cogen in animals in the post absorptive state. A repetition of these experiments on adrenalectomized mice gave the same result. This phenomenon is, therefore, not connected with an increased discharge of epinephrine.

This is a preliminary report.

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Bacteriologic Studies in Acute Rheumatic Fever with Reference to Soluble Toxin Production.

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An extensive comparative study was made of the production of soluble toxins by hemolytic and non-hemolytic streptococci, isolated from a large series of normal and diseased individuals. The author encountered a strongly toxigenic strain of a non-hemolytic, non-methemoglobin-forming, inulin-fermenting, bile-insoluble, gram-positive streptococcus, isolated on March 24, 1926, from blood-cultures of a 5-year-old girl, ill with acute rheumatic fever, endocarditis, myocarditis, pericarditis, and pleurisy. Post mortem, the identical organism was isolated from the vegetations on the mitral valves, as well as from the heart's blood. In subsequent studies, a similar non-methemoglobin-forming streptococcus was regularly isolated from the tonsillar crypts, abscesses, and irregularly from blood-cultures, heart-vegetations, feces and urine, of persons with rheumatic fever and the allied forms of this protean disease. Culturally, serologically, and toxigenically, this new type of non-methemoglobin-forming streptococci was found to constitute a closely related group of micro-organisms, distinguishable from the *Streptococcus viridans* and *Streptococcus hemolyticus* groups. (Table I.) For toxin production, the tryptic medium employed was the original Douglas'[1] tryptic medium digest, modified by Hartley,[2] Watson and Wallace,[3] and the cultures were incubated at 37°

TABLE I.

Biological reactions of non-hemolytic streptococci isolated from patients with history of rheumatic fever.

Strain	Source	Glucose	Lactose	Sucrose	Mannite	Salicin	Raffinose	Inulin	Bile	Toxin	RF.-AT neutral	Agglutination Reactions		
												RF. I Serum	Str. virid. Serum	
													"SV 76"	R. 37
*Beattie—227	Blood	A	A	A	A	A	—	—	—	—	—	—	—	—
*Allen—304	Blood	A	A	A	A	A	—	—	—	—	—	—	—	—
*Swift—A. 49	Blood	A	A	A	—	—	—	—	—	—	—	—	—	1:40
*Swift—B. 38	Blood	A	A	A	—	A	A	A	—	—	—	1:80	—	—
*Swift—38 D.	Blood	A	A	A	—	—	—	—	—	—	—	—	—	—
*Swift—A.179	Heart	A	A	A	—	—	—	—	—	—	—	—	—	1:160
*Swift—W. 72	Nodule	A	A	A	—	—	A	—	—	—	—	1:40	—	—
RF. 1—b.	Blood	A	A	A	—	A	A	A	—	+	+	1:1,280	1:20	1:40
RF. 1—v.	Heart	A	A	A	—	A	A	A	—	+	+	1:2,560	1:20	1:20
RF. 2	Tonsil	A	A	A	—	A	A	A	—	+	+	1:640	—	—
RF. 3—t.	Tonsil	A	A	A	—	A	—	A	—	+	±	1:160	—	—
RF. 3—f.	Feces	A	A	A	—	A	A	A	—	+	±	1:320	1:20	1:40
RF. 4	Tonsil	A	A	A	—	A	A	A	—	±	±	1:40	1:20	1:20
RF. 5	Chorea	A	A	A	—	A	—	A	—	±	+	1:80	—	—
RF. 6	Chorea	A	A	A	—	A	—	A	—	±	+	—	—	1:40
RF. 7	Tonsil	A	A	A	—	A	—	A	—	±	+	—	1:40	—
RF. 8	Tonsil	A	A	A	—	A	A	A	—	±	+	1:80	1:20	—
RF. 9—t.	Tonsil	A	A	A	—	A	—	A	—	±	+	1:160	—	—
RF. 9—f.	Feces	A	A	A	—	A	—	A	—	+	+	1:80	—	1:160
RF. 10—b.	Blood	A	A	A	—	A	A	A	—	+	+	—	—	—
RF. 10—t.	Tonsil	A	A	A	—	A	A	A	—	+	+	1:320	—	—
RF. 10—a.	Abscess	A	A	A	—	A	—	A	—	+	+	1:1,280	1:20	1:40
*RF. 11	Blood	A	A	A	—	—	—	—	—	—	—	—	—	—
RF. 12	Tonsil	A	A	A	—	—	—	A	—	±	±	1:40	1:20	—
RF. 13—a.	Abscess	A	A	A	—	A	—	A	—	+	+	1:640	1:40	1:80
RF. 13—t.	Tonsil	A	A	A	—	A	—	A	—	+	+	1:320	1:40	—
RF. 14	Tonsil	A	A	A	—	—	—	A	—	+	+	1:40	1:20	—
RF. 15	Tonsil	A	A	A	—	A	A	A	—	+	+	—	—	—
RF. 17	Chorea	A	A	A	—	A	—	A	—	+	+	1:640	1:40	1:20
RF. 18	Abscess	A	A	A	—	A	A	A	—	+	+	1:1,280	1:40	—
RF. 21	Tonsil	A	A	A	A	—	—	A	—	±	±	1:320	—	—
RF. 24—t.	Tonsil	A	A	A	—	A	—	A	—	±	±	1:80	—	—
RF. 24—u.	Urine	A	A	A	—	A	—	A	—	—	—	1:160	—	1:160
RF. 36	Tonsil	A	A	A	—	A	A	A	—	—	+	1:1,280	1:80	—
*R 37	Blood	A	A	A	—	—	—	—	—	—	—	1:20	1:20	1:2,560
*SV. 76	Blood (SBE)	A	A	A	—	—	—	—	—	—	—	1:20	1:2560	1:40

*Typical strains of *Streptococcus viridans* type; remaining strains are non-methemoglobin-forming streptococci. T-AT neutral indicates that 0.01 of the RF. I antitoxin (rabbit) is mixed with 0.1 cc. of a 1 to 100 dilution of the toxic filtrate and is injected intradermally. + indicates complete neutralization; ± partial neutralization, and — no neutralization. A indicates acid fermentation without gas-production. SBE implies subacute bacterial endocarditis.

C. over periods varying from 4 to 10 days, when the cultures were filtered through Berkefeld V candles. Ninety-eight strains of hemolytic streptococci and 247 strains of the green streptococci yielded only 4.7 per cent of toxin producing strains. Among 68 strains of the non-methemoglobin-forming streptococcus isolated from rheumatic fever, 49 strains, or 72 per cent were found to produce a demonstrable soluble toxic filtrate, slightly weaker in toxicity than that obtainable from *Streptococcus erysipelatis.*[4] When normal persons without history of rheumatic fever were tested intradermally with a skin test dose of 0.1 cc. of 1 to 100 dilution of the soluble toxic filtrate produced by the non-methemoglobin-forming streptococcus, isolated from rheumatic fever, 18 per cent of adults and 11 per cent of children gave positive skin reactions, similar to the Dick test, and measuring more than 1 cm. in diameter within 24 hours after the injection. Among persons with history of rheumatic fever and tested with 0.1 cc. of 1 to 100 dilution of this soluble toxin, 56 per cent of adults and 76 per cent of children gave positive skin reactions within 24 hours after the injection. When the same persons were tested with a lower dilution of 0.1 cc. of 1 to 10, it was found that 67 per cent of adults and 85 per cent of children gave positive reactions within 12 hours following the intradermal injection. In many instances the local swelling simulated erythema nodosum. Complete neutralization of the toxin was obtained by mixing the skin test dose with 0.01 cc. of the serum drawn from rabbits actively immunized with the toxin or with the serum drawn from individuals actively immunized by intramuscular injections of toxin-antitoxin mixtures.

Thermal inactivation of the toxin produced by the non-methemoglobin-forming streptococcus isolated from rheumatic fever, was first detected after boiling the filtrate for 1 hour. Exposing the toxin to direct sunlight and room temperature for more than 3 months failed to destroy the toxic principle. The active toxic substance was completely removed with the fraction precipitated by 6 volumes of absolute alcohol. The resultant white and flaky precipitate was readily redissolved in normal saline solution and in this manner could be purified and concentrated with great ease.

Intravenous and subcutaneous injections in the rabbit of the living non-methemoglobin-forming streptococcus, invariably produced a non-suppurative polyarthritis, with marked swelling of the joints. The joint fluid was mucoid in appearance, containing numerous polymorphonuclear cells and no bacteria. Subacute bacterial endocarditis and myocarditis, with a unique and regular tendency

to vegetative mitral stenosis, extensive mural vegetations, and occasionally pseudo-nodular arrangements of multinucleated forms of leucocytes, as well as of polymorphonuclear and mononuclear cells, were encountered. Intravenous and subcutaneous injections of the sterile toxic filtrate rapidly developed polyarthritis of the non-suppurative form, after repeated injections of increasing doses. The only changes found in the heart of the rabbits injected with the sterile toxic filtrate, were those of myocardial degeneration.

After seven months of constant intradermal injections of skin test doses of the sterile toxic filtrate of the non-methemoglobin-forming streptococcus, in which time the positive skin reaction remained unchanged in degree of cellular response, the author injected himself intra-articularly in the left wrist and intramuscularly in the right forearm with 1 cc. of the undiluted and sterile toxic filtrate. Twenty-four hours later general malaise developed, and chills, temperature and drenching perspiration preceded irregular joint pains. Within 48 hours after the injections, a typical clinical picture of acute polyarthritis of the rheumatic type developed, attended by hyperpyrexia, rapid pulse, excessive perspiration and migration of arthritis from one large joint to another. On the fourth day of the illness salicylates were taken in large doses and the general condition was rapidly restored to within normal limits. A secondary recrudescence of the lesions in the skin produced by the intradermal injections of the toxins occurred simultaneously with each fresh attack of arthralgia during the entire course of the migratory polyarthritis, similar to the Arthus phenomenon,[5] and the Andrewes, Derick, Swift "secondary reaction."[6] The frequency of positive reactors with the toxin produced by the rheumatic fever non-methomoglobin-forming streptococcus especially among persons stigmatized by rheumatic fever, has suggested to the author a state of allergy in such persons in relation to the toxins produced by the new species of the non-methemoglobin-forming streptococcus described in these studies and no doubt previously encountered by Mandelbaum[7] in 1907, as *Streptococcus saprophyticus,* by Zangemeister[8] in 1910, as *Streptococcus anhemolyticus vulgaris,* by Rosenow[9] in 1914, from the joint fluids in acute rheumatism, articular and muscular, and while my own studies were in progress, by Small[10] as *Streptococcus cardioarthriditis.*

This is a preliminary report.

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Further Observations on Apparent Effect of Diphtheria Toxin on Growth of Bacteria.

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In a previous report[1] we have noted the fact that diphtheria toxin when added to cultures of *Bacillus cereus* in a concentration of 30 m.l.d. per cc. leads to a slight but definite retardation of growth. While our data do not prove that the inhibitory factor for *B. cereus* is in fact the toxin rather than some other by-product of growth of the diphtheria organism, there is some basis for believing that this might be the case. Of particular interest is the fact that the addition of antitoxin appears to counteract this inhibitory action.

In the present paper we wish to record that similar results have been obtained with *Proteus vulgaris* and *Staphylococcus albus*. The apparent inhibitory effect of the diphtheria toxin upon the growth of these organisms is of about the same magnitude as in the case of *B. cereus*.

This is a preliminary report.

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