produced, the virus was derived from the same strain and this strain was passed from man to mosquito and back to man through six generations. There was no evidence that the virus suffered attenuation nor that its virulence was increased as a result of continuous alternate passage through man and mosquito.

The numbers of potentially infected *Aedes* that took blood for infecting purposes in the forty-seven positive cases varied from two to thirty-six, and 50 per cent of the positive cases were bitten by from two to ten potentially infected mosquitoes.

The preliminary periods of isolation and time interval intervening between biting experiments, with one exception, was not less than eight days and did not exceed eighteen days.

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Studies on the biology of the streptococcus erysipelatis. IV. Toxin production of the streptococcus erysipelatis.

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Studies of toxin production by *Streptococcus erysipelatis,* and on neutralization of this toxin by the serum of convalescent erysipelas patients, and by erysipelas antistreptococcic rabbit and donkey sera, which were begun by me in the Medical Clinic at the Johns Hopkins Hospital in the fall of 1924, and continued in this laboratory, add further evidence to my previous reports[1] that a specific relationship exists between *Streptococcus erysipelatis* and erysipelas. The toxins employed in these studies were prepared in 48 hours' tryptic broth medium cultures of *Streptococcus erysipelatis,* incubated at 37° C. Thirty-four strains tested were found to yield uniformly toxic filtrates. The tryptic medium employed was the original Douglas'[2] tryptic medium digest, modified by Hartley,[3] Watson and Wallace.[4] From a large

[1] Birkhaug, K. E., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 292; *Bull. Johns Hopkins Hosp.*, 1925, xxxvi, 248; *ibid.*, 1925, xxxvii, 85; *ibid.*, 1925, xxxvii, 307.

[2] Douglas, S. R., *Lancet,* 1914, ii, 891.

[3] Hartley, P., *J. Path. and Bact.*, 1922, xxv, 479.

[4] Watson, A. F., and Wallace, U., *J. Path. and Bact.*, 1923, xxvi, 447.

series of toxic filtrates incubated over periods varying from six to ninety-six hours, the greatest toxin production was found to occur about 48 hours after inoculation of the medium. A definite decrease in toxin concentration took place about 90 hours after the initial incubation. The curve of toxin production and concentration was determined in the skin of persons susceptible to a skin test dose of 0.1 cc. of a 1:1000 dilution in normal saline solution of the toxic filtrate. The first appearance of sufficient toxin concentration to render a positive skin lesion, measuring more than 5 mm. in diameter, occurred about 12 hours after the inoculation of the medium, and the lesion produced by the toxic filtrate incubated for 24 hours, measured about 1.5 cm. in diameter. The lesion produced by one skin test dose of the toxic filtrates from the cultures incubated for periods of 48, 96, and 120 hours, measured respectively 3.6 cm., 3 cm., and 2.4 cm., in diameter. The lesions were similar to those observed in the Dick test for scarlet fever susceptibility. The skin lesions were read 24 hours after the injection of the skin test dose. The lesion rapidly disappeared about 60 hours after the injection of the skin test dose, and only occasionally was found to leave behind a slightly pigmented area. Complete neutralization of the toxin was obtained by mixing the skin test dose with an equal amount of convalescent erysipelas serum, or with 0.001 cc. of erysipelas antistreptococcic rabbit or donkey sera.

Thermal inactivation of the toxic filtrates was first detected after heating the filtrates at 90° C. for one hour. The lethal dose of the toxin for rabbits varied from 2 to 12 cc. per kilogram of weight, death occurring from 8 to 96 hours after the intravenous, intraperitoneal, or intramuscular injection of the toxic filtrate. Rabbits susceptible by the skin test to one skin test dose of the toxin, succumbed rapidly following the intravenous administration of 3 to 5 cc. of the toxic filtrates. Six rabbits out of 37 animals tested, or 16 per cent, gave uniform skin lesions with one skin test dose. It was clear, however, from testing a large number of laboratory animals that such material was unsatisfactory for the titration of a standard skin test dose. Persons susceptible to the erysipelas streptococcic toxic filtrates were employed for the purpose of titration of an adequate skin test dose.

Eighteen cases of erysipelas injected with a skin test dose of the toxin gave positive lesions on their arrival at the hospital.

During defervescence of the disease and the regression of the erysipelatous lesion, the reaction to one skin test dose of the toxin became rapidly obscured. The shortest period in which a patient's positive skin reaction became negative was 5 days after the onset of the disease, and the longest period recorded was 38 days after admission to the hospital. The blood serum from erysipelas patients with positive skin reactions, when injected intradermally in normal, but susceptible individuals, gave positive skin reactions. When the patient's blood serum was mixed with an equal amount of convalescent erysipelas serum, or with 0.001 cc. of erysipelas antistreptococcic donkey or rabbit sera, the toxin was completely neutralized. During the period of infection in which the patient's blood serum contained enough toxin to produce a positive skin lesion in susceptible persons, the patient's urine contained a similar toxic substance, which was completely neutralized by convalescent erysipelas serum, or by the erysipelas antistreptococcic donkey or rabbit sera. The toxic substance in the urine was obtained by passing the fresh urine through a Berkefeld V candle and by diluting the filtrate in physiological saline solution up to 1:500. A skin test dose of 0.1 cc. of this dilution uniformly produced a positive skin lesion in the patient or susceptible persons.

Erysipelas patients whose skin reactions were positive on admission to the hospital, when treated with therapeutic doses of erysipelas antistreptococcic donkey or rabbit sera (25 to 100 cc.) gave negative skin reactions with multiple skin test doses of the toxin as soon as 12 hours after the intramuscular administration of the serum. If the disease persisted unchecked by the serum therapy, the skin reaction remained positive until defervescence and definite regression of the erysipelatous lesion occurred.

Among 135 hospital patients admitted with other complaints than erysipelas and ranging in ages from 18 to 72 years, thirty-six patients, or 27 per cent, gave positive skin reactions with one skin test dose. Among nineteen patients, with definite histories of erysipelas, from one to twelve years ago, four gave positive skin reactions.

Among 272 normal school children, ranging in ages from 7 to 17 years, fifty-seven persons, or 21 per cent, gave positive skin reactions with one skin test dose. When 251 of the same children were tested with one skin test dose of the Dick scarlet fever toxin, one hundred and thirty-one persons, or 52 per cent, gave

positive skin reactions. Only 25 of these children, or 10 per cent, gave simultaneously positive reactions when tested at once with the erysipelas streptococcic toxin and the Dick scarlet fever toxin.

Neutralization of the erysipelas toxin, as judged by the human skin test, was accomplished by convalescent erysipelas serum and the erysipelas antistreptococcic donkey or rabbit sera, and not by Dochez' scarlatinal antistreptococcic serum, nor by normal rabbit or donkey sera.

Summary.

1. Among 34 strains of *Streptococcus erysipelatis* grown in tryptic medium at 37° C., the maximum toxin concentration was obtained in the lots incubated about 48 hours. 2. A skin test dose of 0.1 cc. of a 1:1000 dilution of erysipelas toxic filtrate produced in the skin of susceptible persons a lesion which measured more than 1.5 cm. in diameter. 3. Complete neutralization of one skin test dose of the erysipelas toxin was obtained by mixing it with an equal amount of convalescent erysipelas serum, or 0.001 cc. of erysipelas antistreptococcic rabbit or donkey sera. 4. During the acute stages of erysipelas the patient's blood serum and urine contained a toxic substance which was completely neutralized by convalescent erysipelas serum and which disappeared from the patient's blood serum and urine as soon as twelve hours after the administration intramuscularly of 25 to 100 cc. of the erysipelas antistreptococcic rabbit or donkey sera. 5. Positive skin reactions were obtained by one skin test dose of erysipelas streptococcic toxin in 27 per cent of apparently normal adults and 21 per cent of normal school children. 6. Among nineteen persons with definite histories of single and recurrent attacks of erysipelas, 4 persons gave positive reactions with one skin test dose of the erysipelas streptococcic toxin. 7. Neutralization of the erysipelas streptococcic toxin was not accomplished by Dochez' scarlatinal antistreptococcic serum, nor by normal rabbit or horse sera.