for humans. Parallel toxicity and neutralization experiments in man, pig and sheep gave virtually the same results, although those in the pig were the most uniformly satisfactory.

It has been found that the toxicity of filtrates varies greatly according to the culture medium used, blood-broth cultures yielding filtrates of highest toxicity. Fresh kidney tissue in parallel experiments markedly reduced the toxicity. In one series of experiments, all but two of thirteen scarlatinal strains yielded filtrates of high toxicity. The two yielding only slightly toxic filtrates were isolated, one four, and the other three and a half years previously. In this same experiment, six of eleven non-scarlatinal strains yielded filtrates of high toxicity whose action was indistinguishable from that of the scarlatinal strains. Control strains of green-producing streptococci never produced filtrates manifesting high toxicity on intracutaneous injection. The immune scarlatinal serum from Horse 10, had marked neutraliz-

ing power, not only over nearly all filtrates from scarlatinal strains, but also over those from non-scarlatinal strains. The serum from Horse 19 had slight neutralizing power over some filtrates; normal horse serum had none. The identity of the toxin produced by the respective strains is further shown by the fact that convalescent scarlet fever serum, and the serum of humans after severe reactions to inoculations of scarlet fever filtrates, had neutralizing power over both sets of filtrates.

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A study of the occurrence of peptide nitrogen in the blood.

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The nitrogen found in tungstic acid blood filtrates, which cannot be attributed definitely to any known nitrogenous substances, has been studied by the following method: To 10 cc. of tungstic acid blood filtrate in a large test-tube graduated to 25 cc. are added 10 cc. of a saturated solution (room temperature) of barium hydroxide. The test-tube is fitted with a reflux condenser, and the contents kept boiling gently for 20 to 24 hours. The solution is then concentrated to 5 cc., a drop of 0.25 percent phenolphthalein is added and sufficient 2 percent sulfuric acid to precipitate the barium. Water is added to the 25 cc. mark, and the contents shaken and filtered. The determination of the free and hydrolyzed amino acids is carried out according to the method of Folin.[1] The difference between the free and total amino-acid nitrogen is considered as peptide nitrogen.

In a recent paper Blau[2] came to the conclusion that the amount of peptide nitrogen is by far too small to account for the undetermined nitrogen. The values found in the table, obtained by the method described, show that the peptide nitrogen makes up a very high percent of the unknown nitrogen. The reason that a more exact check on the total nitrogen is not obtained, is due probably to unavoidable small losses in the various methods and

[1] Folin, O., *J. Biol. Chem.*, 1922, li, 377.

[2] Blau, Nathan F., *J. Biol. Chem.*, 1923, lvi, 873.