

PHAGOCYTTIC IMMUNITY IN STREPTOCOCCUS INFECTIONS.¹

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Study of the means whereby the human organism combats streptococcus has had an important part in the investigation of infectious diseases. Metchnikoff in 1887 first observed phagocytosis of streptococci with erysipelas. This observation, associated with the marked leucocytosis found in infections due to the streptococcus, naturally lead to the opinion that phagocytosis was an important means of overcoming such infections. Bordet,² Denys and Le Clef,³ and van der Velde⁴ have demonstrated that phagocytosis both in the animal body and in test-tube experiments may be a powerful agent for destruction of streptococci. Ruediger⁵ has shown that human serum has no bactericidal action on streptococcus but when leucocytes and serum are incubated with streptococci, destruction of the organism is accomplished. I have demonstrated⁶ that the serum of rabbits immunized to living streptococci acquires a greatly increased power to promote phagocytosis which may be one hundred times that of normal serum and nevertheless the serum has practically no bactericidal activity.

The part of phagocytosis in human infection with streptococcus has been investigated by many workers but quantitative estimation of the power of the blood serum to promote phagocytosis has usually been made by the use of the "opsonic index" of Wright. The results have been inconclusive, since a high degree of immunity has

¹ Received for publication July 10, 1909.

² *Ann. de l'Inst. Pasteur*, 1896, x, 104; 1897, xi, 177.

³ *La Cellule*, 1895, xi, 175.

⁴ *Ann. de l'Inst. Pasteur*, 1896, x, 580.

⁵ *Jour. of the American Med. Assn.*, 1905, xlv, 198.

⁶ *Jour. of Exper. Med.*, 1908, xi, 100.

seldom been observed and the charts which have been published exhibit great variations and daily irregularities which are dependent upon the inaccuracy of the method. Nevertheless they have demonstrated that the activity of phagocytosis is not normal and especially during convalescence from such infection a slight increase of the opsonins for streptococcus may occur.

In immunized animals I have demonstrated a high degree of opsonic immunity by diluting the blood serum and determining at what dilution phagocytosis ceases to occur. In the present work I have applied this method to the investigation of the opsonic immunity which occurs with streptococcus infections in man. The technique employed has been identical with that used in my experiments on animals and in all instances the streptococcus which has been isolated from the infected individual has been used to estimate his phagocytic power. The leucocytes were always obtained from the blood of a normal individual. Since chronic infection affords the best opportunity for observing the course and development of immunity the following case of chronic septic endocarditis was investigated.

HISTORY No. 5166.—W. C., aged 24 years, white, male, was admitted to the Presbyterian Hospital on February 12, 1909, in the service of Dr. James, to whom I am indebted for the privilege of reporting the clinical history.

Personal History.—The patient, a butler, used alcohol in moderation; tobacco in excess. When a child he had mumps and at the age of fifteen he had scarlatina, followed by a severe attack of acute rheumatic fever, lasting six months. Four years later he suffered with a second attack of acute rheumatic fever lasting from four to five months. During the past two years he had two mild attacks of gonorrhoeal urethritis, from both of which he made an apparently complete recovery. Luetic history was denied.

History of the Present Illness.—About January 1, 1909, there was general malaise, anorexia, drowsiness, vertigo, and edema of the eye-lids. At the same time there were pains throughout the extremities but especially about the joints, and the left knee was swollen and reddened. Weakness gradually increased and there was loss of weight. There was considerable sweating and slight mucoid expectoration but no chills, dyspnea, orthopnea or cough.

Physical Examination on Entrance to the Hospital.—The patient is a fairly well-nourished man apparently about 25 years of age and is somewhat anemic. The eyes and ears are normal; the tonsils are not enlarged; the teeth are in very poor condition. The axillary and inguinal lymph nodes are palpable. The thorax is well formed and the subcostal angle is wide.

Impulse at the apex of the heart is visible and palpable in the fifth intercostal space, 8.5 cm. to the left of the mid-sternal line. The limits of the

cardiac dulness are 12 cm. to the left and 4 cm. to the right of the mid-sternal line. The cardio-hepatic angle is slightly blunted. On auscultation there is heard at the apex a loud, harsh, and high pitched systolic murmur which immediately follows the first sound and lasts throughout the systole. This murmur is well transmitted into the axilla. At the base of the heart there is heard a soft blowing systolic murmur different in character from that heard at the apex, and it is faintly transmitted into the vessels of the neck. Over the aortic area there is heard a soft diastolic murmur with the point of maximal intensity in the fourth intercostal space just to the left of the sternum; it is lost towards the apex.

The pulse rate is about 100 to the minute. It is full in systole with a sharp fall in diastole. The tension is not increased and the vessel wall is slightly thickened. A capillary pulse is visible in the finger-nails.

The lungs appear normal. The abdomen is round and symmetrical and exhibits no tenderness. The liver and spleen are not enlarged. There is a very slight mucoid discharge from the urethra in which very few pus cells and no gonococci are found. Urine, acid, sp. gr. 1024, contains a faint trace of albumin and a few hyaline casts. For the temperature curve see Chart I. Table I shows the findings of the various blood examinations.

TABLE I.

Date.	White blood cells.	Hemoglobin.	Red blood cells.	Blood culture.
Feb. 12, '09.	17,700	70 per cent.	4,300,000	Streptococcus. Streptococcus.
" 13.				
" 15.				
" 17.	18,000			
" 24.	23,000			
Mar. 1.	14,600			
" 8.	14,000	68 per cent.	4,500,000	Streptococcus. Sterile.
" 23.	17,200	65 per cent.	3,800,000	
Apr. 5.	14,000			
" 8.				
" 24.	12,400	60 per cent.	3,000,000	Sterile.
May 7.				

Differential Count (Feb. 13).—Polymorphonuclear leucocytes, 73.5 per cent.; transitionals, 2.5 per cent.; large mononuclears, 0 per cent.; lymphocytes, 23.5 per cent.; basophiles, 0.5 per cent.

On February 20, 1909, a vaccine prepared with streptococci obtained from the patient's blood on February 13 was given. For the estimated amount and frequency of administration see Table II.

March 9, 1909.—Signs of fluid were discovered in the right pleural cavity and 25 c.c. of slightly turbid, amber fluid were obtained by exploratory puncture. Its specific gravity was 1021, it formed a large coagulum, and there was little sediment. Albumin was 55 per cent. by volume; reducing bodies were present. Differential count of cells was as follows: polymorphonuclear leucocytes, 30 per cent.; large mononuclear leucocytes, 20 per cent.; lymphocytes, 24 per cent.; eosinophiles, 1 per cent.; endothelial cells, 25 per cent. Media inoculated for cultures remained sterile.

TABLE II.

Number of vaccination.	Date of vaccination.	Amount of vaccine in hundred million streptococci.
1	Feb. 20	one
2	Feb. 24	one
3	Mar. 6	two
4	Mar. 12	two
5	Mar. 16	three
6	Mar. 21	three
7	Mar. 26	three
8	Mar. 31	four
9	Apr. 5	four
10	Apr. 10	four
11	Apr. 15	four
12	Apr. 20	four
13	Apr. 25	five
14	Apr. 30	five
15	May 5	five
16	May 10	five

March 24, 1909.—An ulcerated tooth was extracted and the temperature fell to 101° F., remaining depressed for three days.

May 5, 1909.—The condition has remained almost unchanged but the area of cardiac dulness is a little larger and the murmurs are louder. On this date the patient developed measles and was transferred to the isolation ward.

May 14, 1909.—The patient has complained of pains in the joints but these pains have disappeared; he is feeling better than at any time since entering the hospital and expects to be soon completely recovered.

May 17, 1909.—At 1 A. M. the patient suddenly became nauseated, vomited and complained of headache. His speech was thick and there was hemiplegia of the left side with dilatation of the right pupil and failure of both pupils to react to light. Unconsciousness soon supervened and the patient died after six hours. An autopsy was refused.

Clinical Diagnosis.—Chronic septic endocarditis (streptococcus); chronic valvular disease (mitral and aortic); sero-fibrinous pleurisy; embolism in spleen; embolism of the right middle cerebral artery; measles.

The patient was under observation during a period of three months and during this time the opsonic power of the blood serum was estimated at intervals of from five to seven days. An examination of the chart demonstrates that this property of the blood serum may be greatly increased by chronic infection with streptococcus, reaching a strength fifty times greater than normal. The opsonic power of normal blood serum was frequently determined (see Chart 1) and it does not exhibit the fluctuations which are almost constantly present in curves obtained by use of the ordinary opsonic

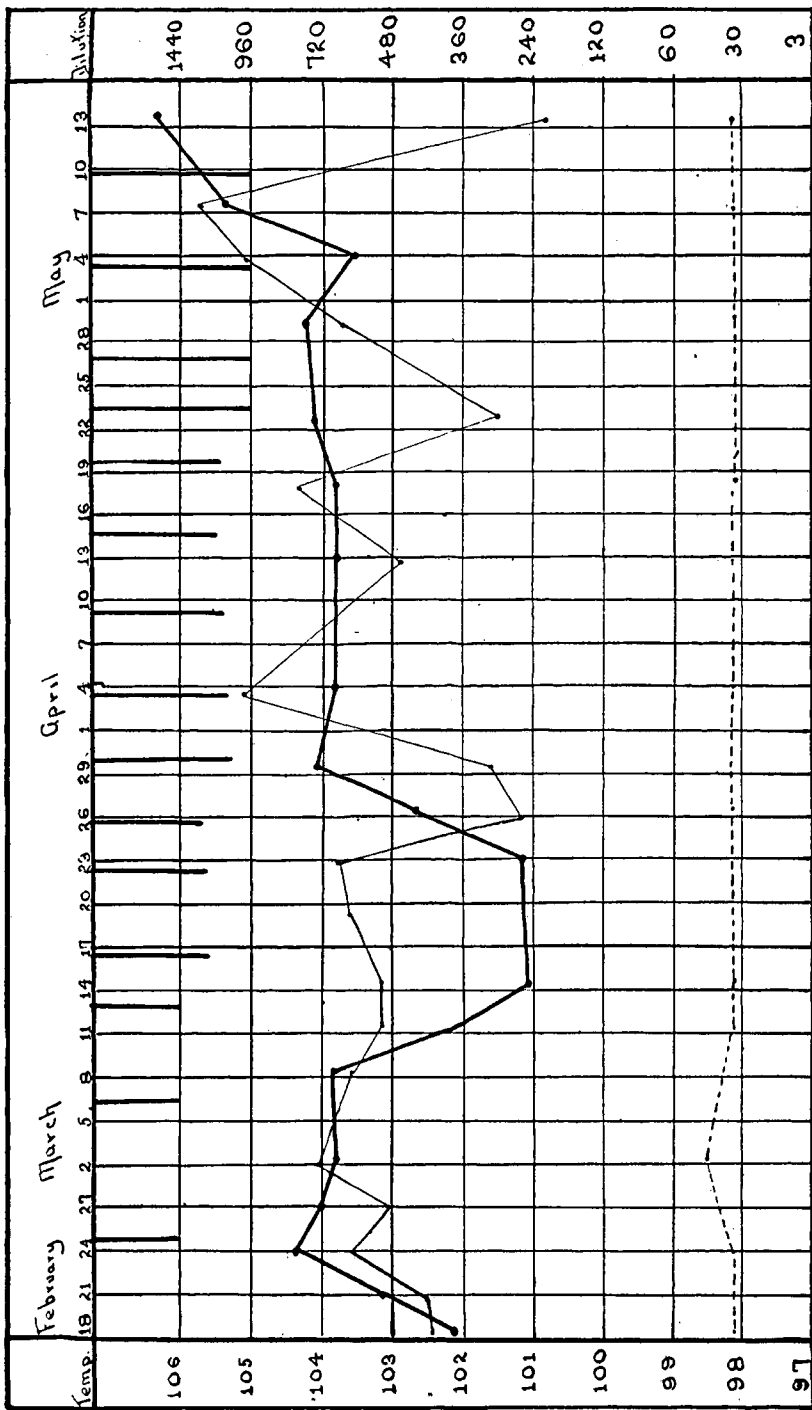


CHART I. Heavy straight line represents opsonic power of patient's bloodserum; dotted line, opsonic power of serum of normal individual; fine straightline, temperature of patient. Perpendicular lines at the top of the chart represent administration and dosage of vaccines.

index. Since fluctuations are slight the possibility of errors of technique may be neglected.

During the period from March 9 to March 23 there was a gradual lessening of the opsonic power from 600 to 250, maintained for a time and followed by a gradual increase. Examination of the history shows that on March 9 the patient first exhibited signs of acute sero-fibrinous pleurisy which had completely disappeared by April 1 when the opsonic power had again returned to its former high level. Whether the pleurisy was the cause or the result of the depression of the opsonic power is uncertain. Since the onset of pleurisy apparently antedated depression of the opsonic power by a few days, and the subsequent rise of the opsonic power was synchronous with the improvement of the pleural lesion, it is probable that the diminution of the opsonins was the result of the pleurisy.

A vaccine prepared from the streptococcus cultivated from the patient's blood was administered by subcutaneous injection at intervals of from five to seven days. The first dose was one hundred million streptococci and this amount was gradually increased until five hundred million streptococci were given at each dose. The patient was vaccinated sixteen times and a total of fifty-four hundred million streptococci was received. The effect of the vaccination is not apparent as the opsonic power of the serum shows no definite change ascribable to the administration of the vaccine. Whether the gradual increase of the phagocytic power during the later part of the illness is due to the increased dosage is uncertain. The condition of the patient did not improve while he was under observation. There was however only moderate loss of weight, the red blood corpuscles diminished in number very slowly, the amount of hemoglobin was very slightly reduced and up to a few hours before death the patient felt well, although streptococci were repeatedly found in the circulating blood.

During the past two years nine cases of chronic septic endocarditis, in which streptococci were isolated from the blood, have been observed in the wards of the Presbyterian Hospital. All of these patients died after a more or less chronic illness. Autopsies were obtained in seven of the cases, in all of which the clinical diagnosis was confirmed.

Five of the patients were treated by the administration of homologous vaccines while in four only symptomatic treatment was employed. The duration of the illness in the cases treated by vaccination averaged sixteen weeks while in the unvaccinated cases the duration of the illness averaged eight weeks. Although the vaccines have not produced any definite improvement in the clinical picture or in the opsonic power there is some indication that they have preserved the patient's strength and prolonged the period of life.

In all the estimations of the opsonic power of the blood serum in the case which has been described the streptococcus isolated from the blood of the patient has been used. In order to ascertain if the opsonic power of the patient's serum was equally active towards other streptococci duplicate experiments were made with other strains of streptococcus. The results demonstrate that the patient's blood may be very powerful towards the homologous strains of streptococcus although towards heterologous strains it may be little stronger than normal serum (see Table III).

TABLE III.

Streptococcus and its source.	Relation of streptococcus to patient's serum.	Dilution of patient's serum at which phagocytosis ceases.	Dilution of normal serum at which phagocytosis ceases (control).	Number of cocci per leucocyte in Ringer's solution.
Strep. C.—Blood; endocarditis.	Homologous.	1 to 720	1 to 30	0.5
Strep. R.—Blood; pelvic cellulitis.	Heterologous.	1 to 45	1 to 30	0.45
Strep. Ca.—Blood; purpuric infection.	Heterologous.	1 to 30	1 to 30	0.6
Strep. K.—Blood; endocarditis.	Heterologous.	1 to 30	1 to 30	0.4

The result of this experiment was so definite that streptococci isolated from the blood or from local lesions of other cases were tested both with the serum of the patient from which the organism was isolated and with the blood serum of other individuals with streptococcus infection.

Two strains (Streptococcus McL. and K.) were phagocyted just as well in Ringer's solution as in serum. Streptococcus K. was taken directly from a bouillon blood culture containing blood of the patient and the organisms were doubtless completely opsonized by

TABLE IV.

No. repre- senting pa- tients from whom serum was obtained.	Streptococcus and its source.	Relation of streptococcus to patient's serum.	Dilution of patient's serum at which pha- gocytosis ceases.	Dilution of normal serum at which pha- gocytosis ceases (control).	Number of cocci per leucocyte in Ringer's solution.
I	Strep. H.—Tonsil; ton- sillitis.	Homologous.	1 to 240	1 to 30	0.7
	Strep. G.	Heterologous.	1 to 30	1 to 30	0.5
II	Strep. Ha.—Tonsil; ton- sillitis.	Homologous.	1 to 150	1 to 30	0.5
	Strep. G.	Heterologous.	1 to 30	1 to 30	0.6
III	Strep. G.—Tonsil; ton- sillitis.	Homologous.	1 to 120	1 to 30	0.5
	Strep. H.	Heterologous.	1 to 30	1 to 30	0.7
	Strep. Ha.	Heterologous.	1 to 30	1 to 30	0.7
IV	Strep. McL.—Tonsil; tonsillitis.	Homologous.	Spontaneous.	Spontaneous.	Spontaneous.
	Strep. Ha.	Heterologous.	1 to 15	1 to 30	0.5
	Strep. H.	Heterologous.	1 to 30	1 to 30	0.6
V	Strep. Ca.—Blood; pur- puric infection.	Homologous.	1 to 480	1 to 30	0.55
	Strep. C.—Blood; en- docarditis.	Heterologous.	1 to 30	1 to 30	0.6
VI	Strep. R.—Blood; pel- vic cellulitis.	Homologous.	1 to 240	1 to 30	0.7
	Strep. C.	Heterologous.	1 to 30	1 to 30	0.5
VII	Strep. K.—Blood; mas- toiditis (blood bouillon medium).	Homologous.	Spontaneous.	Spontaneous.	Spontaneous.
	Strep. K.—(agar-agar medium).	Homologous.	1 to 480	1 to 30	0.6
	Strep. C.	Heterologous.	1 to 30	1 to 30	0.5

opsonins in the patient's serum. The dilution of the blood in the blood culture (5 c.c. of blood in 125 c.c. of bouillon) was one part to twenty-five, a dilution well within the strength necessary to completely opsonize the cocci. A second experiment shows that when the streptococci were grown on plain agar-agar this spontaneous phagocytosis did not occur.

The spontaneous phagocytosis of *Streptococcus* McL. could not be explained as it occurred repeatedly even when the organism was grown on plain agar-agar. The possibility that the leucocytic emulsion which was used might contain sufficient serum to promote phagocytosis was eliminated by drawing blood from a normal individual (as in all the other experiments) into sodium citrate solution,

and washing the corpuscles three times with Ringer's solution. That this procedure was sufficient to remove opsonins was proven by testing the same leucocytic emulsion with other streptococci which were not phagocytosed spontaneously. This observation emphasizes the necessity of making a control experiment to determine if the organism employed undergoes phagocytosis in normal salt solution or other allied fluid such as Ringer's solution which does not contain blood serum; the results of these tests are given in the last column of Tables III and IV.

In all the experiments the serum of the patient infected with streptococcus produced marked phagocytosis of its own streptococcus whereas the serum of this patient was only slightly more active than normal against the heterologous streptococcus. The marked difference in the effect of the serum of a patient on his own streptococcus and on streptococci from other sources and the constancy with which such differences occur show that different strains of streptococci may produce an opsonic immunity for a particular streptococcus which is not effective for other strains. If further experiments prove that this phenomenon is constant, the failure of anti-streptococcic serum to produce satisfactory results may be expected, for the opsonic activity of such sera is the principal, if not only, means they possess for overcoming the streptococcus. Aronson⁷ claims to have produced an anti-streptococcic serum from horses which will protect an animal from a lethal dose of the streptococcus used to produce the immunity. But he found that such a serum was much less active against other strains of streptococci even though these strains were obtained from the same disease in man. This may be explained by the facts demonstrated in the above experiments, namely that the animal organism can produce protective substances, opsonins, which will act upon the infecting strain of streptococcus but are comparatively inactive towards an heterologous strain.

In the cases treated by vaccination there is no direct evidence that the vaccines have been of any value. But the observations described above show that little result may be expected from the use of heterologous vaccines (streptococcus), and if streptococcus vaccines are

⁷ *Berliner klin. Woch.*, 1902, xxix, 981.

employed they should be prepared from the homologous strain of streptococcus.

CONCLUSIONS.

1. In infections with streptococcus in man, such as acute septic endocarditis, and tonsillitis, there is produced a powerful opsonic immunity.
2. This opsonic immunity is effective towards the streptococcus which has caused infection but may be ineffective in the presence of streptococcus from other sources.
3. Little benefit can be expected from streptococcus vaccines unless they are prepared from the streptococcus which has caused infection.