BACTERIOSTATIC EFFECT OF HUMAN SERA ON GROUP A STREPTOCOCCI

III. Interference with Bacteriostatic Activity by Blockage of the Leukocytes

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Plates 5 and 6

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The purpose of this investigation has been to study the antagonistic effect of certain antigenic components of group A streptococci on the bacteriostasis of these bacteria in the presence of sera from patients convalescent from streptococcal infections; particular efforts were directed toward determining the manner in which the interference was effected.

Without fully elucidating how the action took place, many workers have reported inhibition of phagocytosis by products of bacterial metabolism or components of the bacteria in a system in which homologous immune serum or serum containing natural antibodies was present. Since bacteriostasis of Gram-positive cocci depends ultimately upon phagocytosis, any metabolic product or component of the bacterial cell that interferes with the phagocytic function of leukocytes must be considered in studying this phenomenon. Rosenow (1) reported that a sodium chloride extract of virulent pneumococci depressed the phagocytosis of pneumococci but not of staphylococci or streptococci. He thought the extract neutralized the opsonin in the serum; hence the bacteria resisted phagocytosis and growth occurred. A similar activity by cell-free culture broth of virulent pneumococci was reported by Tchistovitch and Yourevitch (2). Wadsworth and Hoppe (3) demonstrated a substance distinct from toxin in the culture filtrate of a variety of toxin-producing and other bacteria, including the strain used as the test organism, which prevented the phagocytosis of staphylococci sensitized with normal horse serum. No specificity was observed, and it was considered that adsorption of the substance on the leukocytes hindered their phagocytic activity.

Sia (4) described an antiphagocytic property of pneumococcal type-specific carbohydrate in the phagocytosis of homologous pneumococci in normal rabbit or cat serum-leukocyte mixtures, but was unable to explain the interference. Phagocytosis of pneumococci occurred in the absence of type-specific carbohydrate. Todd (5), however, clarified this problem somewhat by showing that antigen-antibody precipitates destroyed the bactericidal power of normal human blood. The action of the precipitates was considered a complex phenomenon involving many factors, including the fixation of complement. Wadsworth and Sickles (6) studied a type-specific inhibitory action of pneumococcal culture filtrates on phagocytosis of these organisms,

and suggested that this inhibition was due to an effect on the serum constituents rather than on the leukocytes as had been thought earlier (3).

Cromwell and Centeno (7) further explained the phenomenon by noting vacuolization of leukocytes in fresh citrated blood to which were added antigen-antibody mixtures. The precipitates formed by these mixtures were thought to be associated with vacuolization, which did not occur if the precipitate was removed, and only the supernate was added to the blood. When, on the other hand, washed precipitate was added, leukocytic vacuolization appeared, which suggested that the specific precipitates were phagocytized and that the vacuoles were somehow associated with subsequent digestion of the precipitate.

Materials and Methods

Preparation of Group and Type-Specific Extracts.—A stock solution of group-specific carbohydrate C was made by Fuller's formamide method (8). To determine optimal proportions for precipitation, varying saline dilutions of this solution were mixed with constant amounts of hyperimmune anti-C rabbit serum; 1:120 or 1:240 dilutions of the stock C solution gave the heaviest precipitate.

M extracts prepared according to Lancefield's method (9) from the bacterial sediment of 1,500 cc. of Todd-Hewitt broth cultures were dissolved in 12 cc. of physiological saline solution and stored at 4° C. The M extracts were tested for specificity with absorbed immune rabbit serum of homologous and heterologous types, and were also shown to be free of group-specific C substance by precipitin tests with suitable antisera.

Sera.—Sera from the 3 adults convalescent from streptococcal infections described previously (10) were used for the bacteriostatic and precipitin tests. Hyperimmune rabbit serum was employed to prepare the precipitates.

Precipitin Test.—The precipitin tests were performed by adding 0.1 cc. of serum to 0.15 cc. of varying dilutions of C or M extracts in a series of tubes, which were then shaken and heated for 2 hours in a water bath at 37° C. followed by overnight refrigeration at 4° C. Readings were made at the end of this period, and the degree of precipitation was recorded on a ++++ to + scale.

Suspensions of Specific Precipitates and of Non-Specific Particles.—The specific precipitates obtained by mixing C or M extract with their respective homologous immune rabbit sera were washed 3 times in saline solution, broken into fine particles by repeated aspiration in capillary pipettes, and resuspended in saline solution. Collodion particles, approximately 1 to 2 micra in diameter, were prepared by Cannon and Marshall's technique (11). Charcoal (Darco) was suspended in physiological saline solution and large particles removed by centrifugation. Higgins' American India ink was freed from extraneous material as described by Victor *et al.* (12). Finely divided coagulated particles of normal human plasma and ascitic fluid were prepared by passing these substances through a hot glass capillary tube as performed by Todd (5). Microscopically these particles in normal saline suspensions appeared much like antigen-antibody precipitates. The final turbidity of the suspensions was adjusted to MacFarland scale No. 3.

Bacteriostatic Test.—The details of the bacteriostatic method have already been described (10). The strains of streptococci were isolated from the nasopharynges of the patients furnishing the respective sera. Each test sample contained 0.05 cc. of a broth culture dilution of streptococcal cells, 0.05 cc. of undiluted convalescent human serum, 0.05 cc. bacterial extract or precipitate suspension, and 0.25 cc. of heparinized blood from afebrile children 3 to 9 years of age.

In preparing material for microscopic study, films from samples of different mixtures were stained successively with Wright and Giemsa solutions, which stained both leukocytes and precipitates.

EXPERIMENTAL FINDINGS

Inhibition of Bacteriostasis by M and C Streptococcal Extracts.—Sera from 3 adults convalescing from type 6, 19, or 26 streptococcal infections, respectively, were mixed with corresponding dilutions of type 6, 19, or 26 M extract or with saline solution as a control. The dilution of M extract which was used gave maximal precipitation. To each tube was then added a dilution of streptococcal culture, homologous with respect to each serum, and whole blood. Table I shows that bacteriostasis was inhibited wherever precipitation was found in the corresponding precipitin test; for example, with type 6 serum, inhibition occurred with all the extracts; with type 19 serum, inhibition appeared with type 6 and 19 M extract and slightly with type 26 M extract. Where the M extract and serum did not form a precipitate, bacteriostasis paralleled that in the saline control containing no M substance. The M extract without antibody was not in itself inhibitory. The inhibition was not type-specific as type 6 convalescent sera formed precipitates with all the extracts employed.

A similar experiment was performed with the C substance of group A streptococci. Serum of the patient convalescing from a type 6 streptococcal infection was selected because it was known to contain anti-C precipitin. The C extract was tested in different dilutions to find the range of maximal precipitation. Table II shows that the inhibition of bacteriostasis closely paralleled the formation of precipitates in the C-anti-C systems. A definite prozone, both in the precipitin test and inhibition of stasis was observed in the 1:60 dilution of C substance and less so in the 1:120 dilution. Bacteriostasis occurred normally in tests with many other samples of sera containing no detectable anti-C precipitin. Occasionally, however, slight interference occurred in the absence of visible precipitate; but one might suspect that a macroscopically invisible precipitate was responsible.

Since complement is essential in these systems and since it is known that precipitates adsorb complement, this substance was measured under the conditions of the test. The same experiments as those just described were repeated, and the supernate was pipetted from all of the mixtures at the end of the test; those from the antigen-antibody mixtures showing precipitates were compared with fluids from systems having no antigen or showing no precipitate. The complement in these fluids, tested with sensitized sheep erythrocytes, was consistently only one-third less in tubes containing precipitates than in those without them; but in all cases the residual complement was still sufficient for

TABLE I

Inhibition of Bacteriostasis by M Substance of Group A Streptococci Correlated with Formation of Precipitates

Convalescent sera from patients with:		Type 6 M extract				Type 19 M extract				Type 26 M extract				Control: saline			
		Dilution of culture of group A type 6 streptococci															
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Type 6 infection	Bacteriostatic test																
	Undiluted serum	++++	++++	+++	+++	++++	++++	+++	+++	++++	+++	+++	+±	1	1	0	0
	Control: no serum	++++	++++	+++	++	++++	++++	+++	++	++++	++++	+++	++	++++	++++	+++	+++
	Precipitin test																
	Undiluted serum		++				++				++				0		
		Dilution of culture of group A type 19 streptococci															
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Type 19 infection	Bacteriostatic test																
	Undiluted serum	++++	++++	+++	9	++++	++++	+++	+	++±	+±	1	0	7	1	0	0
	Control: no serum	++++	+++	+++	+	++++	++++	+++	6	++++	++++	+++	++	++++	++++	+++	+
	Precipitin test																
	Undiluted serum		+				++				0				0		
		Dilution of culture of group A type 26 streptococci															
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Type 26 infection	Bacteriostatic test																
	Undiluted serum	9	0	0	0	+	0	0	0	++++	+++	++	1	++±	2	0	0
	Control: no serum	++++	++++	++	+	++++	+++	++	+	++++	++++	++±	+	++++	+++	++±	+±
	Precipitin test																
	Undiluted serum		0				0				+				0		

In bacteriostatic tests degree of growth is indicated on a ++++ to + scale; fewer than 10 colonies are represented in arabic numerals; 0 indicates no growth.
In precipitin tests the degree of precipitation is indicated on a ++++ to + scale; 0 represents no precipitation.

bacteriostasis. It is unlikely, therefore, that the antigen-antibody mixtures fixed complement sufficiently to inhibit bacteriostasis.

The next experiment was undertaken to investigate whether the observed antagonistic effect resulted exclusively from the precipitate. Preformed washed immune precipitates were introduced into a set of tubes; and the supernatant fluid in which one of the precipitates had formed was introduced into another set. These respective substances were added to the bacteriostatic systems in quantities equivalent to the amount of antigen extract added in the previous experiment.

Table III illustrates that interference with bacteriostasis took place whereever precipitate was added and that this inhibition was non-specific with respect to streptococcal types. In this experiment the C-anti-C precipitate formed small particles; but in another, not detailed here, large particles of comparable precipitate did not inhibit bacteriostasis. This suggested that the size of the particles was a significant factor in the underlying inhibitory mechanism. Moreover, the fact that bacteriostasis was not inhibited in the system containing supernatant fluid from which the precipitate had been removed indicated that the effect came from the precipitate and not from other products of the antigen-antibody combination. Furthermore, precipitates formed by mixing type-specific carbohydrate of pneumococcus types I and III with their respective hyperimmune rabbit sera also inhibited the bacteriostasis of the streptococci.

The Effect of Particulate Matter Other Than Antigen-Antibody Aggregates.—Suspensions of finely divided collodion, charcoal, India ink, and heat-coagulated human plasma and ascitic fluid particles were used in this experiment. The observations recorded in Table IV reveal that inert particulate matter other than the coagulated particles of plasma or ascitic fluid failed to inhibit bacteriostasis of the streptococci. Untreated normal human plasma or ascitic fluid was not antibacteriostatic. Particle size apparently did not play a rôle in the results of this experiment since all particles used were of approximately the same dimensions.

Preliminary Blocking of Leukocytes by Antigen-Antibody Mixtures.—An experiment to detect whether the combined action of antigen and antibody mixtures was on the white blood corpuscles is summarized in Table V. To different mixtures comprising 5 parts of normal whole blood and one part of serum from a patient recovering from a type 19 streptococcal infection was added one part of type 3, type 19, or type 26 M extract; and to control tubes physiological saline solution was added instead of M extract. This serum was known to form a precipitate with homologous M extract but not with type 3 or type 26 M. The tubes containing these mixtures were rotated for 30 minutes at 37° C. Blood films from the various samples at this stage of the experiment (Figs. 1 to 4) are described below.

TABLE II

Inhibition of Bacteriostasis by C Substance of Group A Streptococci Correlated with Formation of Precipitate

Convalescent serum from patient with type 6 infection	Dilution of C extract of group A streptococci																			
	1:60				1:120				1:240				1:480				1:960			
	Dilution of culture of group A Type 6 streptococci																			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Bacteriostatic test																				
Undiluted serum....	+	0	0	0	++±	4	0	0	+++±	+++±	++	+	++±	++	0	0	+	0	0	0
Control: no serum...	++++	+++±	+++	++±	++++	++++	+++	++	++++	+++±	+++	++	++++	+++	+++	+±	++++	+++	++++	+++
Precipitin test																				
Undiluted serum....	0				+				+±				+				0			

Same symbols for bacteriostatic and precipitin tests as used in Table I.

In both bacteriostatic and precipitin tests the controls without C substance gave same readings as tubes with 1:60 dilution of this substance.

TABLE III

Inhibition of Bacteriostasis by Addition of Preformed Precipitates or Supernatant Fluid

Convalescent serum from patient with type 19 infection	Precipitates from mixtures of antigens with homologous rabbit-antisera												Supernatant fluid after removal of precipitate from mixture of type 19 M extract and anti-M serum			
	Type 6 M extract + anti-M serum				Type 19 M extract + anti-M serum				C extract + anti-C serum							
	Dilution of culture of group A type 19 streptococci															
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Undiluted serum.....	++++	+++	+++	++	++++	+++	+++	++±	+++	+++	++	+	9	0	0	0
Control: no serum.....	++++	+++	+++	++	++++	+++	+++	++	++++	+++	+++	+	++++	++++	+++	+±

Same symbols for bacteriostatic tests as used in Table I.

Control without preformed precipitate or supernatant fluid gave same reading as tubes with supernatant fluid.

Following centrifugation of the tubes at 1,200 R.P.M. for 5 minutes, the supernatant fluid was discarded. The corpuscles were washed 3 times with 5 volumes of Locke's solution and resuspended in normal human plasma to the original volume of the whole blood; and one part of serum from a patient convalescent from a type 6 infection and one part of homologous bacterial cell dilution were added to 5 parts of the previously treated blood. The remainder of the experiment was carried out as previously described. (Figs. 5 to 9 are photomicrographs of blood films taken at completion of this experiment.) Table V shows that inhibition of bacteriostasis occurred only with mixtures that contained type 19 M extract and homologous serum. This was associated with the vacuolization of leukocytes illustrated in Figs. 7 to 9 and with the failure of these cells to phagocytize the streptococci in the bacteriostatic phase of the experiment, which thus permitted growth of the microorganisms.

Microscopic Appearance of Leukocytes from Various Systems in the Bacteriostatic Test—In stained preparations the leukocytes revealing the most marked changes in the presence of antigen-antibody precipitates were the polymorphonuclear leukocytes and the monocytes. The polymorphonuclear leukocytes (Figs. 3, 4, 7–9) were very edematous as compared with the non-vacuolated cell in Fig. 1; but the cell membranes were intact and the normal staining properties were retained. The greatest abnormality was the presence of different sized vacuoles throughout the cytoplasm which often forced the nucleus to the periphery (Figs. 3, 4, 7, and 8). Vacuoles were also found in monocytes (Fig. 2). Within some vacuoles there was ill defined light blue staining granular material (Figs. 3 and 4). Cells from systems containing streptococci but no precipitate showed well stained chains of cocci, also within vacuoles (Figs. 5 and 6). In experiments where preformed precipitates were used the material within the vacuoles was more readily recognized as precipitate (Fig. 10). When both precipitate and microorganisms were present, they were frequently observed in the same cells. Extracellular streptococci were rarely seen in films taken from samples which contained no precipitates (Figs. 5 and 6), but many extracellular cocci were observed in those with precipitates (Figs. 7 to 10) even though numerous cocci were adherent to the surface of the vacuolated cells (Fig. 8). The few eosinophils and basophils studied revealed no abnormal changes. An occasional cell, thought to be a lymphocyte, was seen which contained microorganisms or possible vacuoles but these cells may have been monocytes.

Ingestion of Streptococci and Precipitates by Living Leukocytes—Because the streptococci and ingested precipitates were found within the vacuoles of the leukocytes in stained preparations, living cells were studied to observe the phagocytosis of these substances. Dr. Michael Heidelberger kindly supplied us with a dye-protein R-salt azo-benzidine-azo-egg albumin in solution and an antiserum prepared against it (13). Sabin (14) used an alum-precipitated form of this colored antigen to study cellular reactions *in vivo*. When the dye-protein

TABLE IV

Effect on Bacteriostasis by Different Particulate Substances

Convalescent serum from patient with type 19 infection	Suspensions												Finely coagulated particles formed by heating							
	Collodion				Charcoal				India ink				Human plasma				Human ascitic fluid			
	Dilution of culture of group A type 19 streptococci																			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Undiluted serum	9	0	0	0	3	0	0	0	6	0	0	0	++±	++	++	+±	+++	++±	++±	++
Control: no serum	++++	+++	+++	++	++++	+++	+++	+±	++++	+++	++	++	+++	++	++	++	+++±	+++	+++	+++

Same symbols for bacteriostatic tests as used in Table I.

Control without particulate substance gave same reading as tubes with the collodion suspension.

TABLE V

Blocking of Leukocytes with Antigen-Antibody Mixtures before Bacteriostic Test

	Normal whole blood mixed for 30 min. with convalescent human serum (type 19 infection)			
	+ saline	+ heterologous type 3 M extract	+ homologous type 19 M extract	+ heterologous type 26 M extract
	After centrifugation, supernatant discarded; blood cells washed and resuspended in normal plasma to original volume of whole blood. These samples used in following bacteriostatic test			

Convalescent serum from patient with type 6 infection	Dilution of culture of group A type 6 streptococci															
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Undiluted serum	+±	1	0	0	++	7	0	0	++++	+++	+++	+++	++	5	0	0
Control: no serum	++++	+++±	+++	++	++++	+++	++±	++±	++++	+++	+++	++±	++++	+++	++±	++±

Same symbols for bacteriostatic test as used in Table I.

interacted with its antibody, a flocculent purplish-red precipitate formed which could easily be detected by its color. This provided a visible "tagged" precipitate for study with living cells. The precipitate was washed thoroughly with saline solution until the washing fluid was colorless; then was broken into very fine particles and suspended in saline solution.

Unstained wet preparations of mixtures of 5 parts of normal human blood and one part each of colored precipitate, convalescent human serum, and homologous streptococci were observed with a microscope placed in a warm box. When the leukocytes started to phagocytize colored particles, the progressive motion of these cells stopped and all cellular activity was apparently directed toward engulfing the "tagged" precipitate, which was soon segregated in vacuoles and separated by a clear zone from the cytoplasm. This zone widened as the precipitate became smaller, but the "tagged" precipitate was not seen to disappear completely. Some of the leukocytes which were phagocytizing the precipitate also engulfed streptococci, but once the cells had taken up a moderate amount of material, either precipitate or streptococci, further phagocytosis was not observed. No selectivity of the leukocytes for microorganisms or flocculent material was noted. It was clear, however, that during comparable periods more streptococci were engulfed in preparations without precipitate than in those with it. Control preparations with streptococci and colored antigen or antibody contained no flocculent granular material which could be interpreted as precipitate.

Supravital preparations made on slides stained with neutral red and Janus green showed that neutrophilic leukocytes ingested both the stained precipitate and microorganisms; but the functional activity of the lymphocytes could not be determined. Eosinophils did not take up precipitate and only rarely engulfed bacteria; basophils showed no phagocytic activity. The neutral red in the rosettes of the monocytes occasionally confused the picture and at times also obscured the precipitate.

From these studies of living cells it appeared that phagocytosis of the precipitates took place and that no predilection for the streptococci or flocculent material was observed. Adding precipitates to these systems increased the foreign material to be ingested, and complete removal of both the streptococci and the precipitates was impossible because of the limited number of leukocytes; therefore, the non-phagocytized streptococci were able to multiply.

DISCUSSION

The present data provide experimental evidence that both type-specific M substance and group-specific C carbohydrate have a definite antagonistic action on bacteriostasis when they form precipitates with the serum used in the test. In the absence of precipitate normal bacteriostasis is observed, a fact which suggests that the antigenic components in themselves cause no interference.

Although the inhibition of bacteriostasis by the M extracts in the presence of convalescent sera is not type-specific, and is associated only with formation of precipitate, the bacteriostasis of the streptococci, which depends upon sensitization with samples of the same sera, is type-specific, as has been demonstrated in the first report (10). In experiments, however, with the type-specific polysaccharide of the pneumococcus, the inhibition produced is specific with respect to the pneumococci employed (4, 15, 16), as might be inferred, because a precipitate is formed only by the interaction of the type-specific polysaccharide and its homologous antiserum. On the other hand, the lack of specificity of the M extract in inhibiting bacteriostasis is correlated with the many cross-reactions observed in precipitin tests with these extracts and human convalescent sera. Cross-reactions by the M extracts may be explained on the basis that they probably also contain non-type-specific substances which precipitate corresponding antibodies in the patients' sera. That inhibition of bacteriostasis is merely associated with the formation of precipitates is supported by the observation that the test is also inhibited by washed preformed precipitates resulting from antigen-antibody combinations from extracts of various microorganisms and their respective antisera, as well as by finely divided coagulated particles of human plasma or ascitic fluid.

Under certain conditions, the particles of C-anti-C precipitate are large and do not cause interference, but finely divided suspensions of the same material cause inhibition of bacteriostasis. The failure of collodion, India ink, or charcoal suspensions to inhibit bacteriostasis cannot be explained on the basis of particle size. The observations of Lucké *et al.* (17), who reported that macrophages or mononuclear cells phagocytize collodion particles far better than do microphages or neutrophilic leukocytes, perhaps explain why these suspensions did not interfere with the phagocytosis of the microorganisms. This assumption appears logical since the blood cell mixtures in the present study contained approximately 10 polymorphonuclear leukocytes to one monocyte.

Because no antibacteriostatic effect was exerted by the clear supernatant fluid from the antigen-antibody mixtures after the precipitate had been removed, and because the washed precipitates interfered markedly with the bacteriostasis of streptococci, it appeared that the interference was produced by the immune precipitate alone and not by other products of the interaction of antigen and antibody.

In the second paper of this series it was shown that leukocytes, complement, and a relatively heat-stable factor of normal human blood were essential for bacteriostasis under the conditions of these experiments. Since it had been demonstrated that precipitates interfere with bacteriostasis, it was desirable to determine on which of these components the precipitates acted. Reduction of complement by the precipitate did not explain the interference phenomenon because enough complement still remained in the systems to promote bacterio-

stasis. Similar observations and interpretations were reported by Ward on studies of phagocytosis of pneumococci (18).

That the antagonistic effect was on the leukocytes and not on any serum component was best demonstrated by treating leukocytes in the preliminary phase of an experiment with antigen-antibody mixtures which formed precipitates. These cells were then washed thoroughly and resuspended in untreated normal human plasma, but they failed to phagocytize the streptococci in homologous serum, while leukocytes treated with antigen-antibody mixtures that formed no precipitate, phagocytized the streptococci. Microscopic studies of stained films taken after the cells were mixed with homologous antigen-antibody combinations revealed enlarged vacuolated leukocytes and confirmed Cromwell and Centeno's observations (7). Further studies at the completion of the test showed that the vacuolated cells had not taken up the bacteria. When preformed precipitates were used, it was possible to demonstrate phagocytized precipitate within the vacuoles of neutrophilic leukocytes and monocytes.

Studies of living cells indicated that the inhibition of bacteriostasis was due to blocking of the leukocytes by the precipitates, which thus prevented complete phagocytosis of the bacteria by the limited number of polymorphonuclear leukocytes and monocytes in the systems during a 3 hour period. It is not evident from these data whether during a longer period the cells would have been able to phagocytize the streptococci after the engulfed precipitate had been digested.

By analogy these data suggest that leukocytes of circulating blood may phagocytize precipitates formed by the union of antigen and antibody in the tissues. The work of Opie (19) indicates that such a phenomenon evidently takes place, since he observed that injected precipitates were strongly chemotactic for leukocytes in animal tissues. In infected tissues of the body where the blood supply is diminished, the blocking of leukocytes by precipitates resulting from the combination of precipitinogen, liberated by the growth of the organism and precipitin in the serum from a past infection or other cause, would probably favor the spread of the local lesion.

In the light of these findings and those of others, it is therefore probable that the antiopsonic, or antiphagocytic effects reported by earlier workers were the result of the formation of precipitates which filled the leukocytes and blocked further phagocytosis.

SUMMARY

1. Type-specific M extracts and group-specific C carbohydrate of group A streptococci inhibited bacteriostasis of these microorganisms in the presence of normal whole blood and of sera from patients convalescent from streptococcal infections. The inhibition was not specific with respect to streptococcal types and depended merely on the formation of precipitates in the system. The extracts had no antagonistic action in themselves.

2. Preformed precipitates derived from the interaction of an antigen and its homologous antibody or from finely divided coagulated particles of human plasma or ascitic fluid also interfered with the bacteriostasis. The supernatant fluid in which one of these precipitates was formed did not inhibit bacteriostatic activity; therefore, it seems that other possible products of the antigen-antibody reaction were not inhibitory. The relative size of the precipitate particles was a conditioning factor since small particles of one precipitate inhibited bacteriostasis, but large ones of the same precipitate failed to do so.

3. Stained films of blood cells treated with antigen-antibody mixtures which formed a precipitate revealed large cytoplasmic vacuoles containing precipitates in the polymorphonuclear leukocytes and monocytes; such engorged cells subsequently failed to phagocytize streptococci in homologous serum. Blood cells treated in the same manner, except that the antigen-antibody mixtures formed no precipitate, contained no vacuoles, and these cells were able to phagocytize the streptococci.

4. Leukocytes studied in the living state in the presence of colored precipitate and streptococci sensitized by convalescent human serum showed unselective phagocytosis of both precipitate and bacteria. The capacity of these leukocytes to ingest material however was limited.

5. As a result of non-selective saturation of their phagocytic capacity in the bacteriostatic systems containing both streptococci and precipitate, the limited number of leukocytes phagocytized only a fraction of the streptococci; consequently the remainder were able to multiply.

We greatly appreciate the technical assistance of Miss Grace Vanderhoff during the course of these investigations.

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EXPLANATION OF PLATES

PLATE 5

All films stained with Wright and Giemsa solutions. × 1000.

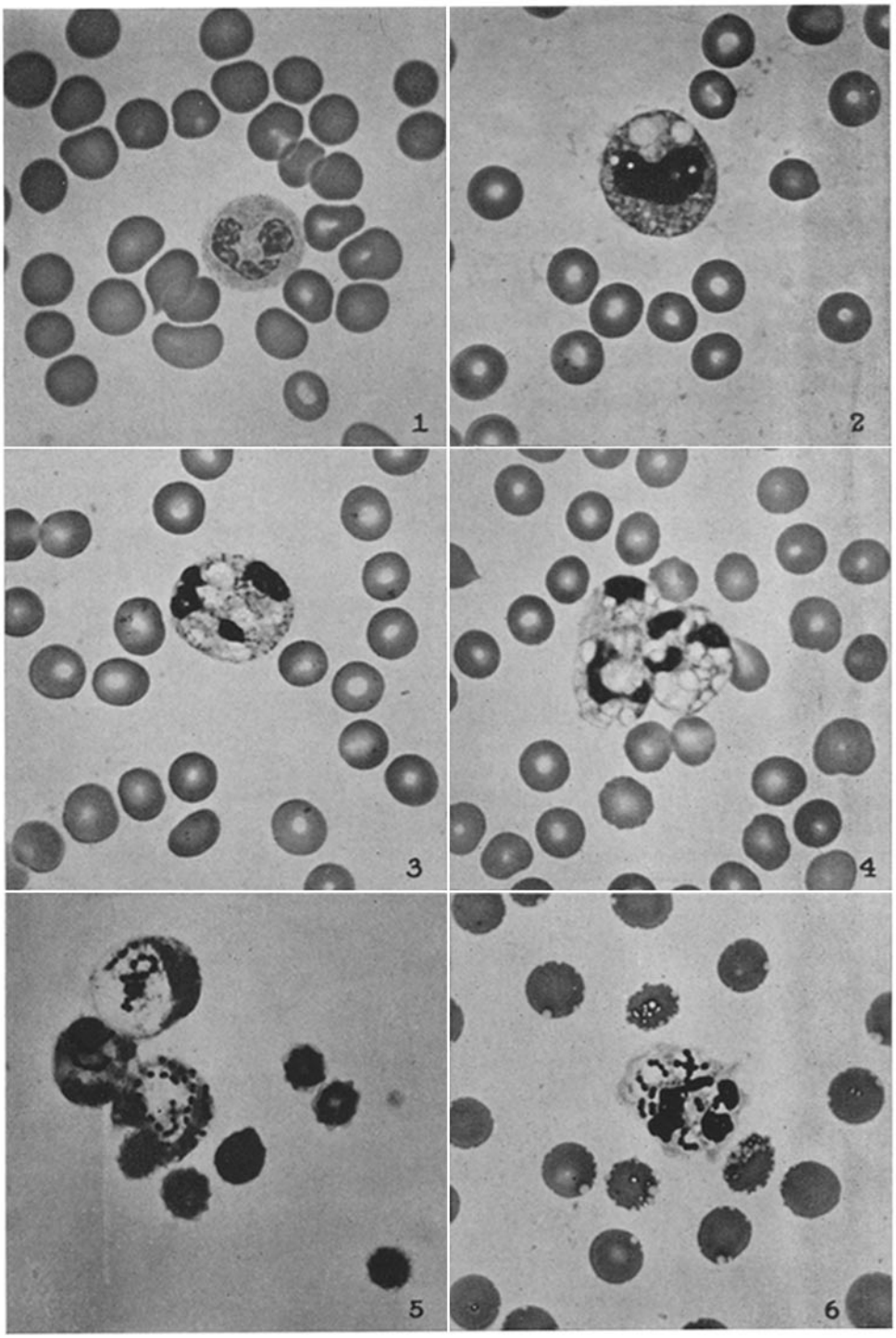
The photographs were made by Mr. Joseph B. Haulenbeek.

FIG. 1. Normal polymorphonuclear leukocyte in a system containing serum from a patient convalescing from a type 19 streptococcal infection, type 26 M extract, and normal human whole blood after 30 minutes rotation at 37° C. No precipitate was formed by interaction of this serum with the type 26 M extract.

FIG. 2. Vacuoles in the cytoplasm of a monocyte in a system containing serum from a patient convalescing from a type 19 streptococcal infection, homologous M extract, and normal human blood after 30 minutes rotation at 37° C. Precipitate was formed by interaction of this serum with the homologous M extract.

FIGS. 3 and 4. Extensive vacuolization of polymorphonuclear leukocytes taken from the same system as the monocyte shown in Fig. 2. The shadows of the ingested precipitate can be seen within some of the vacuoles in these cells.

FIGS. 5 and 6. Polymorphonuclear leukocytes containing streptococci within cytoplasmic vacuoles. These leukocytes were taken from the same system as that in Fig. 1. They had been washed, resuspended in normal human plasma to the original volume of whole blood, and rotated at 37° C. for 3 hours in a second system containing serum from a patient convalescing from a type 6 infection and homologous streptococci.

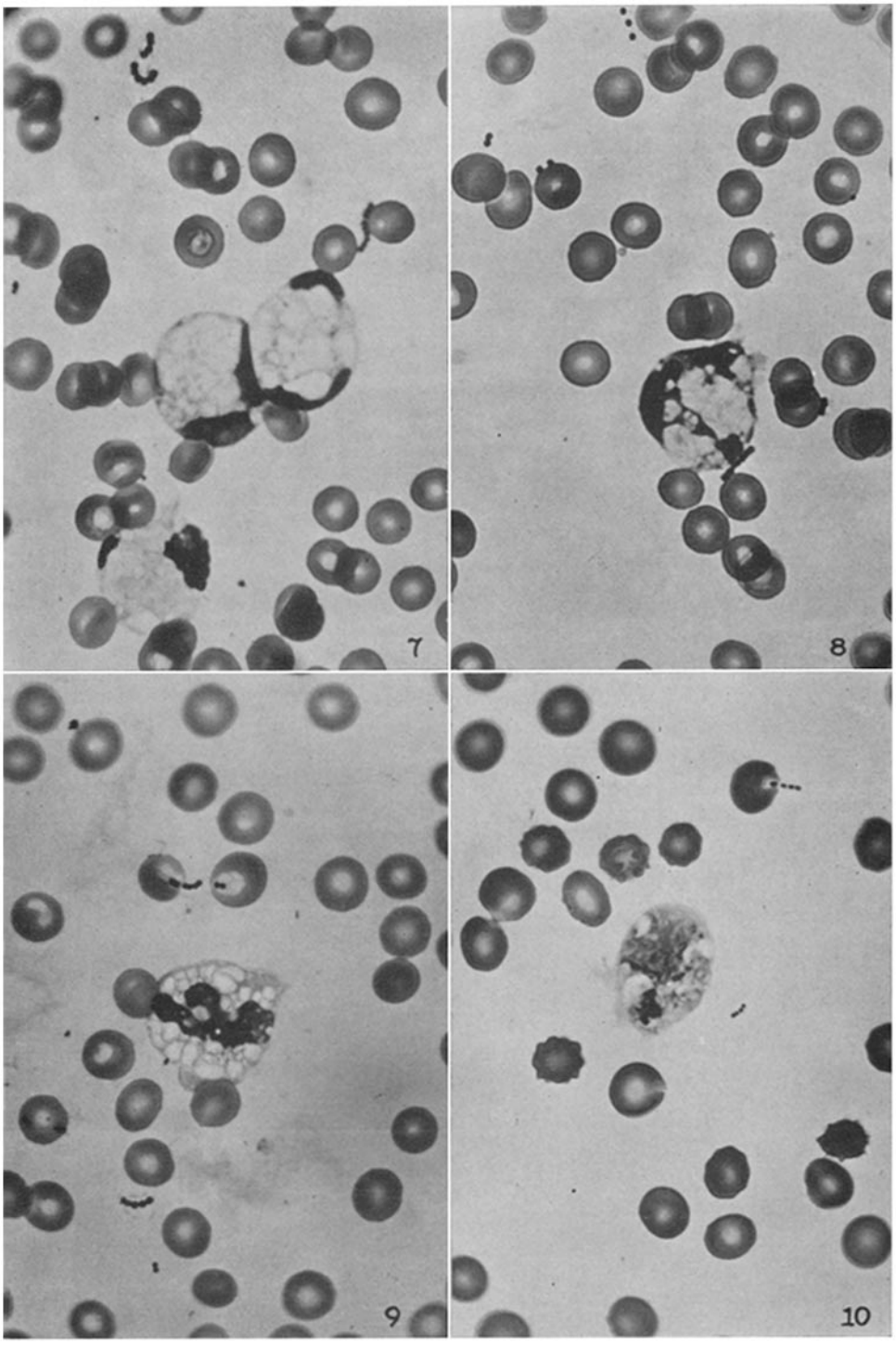


(Rothbard: Effect of human sera on group A streptococci. III)

PLATE 6

FIGS. 7, 8, and 9. Vacuolated polymorphonuclear leukocytes showing shadows of precipitate within the vacuoles and unphagocytized chains of streptococci. In Fig. 8, note streptococci adherent to the surface of the cell. In Fig. 9, extracellular precipitate is clearly visible. These cells were taken from the same system as the leukocytes shown in Figs. 2 to 4. They had been washed, resuspended in normal human plasma to the original volume of whole blood, and rotated at 37° C. for 3 hours in a second system containing serum from a patient convalescing from a type 6 infection and homologous streptococci.

FIG. 10. Preformed precipitate visible as irregularly shaped, dark staining material within a vacuole in a polymorphonuclear leukocyte. The nucleus has been forced to the lower portion of this cell and is not clearly outlined. Intracellular and extracellular streptococci may be seen. This cell was taken from a system containing serum from a patient convalescing from a type 19 streptococcal infection, homologous streptococci, and normal human whole blood; to this system has been added preformed washed precipitate produced by the interaction of type 19 M extract and homologous rabbit antibody.



(Rothbard: Effect of human sera on group A streptococci. III)