STUDIES OF GONOCOCCAL INFECTION. II. THE BACTERIOLYTIC POWER OF THE WHOLE DEFIBRINATED BLOOD OF PATIENTS WITH GONOCOCCAL ARTHRITIS [1]

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In the previous paper (1), we presented evidence that the bactericidal action of whole defibrinated blood for the gonococcus was the same as that of plasma or serum. It was demonstrated that phagocytosis of the gonococcus by the polymorphonuclear leukocytes played little or no part in the destruction of the organisms *in vitro*. The experiments supported the conclusions that the gonococcus was killed *in vitro* by bacteriolysis, and that this was accomplished by a sensitization of the organisms by antibody; the lysis was completed by the action of complement. There was no evidence that intracellular digestion of the organisms by leukocytes took place, in spite of the fact that active phagocytosis could be demonstrated.

We then proceeded to study the variations in the bactericidal action of the whole defibrinated blood of 29 patients with gonococcal infections. Sixteen had local infections, notably urethritis, endocervicitis, or conjuctivitis, and the remaining 13 individuals had gonococcal arthritis.

METHODS

The bactericidal action of whole blood was determined according to the method described in the preceding paper (1). A control was included at the time of each test so that the patient's blood could be compared with the blood of a non-infected individual. The same control was always used for a given patient. In all, there were 210 tests carried out on patients and 220 on controls. For purposes of discussion we have divided the cases into those with local lesions and those with arthritis. In order that the bacteriolytic power of a patient's blood could be determined against his own strain bacteria as well as other strains, the tests in some patients were carried out with more than one strain of gonococci. The same procedure was carried out with different controls.

The results are recorded in accordance with the number of organisms killed by 0.5 cc. of blood. That is to say, if there was growth in the tube containing 0.1 cc. of a 10^{-1} dilution of the culture and none in the tube containing 0.1 cc. of the 10^{-2} dilution, it was first recorded as killing power in 10^{-2}; then the number of organisms was calculated from the quantitative cultures in the dilution of 10^{-6}. For example, if 1 cc. of a 10^{-6} culture contained 10 organisms, then the number contained in the 0.1 cc. of the 10^{-2} dilution would be 10,000 organisms.

RESULTS

Bacteriolytic titer of the whole defibrinated blood of patients with local lesions and arthritis

1. Controls. Patient's organism versus whole defibrinated blood of controls. One-half a cubic centimeter of a normal individual's whole blood was mixed with different dilutions of a 24-hour ascitic fluid broth culture of the gonococcus isolated from the patient and incubated as described under methods of study. The same individual was used as a control every time the patient's blood was studied. In this way a number of observations were obtained that permitted one to determine the fluctuation of the titer of the normal blood from time to time, and to compare the bacteriolytic action of non-infected individuals with that of patients with infections. We also studied the bacteriolytic action of the same blood against several different strains. In addition, in some of the cases, the bacteriolytic action of several controls was tested for the same organism. This was done in order to determine the difference in the bacteriolytic titer of the blood of various normal individuals for the same strain. It was found that the titer of the normal individual's

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blood did not fluctuate more than a 100-fold dilution, and in many instances it was either constant or varied less than 100-fold. There was evidence, however, that the titer varied for individual strains. The results are summarized in Tables I and II. From Table I, it can be seen that the blood of normal individuals, when tested against the majority of strains of gonococci, could only kill relatively small numbers of organisms, although there were a few susceptible strains of which more than 10,000 were killed by 0.5 cc. of blood. That this was due to the variations in the strains of organisms rather than variations in the activity of blood from different individuals is borne out by the information summarized in Table II. Here, it is ascertained that the variations in the bacteriolytic power of the blood of the different controls for single strains was slight. There were a few exceptions, but from these observations it would seem justifiable to say that control bloods were able to kill varying numbers of gonococci, depending upon the strain and the individual. In general, the control bloods, 0.5 cc., were able to kill less than 100 organisms of 65 per cent of the strains tested. It was also found that the controls were able to kill more organisms derived from patients with local lesions than those obtained from patients with arthritis. Insofar as one can determine from experiments of this kind, it may be contended that certain normal individuals possess natural bacteriolysins against some strains of gonococci. It cannot be maintained

TABLE I

Maximum number of gonococci killed by 0.5 cc. of blood from controls

Control number	Number of strains studied	Less than 100	100 to 10,000	Over 10,000
1	25	17	6	2
2	11	7	2	2
3	8	8	0	0
4	5	4	1	0
5	4	4	0	0
6	4	3	1	0

from such studies alone, however, that the organisms vary in their ability to invade the tissues freely, although it is suggestive that strains which cause arthritis are less often killed in large numbers by normal individuals.

2. Patient's organism and whole defibrinated blood of patients. When the bacteriolytic titer of the patients' blood was studied in relation to the organism that had been isolated from them, it was found that the number of organisms that were killed by the blood varied considerably. The results can be discussed more easily by dividing the cases into two groups: *A*, those with local lesions; *B*, those with arthritis.

TABLE II

Maximum number of gonococci killed by 0.5 cc. of blood from various controls when the same strain was used

Strains	Controls					
	1	2	3	4	5	6
1	10	25		25	1	7
2	10	3	100	8	3	3
3	18	7	300	7	18	2
4	1,400	35	35	7	18	2
5	400,000	97,000			9,700	9,700
6	1	25				
7	700	150				
8	6		5			
9	6		3			
10	400		800			
11	1,000		10			
12	100,000		40,000			

A. Patients with local lesions. The results of the studies of the bacteriolytic titer of the blood are shown in Figure 1. The maximum number of organisms that were killed by the patient and the controls during the period of observation are charted. Specimens of blood from five of the sixteen patients were capable of killing many more organisms than were those from controls, whereas in the remaining eleven instances the titers of specimens from the patients and controls were approximately the same. These observations indicate that a local infection may be caused by an organism that can be killed in large numbers by normal, non-infected individuals. Moreover, when an infection takes place with an organism which cannot be killed in large numbers by normal individuals, it is common to observe an increased bacteriolytic titer of the patient's serum as the disease advances. In Figure 2 the bacteriolytic titer of the blood of 3 patients with local lesions is charted.

B. Patients with arthritis. When the blood of patients with arthritis was studied and the results charted (Fig. 3), it was found that 8 of the 13 patients had a higher bacteriolytic titer than did

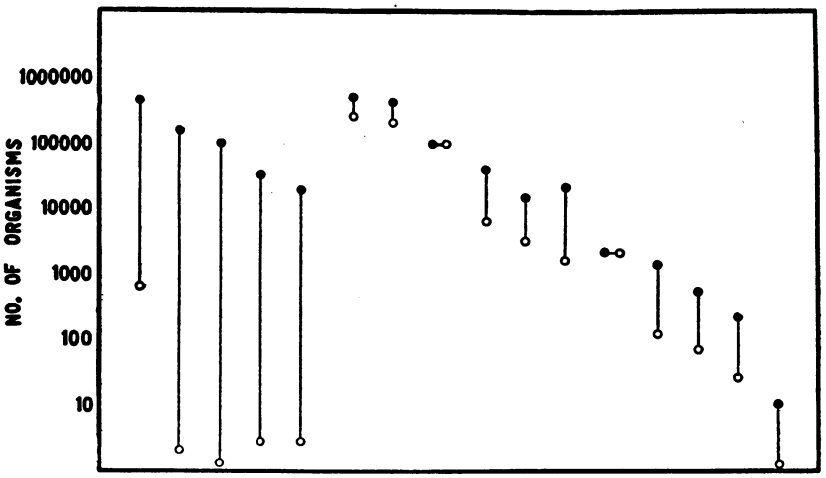

FIG. 1. THE MAXIMUM NUMBER OF ORGANISMS KILLED BY 0.5 CC. OF WHOLE DEFIBRINATED BLOOD DURING THE NATURAL COURSE OF LOCAL GONOCOCCAL INFECTIONS

The dots represent the maximum number killed by the patients, the circles the maximum number killed by normal controls. Each specimen of blood was tested against the organism derived from the patient.

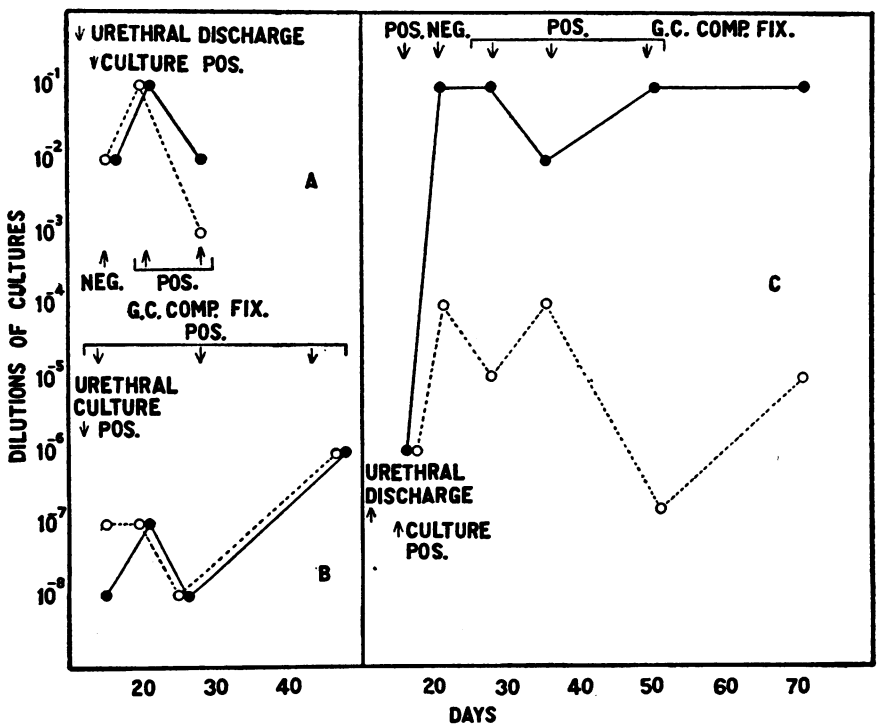

FIG. 2. THE BACTERIOLYTIC TITER OF THE BLOOD IN 3 PATIENTS WITH GONOCOCCAL URETHRITIS

○----------○ Control.
●——————● Patients.

the controls, the remaining 5 were unable to kill many more organisms than the controls. It is worthy of comment, however, that 0.5 cc. of blood from our controls was incapable of killing more than 10,000 organisms. When organisms from urethritis were studied, it was found that a number of controls were able to kill more than 10,000 of these organisms. This is emphasized more clearly in Table III. This table was constructed from the results of 210 bactericidal tests in 29 patients and from 220 tests in a group of controls. The bacteriolytic power shown in the table represents the maximum number of organisms that were killed during the course of the disease. The bacteriolytic power of the patient's blood was determined against his own strain. The same control was always used for a given patient and the

TABLE III

Maximum number of gonococci killed by 0.5 cc. of blood from patients and controls

	Total number	Less than 100	100 to 10,000	Over 10,000
Patients with arthritis	13	0	8	5
Control observations	13	10	3	0
Patients with local lesions	16	2	4	10
Control observations	16	9	5	2

killed by normal control plasmas. It is evident, then, that an active arthritis may be present with or without a high bacteriolytic titer, but it would seem that the organisms in patients from arthritis are more likely to be resistant to destruction by the blood of normal individuals.

We have already stated that the bacteriolytic

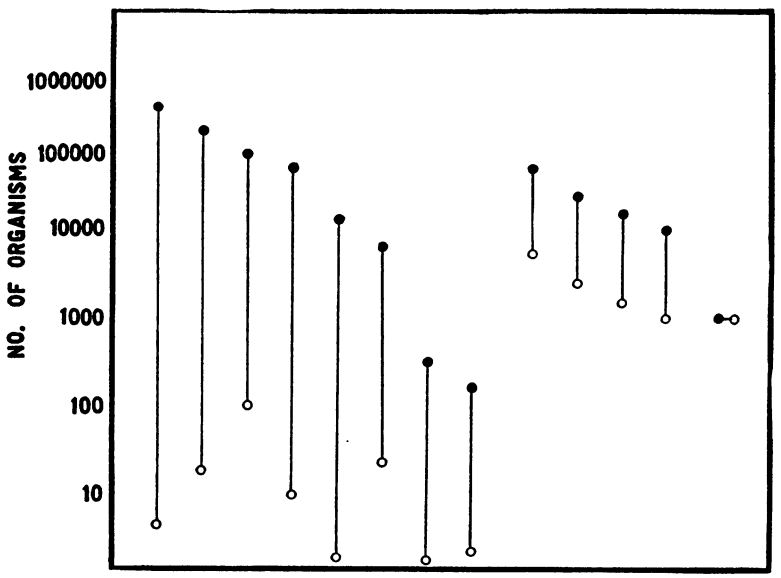

FIG. 3. THE BACTERIOLYTIC TITER OF THE WHOLE BLOOD IN PATIENTS WITH GONOCOCCAL ARTHRITIS. THE MAXIMUM NUMBER OF ORGANISMS KILLED BY 0.5 CC. OF WHOLE DEFIBRINATED BLOOD DURING THE NATURAL COURSE OF GONOCOCCAL ARTHRITIS

The dots indicate the maximum number killed by the patients, the circles, the maximum number killed by normal controls. Each specimen of blood was tested against the organism derived from the patient.

organism was derived from the patient. From the results shown in Figure 3 and Table III, it is fair to say that patients with gonococcal arthritis usually have a higher bacteriolytic titer in the blood plasma than do normal controls. Moreover, very few organisms of these strains are

titer of the blood plasma increased during the course of the illness in some patients with either local infections or arthritis, and while this titer may be maintained for several weeks, it frequently falls off gradually (Fig. 4). It was of interest, therefore, to compare the titer of the

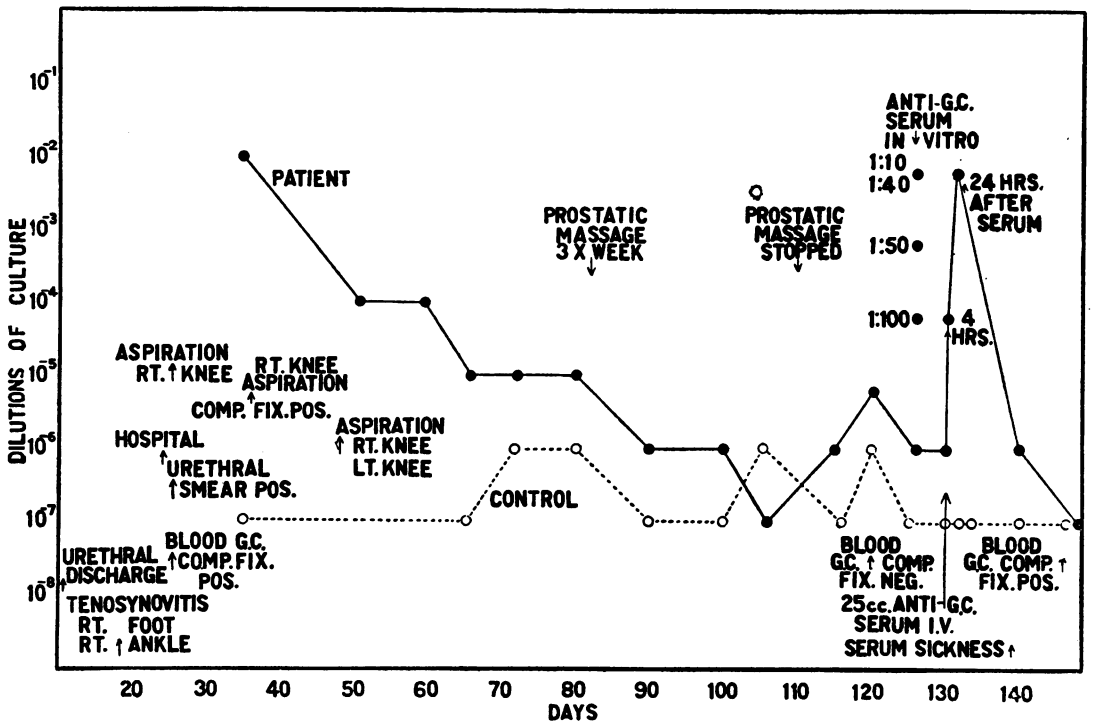

FIG. 4. THE COURSE OF THE BACTERIOLYTIC TITER OF THE WHOLE DEFIBRINATED BLOOD IN A PATIENT WITH GONOCOCCAL ARTHRITIS, SHOWING THE EFFECTS OF INJECTING SERUM INTRAVENOUSLY

blood from several patients against their own organism and against strains derived from other patients. The pertinent information is tabulated in Table IV. The patients' blood was capable of killing many more of their own organisms than other strains. There were, however, several exceptions. Strain number 5 was obtained from a case of urethritis, and specimens of blood from all of the controls and patients were able to kill large numbers of these organisms. It appears, then, that the immune reactions tend to be specific, although there are some virulent strains which are killed in large numbers by blood from normal as well as from individuals infected with gonococci.

TABLE IV

*Comparison of patient's bacteriolytic titer against his own and other strains of gonococci**

Patient number	Own strain	Other strains				
		1	2	3	4	5
1	500	720	140			20,000
2	13,000	6,000		3	96	200,000
3	720,000		1	3		200,000
4	510,000	7	130	6		
5	10	35	3	0		97,000
6	70,000	3	7	2	5	3,100
Controls		2	3	3	6	400,000

* The number of organisms indicates the maximum number killed in 0.5 cc. of whole blood.

Complement fixation

Repeated complement fixation reactions were done on the blood serum of the patients. Our previous experience (2) indicated that the gonococcal complement fixation test was of value in the diagnosis of gonococcal arthritis, inasmuch as it was positive in 85 per cent of the cases studied. In this study, it was found to be positive in 11 of the 16 patients with local infections and in 10 of 15 patients with arthritis.

The reactions were positive in some patients as early as 7 days after the onset of the signs of acute infection, and in a few it appeared before there were any signs of an increased bacteriolytic titer in the blood. In several it was possible to demonstrate a positive complement fixation reaction when the bacteriolytic titer failed to increase. The two reactions did not parallel each other, but it was not uncommon to find a positive

complement fixation test in a patient who was infected by a strain that could be killed in large numbers by the blood of controls. It is indicated from our observations that this test was helpful in diagnosis, but it was of little value in assessing the immune reactions in a quantitative fashion.

Once the complement fixation test becomes positive it may remain so for as long as 3 months. Indeed, we have observed some cases in which it was positive one year after an acute arthritis had subsided.

Agglutination reactions

The blood serum from the patients was tested for agglutinins against homologous organisms at various intervals of time during the course of the disease. In no case did we observe the appearance of agglutinins. Thus, the use of the agglutination test as a means of diagnosis or as a method for the study of immune reactions in patients was of no assistance.

Titration of complement in patients' blood

In the previous paper (1) referred to, it was demonstrated that complement was necessary for the lysis of organisms after they had become sensitized by the antibody. The titer of complement was determined in the blood serum of patients with gonococcal arthritis. The purpose of this was to detect fluctuations from the normal. In other studies of complement we found that normal individuals did not show fluctuations of more than 0.1 cc. of complement on repeated examinations although the differences in titer from one individual to another were greater than 0.1 cc. The complement titer varied from 0.02 to 0.09 cc. in all examinations but one. In this instance it was 0.2 cc. Moreover, during the course of the disease, the widest fluctuation in any single case was from 0.2 cc. to 0.07 cc. It is clear, then, that there is an adequate amount of complement present in the blood of people with gonococcal infections to complete the lysis of organisms, provided antibody is available.

DISCUSSION

From the data presented, there is a fair amount of evidence that the blood of normal individuals is capable of destroying varying numbers of some strains of gonococci isolated from different types of gonococcal infections. It is more common for the blood of normal individuals to destroy large numbers of organisms obtained from local lesions than it is to kill large numbers of bacteria isolated from patients with arthritis. Observations of this kind would indicate that some strains are more invasive than others and while invasiveness cannot be correlated entirely with the absence of antibodies in the circulating blood, it does not appear unlikely that the presence or absence of bacteriolytic antibody in the blood is a factor of importance. It should be added, however, that infection with an organism that can be killed in moderate numbers by the blood of normal individuals may be followed by arthritis, but, in our experience at least, it was somewhat more common to observe arthritis when the organisms were not killed in large numbers by the blood of normal individuals. Attention should be called to the fact that active infection of the urethra can continue in the presence of an excellent bactericidal titer of the blood; this may also be said of the cases in which the joints are involved.

When patients with arthritis and those with local lesions without arthritis are studied for differences in bacteriolytic titer, it becomes manifest that those with arthritis develop a higher titer for their organism more often than do those with only a local lesion. This is what might be expected, since in other infections it is the rule to observe higher bactericidal power in the blood when metastatic lesions are present.

The complement in the blood of patients with gonococcal arthritis was at the same level as it is in normal individuals, so that there is no evidence of a lack of this substance which is so important in the antigen-antibody reaction. The complement fixation test was found to be of aid in diagnosis but it could not be correlated with the titer of the bacteriolytic test. Information was obtained indicating that the complement fixing antibodies appeared earlier than did other antibodies.

The agglutination reaction was negative in all cases and was, therefore, of no aid in diagnosis.

It is not possible, from these studies alone, to state the relative importance of bacteriolysins and local tissue reactions in the mechanism of recovery from gonococcal arthritis. The factors that localize the organisms, suppress their growth, and

finally destroy them are probably multiple. There can be no question, however, that active infection may go on in the face of a high bacteriolytic titer of the plasma, and it is also obvious that invasion from the local focus does not always take place when one fails to demonstrate bacteriolytic substances in the blood. When bacteriolysins are present in the blood serum they undoubtedly aid in the destruction of organisms and probably assist in the localization of infection. The process of lysis is certainly operative to some extent when the organisms localize in an area that can be reached by the blood plasma in large amounts, such as occurs in the case of arthritis. The precise mechanism for the destruction of organisms in various tissues remains obscure. The recent important studies of McMaster and Hudack (2) on the local formation of antibodies in lymph nodes are of significance in emphasizing the importance of antibody formation in tissues before they can be demonstrated in the circulating blood. Such observations suggest, at least, that the destruction of organisms in some tissues is due in part to the development locally of specific antibodies. There is no doubt that the appearance of antibodies in the circulating blood can be taken as an indication of their formation elsewhere and of their existence in some tissues. There is abundant evidence that a part of immunity to infections depends on specific antibodies and that these antibodies have a profound effect on local cellular reactions. It is not possible to say at present how much of a rôle fixed antibody in the tissues plays in the defense mechanism in gonococcal infections; but we have no reason for assuming that it does not play a part since recovery from local infections may occur without the demonstration of specific bacteriolysins in the circulating blood. For the above reasons, it does not seem improper to regard the presence of bacteriolysins as an immune response of significance in the destruction of organisms, in spite of the fact that their relative importance in the mechanism of recovery cannot be evaluated with precision at the present time.

In order to explain the appearance of arthritis in cases of gonococcal infection, it is necessary to understand how the organisms invade and why they survive in the synovial tissues. The present investigation suggests that invasion of the tissues is dependent in part upon: 1, the virulence of individual strains; 2, the antibody content of the host's blood. There are undoubtedly other factors which are not well defined, notably the effects of local inflammation and the rôle of the leukocyte in the fixation of the infection. Why the organisms seem to survive in the synovial cavities will be taken up in a subsequent paper.

SUMMARY AND CONCLUSIONS

1. The whole blood from normal individuals is capable of destroying varying numbers of gonococci obtained from patients with infection. Strains of gonococci obtained from local lesions, such as urethritis, are often killed in larger numbers than are those derived from patients with arthritis.

2. Destruction *in vitro* occurs by lysis and this is a function of the blood plasma and not of the polymorphonuclear leukocytes.

3. During and following the course of a gonococcal infection, especially arthritis, there is evidence of an increase in the bacteriolytic titer of the blood plasma. This is an immune response that can be regarded as an aid in the destruction of the organism.

4. Patients tend to develop a higher bacteriolytic titer in their serum against their own organism than against other strains.

5. The gonococcal complement fixation reaction is a valuable method in the diagnosis of arthritis caused by the gonococcus.

6. The titer of complement of the blood serum is not depressed during the course of gonococcal arthritis.

7. Agglutination tests were of no value in diagnosis since in our experience they were invariably negative.

8. The possible significance of bacteriolysins in gonococcal infections is discussed.

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