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Research Article

A STUDY OF THE RELATIONSHIP OF THE NORMAL BACTERICIDAL ACTIVITY OF HUMAN SERUM TO BACTERIAL INFECTION *

By ROBERT J. ROANTREE † AND LOWELL A. RANTZ

(From the Department of Medical Microbiology and Department of Medicine, Stanford University School of Medicine, Stanford, Calif.)

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The potent heat-labile bactericidal property of fresh mammalian defibrinated blood or serum *in vitro* has been known since the studies of Nuttall (1) and Buchner (2) over 70 years ago. By 1928, it had been shown that serum components very like those of complement were necessary for this action (3). Although a number of investigators in the fields of bacteriology and immunology have interested themselves in this bactericidal power of fresh serum, little notice has been taken of it in clinical medicine, probably, as noted elsewhere (4), because it has been believed to be of doubtful effect *in vivo*.

Interest has been revived in the bactericidal activity of serum by the work of Pillemer, Wardlaw and associates (5, 6). They described a normally occurring serum protein of large molecular weight which they named properdin. They attributed the bactericidal property of serum, in part at least, to the presence of properdin, which acted with the four components of complement in the presence of magnesium to kill the bacteria.

The findings of Wardlaw and Pillemer (6) confirmed those of Mackie and Finkelstein (7) that the bacterial strains sensitive to the effect of serum are principally from species of gram-negative bacilli and that the degree of sensitivity is a characteristic of the strain rather than of the genus. However, whereas Wardlaw and Pillemer have described the action of the properdin system as being nonspecific and unrelated to antibody, Mackie and Finkelstein presented data showing that the absorption of a serum in the cold by a sensitive strain resulted in the removal of the killing effect only for that strain, leaving the effect against other strains intact. The question as to whether the complement-dependent bactericidal effect depends wholly upon a system including properdin or one including an antibody-like substance, or whether it may depend partially on both, is not as yet decided.

The belief that the properdin system plays a role in the native resistance of animals to bacterial infection is based upon the finding that an induced decrease in the serum properdin level of laboratory animals is associated with reduced resistance to bacterial challenge, while increased properdin levels correlate with increased resistance (8–10).

What relationship such changes in properdin level *in vivo* bear to the bactericidal effect of serum *in vitro* is unclear. Nearly all the work on properdin levels and resistance to bacterial challenge has utilized the mouse, an animal whose serum is nonbactericidal *in vitro* owing to the lack of the second component of complement (11, 12). Studies using human sera *in vitro* have indicated that properdin levels of 1 to 10 units per ml. of serum show an equal and optimal activity, while increasing the properdin above this range leads paradoxically to a lesser bacterticidal activity (6). Although properdin levels less than 1 unit per ml. have been reported in human disease, it has not been stated whether these levels altered the bactericidal power of the serum (13).

The present study was initiated to determine whether a lessened bactericidal activity of undiluted serum for gram-negative bacilli could be demonstrated using sera from patients with serum protein abnormalities or with diseases commonly associated with bacterial infections. It soon became clear that such a lack of bactericidal effect rarely, if ever, played a role, and that once the sensitivity of a given bacterial strain was determined to any individual serum, it had a very similar sensitivity to the great majority of other human sera, whether from normal or pathological

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subjects. Thus all bacterial strains used could be classified as to their degree of serum sensitivity. The problem as to whether strains resistant to the killing effect of serum were more often cultured from the blood of bacteremic patients than might be expected was then investigated.

MATERIALS AND METHODS

Serum. The human blood specimens used in this study were collected aseptically, allowed to stand at room temperature for about an hour, rimmed and centrifuged. The sera were frozen at −20° C. in 6.5 ml. amounts. The whole procedure was completed within two hours of the time of bleeding.

Culture medium. Peptic digest of liver broth was employed as a medium for preparing frozen stock cultures and for the 20 hour cultures used for inoculation. This broth was prepared by mixing 1,000 Gm. ground hog's liver, 1,000 Gm. ground hog's stomach and 100 ml. of concentrated HCl in 10 L. of distilled water and digesting at 50° C. for 18 to 24 hours. This mixture was boiled for five minutes and filtered through cotton. Four Gm. KH_2PO_4, 3 Gm. glucose and 0.1 Gm. *p*-amino benzoic acid (PABA) were added per L. of this filtrate and the pH was adjusted to 7.6 by the addition of 20 per cent NaOH. This 0.3 per cent glucose peptone-rich broth with a slightly alkaline pH includes the main requisites of a medium in which a bacterial strain can develop its maximum resistance against the heat-labile bactericidal effect of normal serum (14, 15).

Bacteria. The bacterial strains used in the study, with the exception of the standard *Shigella dysenteriae,* were isolated from various human sources by the Infectious Disease Laboratory of Stanford University Hospitals. They were taken from single colonies of the original isolation, grown overnight in peptic digest of liver broth, and then this culture was divided into the desired number of samples and frozen at −20° C. The standard *Shigella dysenteriae* was a laboratory strain agglutinating specifically with antiserum to its species. It was selected because previous workers had used this species (6) and because the sera of few patients in the San Francisco area possess agglutinins against it. It was grown for 24 hours in 150 ml. of peptic digest of liver broth. This culture was divided among 125 test tubes and frozen at −20° C. for use as starter culture for each of the tests to be performed with it.

All strains used were of smooth colonial form and were able to remain in uniform suspension in 0.85 per cent saline at 37° C. for a 24 hour period.

The test for bactericidal activity of serum used throughout this study was set up as follows: A 24 hour broth culture of the chosen test organism was diluted in physiologic saline solution by serial 10-fold dilutions to 10^{-6}. Then 0.1 ml. aliquots of each of the six dilutions, 10^{-1} to 10^{-6}, were distributed into three or four 10 × 1 cm. test tubes. This distribution resulted in a pattern of test tubes in the rack similar to the pattern of the results of two such tests recorded in Table I. Each of the freshly thawed test sera (10 minutes in a 19° C. water bath) was distributed in 1 ml. amounts through one row of the serially diluted inocula. In each test, a serum heated to 56° C. for 20 minutes was tested with the 10^{-4} to 10^{-6} dilutions as shown in Table I to serve as a control to indicate whether the killing in the test was indeed due to a heat-labile factor. Pour plate counts were done on duplicate 0.1 ml. amounts of the 10^{-6} dilution. From these counts the number of bacteria in each inoculum could be calculated. The rack of tubes containing the serum and bacteria was shaken well and placed in a 37° C. water bath for two hours. Loop subcultures were made from each tube to a one-sixth section of a standard Petri dish containing nutrient agar. The same 2 mm. diameter loop which delivered approximately 0.002 ml. of serum was used throughout. Special care was taken to insert the loop to the center of the test tube bottom and withdraw it without touching the sides. If the section was completely covered by growth, the result was recorded as 4+; if one quarter was covered, as 1+; and if less than 50 colonies appeared, these were counted.

TABLE I

Results of two typical bactericidal tests

Size of inoculum*	Panel of fresh active sera from three human donors			Heated serum
	J	G	B	
	Klebsiella 32			
1.6×10^7	1+	1+	1+	
1.6×10^6	11	2	0	
1.6×10^5	0	0	0	
1.6×10^4	0	0	0	2+
1.6×10^3	0	0	0	26
1.6×10^2	0	0	0	2
	E. coli 34			
2.8×10^7	4+	4+	4+	
2.8×10^6	1+	12	10	
2.8×10^5	5	2	2	
2.8×10^4	0	0	0	2+
2.8×10^3	0	0	0	28
2.8×10^2	0	0	0	0

* Determined by plate counts on duplicate aliquots of the 10^{-6} dilution.

Only the tube with the greatest inoculum giving a sterile subculture was taken into account when the sensitivity of a particular bacterial strain was determined or the potencies of the various sera were compared. For determining the sensitivity of a strain, the majority result from the three or four sera tested was taken as the final reading. For instance, in Table I it is estimated that the rate of killing of *Klebsiella 32* is 1.6×10^6 organisms per ml. of serum per two hours even though one of the three sera shows a sterile subculture for an inoculum of 1.6×10^6. As will be seen from the discussion of results, agreement among the rates of killing by various sera was good and little difficulty was experienced in deciding upon the

end point. For the purpose of classifying the sensitivity of the bacteria in those few tests in which a majority of sera was not in agreement the test was rerun, and in each case there was a majority agreement on the second run. In each test at least one of the sera included was from one of five healthy donors whose sera had shown good agreement with each other in bactericidal effect in preliminary tests. This normal serum was never at more than a one tube variance with the majority.

Blood culture technique. Blood cultures from patients were obtained by inoculating 10 ml. of freshly drawn blood into 100 ml. of peptic digest of liver broth. The time of transfer of blood from the vein to the broth did not exceed one minute. Tests *in vitro* indicated that inocula of from one to three bacteria of a sensitive strain remained viable after such treatment when amounts of active serum twice that contained in the blood were present.

RESULTS

I. A comparison of the bactericidal rates of various human sera

Sera from 43 patients and 36 healthy adults were tested by the above method. In every test the heated serum control failed to kill the 1,000 to 4,000 bacteria present in the 10^{-5} dilution of the seed culture. Often an inoculum of 100 to 400 bacteria gave a positive subculture after two hours' incubation in the heated serum.

Except for three instances to be described below, all active sera whether from the healthy donors or patients showed a remarkable uniformity in the rate of killing any particular test strain. The clinical states of the 40 patients whose serum showed no defect in bactericidal power are listed in Table II.

TABLE II

*Clinical state of patients from whom test sera were obtained**

Widespread cancer	5	Lupus erythematosus	2
Lymphomatosis and Hodgkin's disease	4	Weber Christian's disease	1
Myelocytic leukemia	3	Hypogammaglobulinemia, adult transitory	1
Lymphocytic leukemia	3	Recurrent bacterial infections	1
Diabetes	4		
Pyelonephritis and bacteremia	3	Idiopathic leukopenia	1
Uremia, terminal	2	Infectious mononucleosis	1
Nephrosis	2	Congestive heart failure	1
Cirrhosis	2	Fever, unknown origin	1
Infectious hepatitis	2	Anorexia nervosa	1

* Cases 1, 2 and 3 are excluded.

Except for Case 1 (described below), every serum showed the ability to kill the standard strain of *Shigella dysenteriae* at a rate of 1×10^7 to 1.5×10^8 organisms per ml. of serum in two hours. Since these data were so uniform, they are not included in the tables.

Forty of the 43 sera from patients and all the sera from healthy donors were tested against at least one other bacterial strain, and most were tested against more.

TABLE III

Degree of internal agreement in tests using panels of sera from healthy donors and in those using sera from healthy donors and patients

	Tests utilizing only normal serum samples	Tests utilizing normal and patient serum samples		
		Total	Normal	Patient
No. of samples tested	326	162	89	73
No. of disagreements	65	36	14	22
Disagreements more bactericidal	29	17	5	12
Disagreements less bactericidal	36	19	9	10
% Disagreement	20	22	17	30
No. of two tube disagreements	6	5	1	4
Two tube disagreements more bactericidal	2	0	0	0
Two tube disagreements less bactericidal	4	5	1	4
% Two tube disagreements	2	3	1	5.5

Table I indicates the type of result observed in the great majority of tests. As may be noted, a one tube disagreement might well be expected as a matter of chance. The degree of internal agreement in tests using panels of sera obtained from healthy donors and that of tests using panels of both patients' and normal sera is shown in Table III. Data from the three cases to be described have not been included.

Of the 488 serum samples tested, 101 or 20.7 per cent showed a disagreement with the majority result. Only 11 or 2.3 per cent of the total were two tube disagreements; none was greater. None of the serum samples showed a consistent

difference from the majority result when tested with different test strains. When retested against the same strain, the difference was not usually reproducible.

Although the results from tests using sera from patients showed a slightly greater percentage of total differences from the majority result than did those for tests in which normal sera were employed, it was not a significant difference. Furthermore, the variances that did occur show an almost equal distribution of those manifesting more or less bactericidal activity. There are too few two tube variations to attach significance to the greater number occurring using patient sera.

In three cases the patients' sera were definitely less bactericidal than normal sera, but only in Case 1 was this deficiency a general one. In Cases 2 and 3, it was a defect specific for the organism isolated from the patient's own blood stream which was resistant to the patient's serum but not to other human sera.

Case 1. E. C., a 62 year old white man, had plasma cell leukemia diagnosed at autopsy. He was uremic because of leukemic involvement of the kidneys. His history was not noteworthy for frequent infections. Serum electrophoresis showed a typical myeloma peak in the γ-globulin region and the albumin: globulin ratio was 2.0: 7.5. The only infection recognized clinically during his month long stay was a pneumococcal lobar pneumonia with bacteremia controlled promptly by penicillin. At death, both lower lobes showed consolidation. Coccal and bacillary forms were noted microscopically.

His serum, drawn before the lobar pneumonia occurred, was tested three times with the standard strain of Shigella and once each against one sensitive Klebsiella and two moderately sensitive strains of Proteus. Against none of these strains was a bactericidal effect demonstrated. This serum was found to be anticomplementary.

Case 2. O. B., a 67 year old white woman, was admitted with a history of low grade fever and a recurring urinary tract infection for four months. Despite antimicrobial therapy, *Escherichia coli* of identical antibiotic sensitivity patterns grew from three blood cultures over a span of 23 days. At laparotomy six weeks after entry, an adrenal tumor with an abscess containing *E. coli* was removed.

E. coli from two of the blood cultures were tested with seven normal human sera and were found to be moderately sensitive, as shown in Figure 1 where the black circle labeled 3 depicts one of the strains. The upper circle represents the sensitivity of this strain as tested against the seven sera from other donors. The lower circle at the tip of the arrow is the level of bactericidal effect of the patient's own serum. This latter result was obtained from three separate tests on serum samples obtained within several days of the positive blood cultures. Thus, serum other than the patient's could kill this strain at a rate of 2×10^5 bacteria per ml. of serum in two hours while her serum could kill only 200, or in reality may have had no bactericidal effect at all since such a low count would not always be detected by the method used. This patient's serum was, however, fully effective against three other strains of *E. coli,* the standard strain of Shigella and one strain of Proteus.

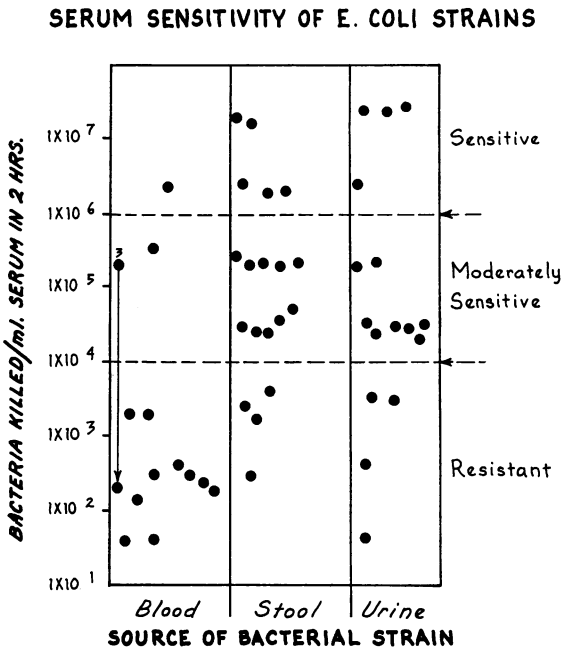

FIG. 1. COMPARISON OF THE DEGREES OF SENSITIVITY TO SERUM BACTERICIDAL EFFECT OF STRAINS OF *E. COLI* ISOLATED FROM THE BLOOD, STOOL AND URINE

Three months later, the patient was readmitted because of recurrent carcinoma and a staphylococcal subdiaphragmatic abscess. At this time her serum, as tested three times, was just as bactericidal for Strain 3 from her previous bacteremia as were the sera from the normal donors.

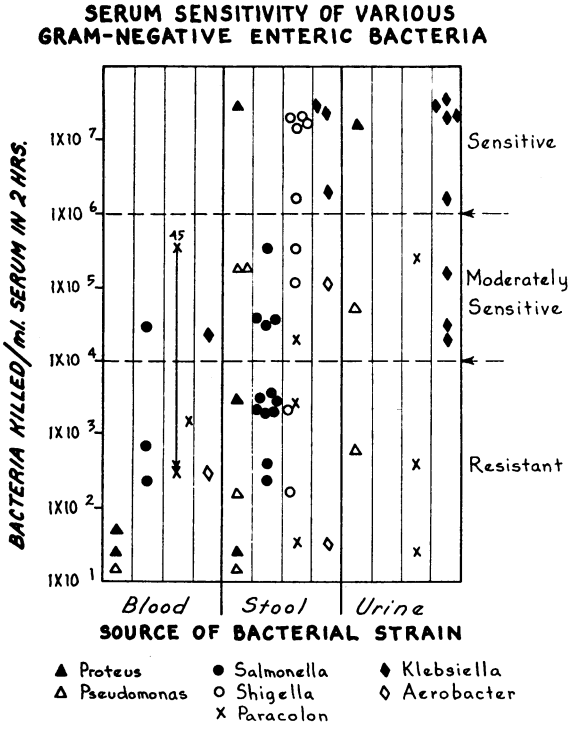

FIG. 2. COMPARISON OF THE DEGREES OF SENSITIVITY TO SERUM BACTERICIDAL EFFECT OF STRAINS OF ENTERIC BACILLI OTHER THAN *E. COLI* ISOLATED FROM THE BLOOD, STOOL AND URINE

Case 3. A. D., a 47 year old white woman, was admitted because of cirrhosis and right pyelonephritis. At entry a strain of "paracolon" bacillus was isolated from the urine and blood. She was treated with tetracycline and chloramphenicol. Her fever subsided after 17 days of treatment. Twenty-two days after entry, an antibiotic-free serum was drawn to test its bactericidal qualities.

The **X** which is labeled 45 in Figure 2 illustrates a situation analogous to that in Case 2. The serum of three normal donors killed at the rate of 4×10^5 and two at a rate of 4×10^6 organisms per ml. of serum in two hours, whereas the patient's own serum was able to kill only 400 organisms, a number barely detectable by this technique. The patient's serum was fully as effective as normal serum against the test strain of Shigella and sensitive strains of Klebsiella and *E. coli.*

In addition to Cases 2 and 3 described above, antibiotic-free sera from five other patients with gram-negative bacillary bacteremia were available for study. Their sera were fully active against sensitive and moderately sensitive test strains.

TABLE IV

Disease states associated with bacteremia from which bacterial strains studied were obtained

	No. of cases		No. of cases
E. coli		Salmonella	
Pyelonephritis	6	Disseminated lupus erythematosus	1
Carcinoma	2	Lymphocytic leukemia	1
Lymphoma	2	Unknown (stool negative)	1
Lymphocytic leukemia	1		
Acute yellow atrophy, liver	1	Paracolon group	
Acute cholecystitis (?)	1	Agranulocytosis	1
Klebsiella		Cirrhosis of liver	1
Lymphoblastic lymphosarcoma	1	Pseudomonas	
Aerobacter		Lymphocytic leukemia	1
Multiple myeloma	1	Proteus	
		Pyelonephritis	2

However, the strains obtained from their blood were resistant to normal serum as well as to their own and thus were not suitable for detecting such a phenomenon as described in Cases 2 and 3.

Thus, in this investigation of differences in the ability of sera from different individuals to kill gram-negative bacteria, only three instances of major differences were found. In one of these it was a lack of killing effect for all test strains and was probably caused by the anticomplementary action of the serum. The other two instances showed a defect specific for the organism with which they were infected and the possible explanation for this phenomenon will be discussed later.

II. A comparison of the sensitivity to serum of bacteria isolated from the blood with those isolated from the stool or urine

Throughout the preliminary stages of the study, it was found that the various test strains evidenced great differences in their sensitivity to the complement-dependent bactericidal effect. Once the sensitivity of a particular strain to one serum was determined, it could be predicted that it would have nearly the same sensitivity to the great majority of other human sera.

It seemed then that a comparison of the sensitivities to serum of bacillary strains isolated from the blood of bacteremic patients to those of strains isolated from the stool or urine might throw some light upon whether the complement-dependent bactericidal effect played any role in native immunity.

Table IV shows the type of disease associated with the bacteremias investigated. The majority of organisms from the stool came from routine cultures and those from the urine were isolated from consecutive positive cultures with no attempt made to distinguish pyelonephritis from cystitis. Neither stool nor urine cultures were from cases with known bacteremia and care was taken to be sure that each specimen came from a different individual.

Figures 1 and 2 contain all the data on the serum sensitivities of the strains of gram-negative bacilli investigated. Three arbitrary zones of sensitivity have been defined, and these form the basis for the categories presented in Table V.

Figure 1 compares the serum sensitivities of strains of *E. coli* isolated from the blood to those of strains from stool and urine cultures. (Strain 3 has been discussed with Case 2 and will not be included in the interpretation of these data.) It will be noted that 10 of the 12 strains isolated from the blood are in the resistant category. Four of 19 strains from the stool, and four of 16 strains from the urine are also resistant. If the Wilcoxon 2 sample test (16) is used to analyze the data presented in Figure 1, the probability of such a distribution's happening by chance is 0.1 per cent.

Figure 2 presents the data on bacterial strains other than *E. coli*. Organism 45 has been discussed in Case 3 and will not be included in the interpretation of these data.

As may be noted from Table V, the various bacterial genera differ markedly as to the percentage of strains in any particular category of sensitivity. The Shigella and Klebsiella groups show a higher percentage of sensitive strains than do the others whereas there is a higher percentage of resistant strains of Salmonella.

TABLE V

Comparison of the degree of sensitivity of bacterial strains isolated from the blood, stool or urine

	Blood	Stool	Urine		Blood	Stool	Urine
E. coli				Aerobacter			
Sensitive	1	5	4	Sensitive	0	0	0
Moderately sensitive	1	10	8	Moderately sensitive	0	1	0
Resistant	10	4	4	Resistant	1	1	0
Total	12	19	16	Total	1	2	0
Salmonella				Proteus			
Sensitive	0	0	0	Sensitive	0	1	1
Moderately sensitive	1	4	0	Moderately sensitive	0	0	0
Resistant	2	8	0	Resistant	2	2	0
Total	3	12	0	Total	2	3	1
Shigella				Pseudomonas			
Sensitive	0	5	0	Sensitive	0	0	0
Moderately sensitive	0	2	0	Moderately sensitive	0	2	1
Resistant	0	2	0	Resistant	1	2	1
Total	0	9	0	Total	1	4	2
Klebsiella				Paracolon group			
Sensitive	0	3	5	Sensitive	0	0	0
Moderately sensitive	1	0	3	Moderately sensitive	0	1	1
Resistant	0	0	0	Resistant	1	2	2
Total	1	3	8	Total	1	3	3

No strain of Shigella from a bacteremia was studied. The percentage of resistant strains of Salmonella isolated from the blood compared to that from the stool was exactly the same. Of the remaining five bacterial groups studied, those strains isolated from the blood showed a higher proportion of resistant strains than those from the stool or urine. Such results agree with the findings from the more extensive study on the strains of *E. coli.*

Considering all the bacteria studied, and excluding the strains of Cases 2 and 3, one finds that there were 21 strains of gram-negative bacilli isolated from the blood. Of these, four were sensitive or moderately sensitive. The two such strains of *E. coli* in Figure 1 came from a case of lymphocytic leukemia and one of diabetes with pyelonephritis. Antibiotic-free serum from neither patient was available for testing. Of the strains of moderate sensitivity depicted in Figure 2, the Salmonella came from a case of lymphocytic leukemia whose serum was not obtained. The strain of Klebsiella was cultured on two different occasions from a case of lymphoblastic sarcoma. This patient's serum was studied and proved to be just as bactericidal for this strain of Klebsiella as was normal serum. His serum was also normally active against two other test organisms.

DISCUSSION

This study was initiated to determine whether major differences in the heat-labile bactericidal effect of individual human sera can be demonstrated and, if such differences exist, whether they can be correlated with an altered susceptibility to bacterial infection. The bactericidal effect was measured by adding bacterial inocula containing varying numbers of bacteria to undiluted serum and noting the surviving organisms after two hours. This method was adopted rather than one using a standard inoculum and varying dilutions of serum because it seemed to simulate more closely the natural conditions under which an infection, and particularly a bacteremia, might occur. Furthermore, since a deficiency of any one of at least six different components will limit the activity of a serum (6, 17), a result from a diluted serum may not reflect the activity of the whole serum. Although the technique employed would not detect minor dissimilarities among sera from various individuals, it seemed evident early that small differences should play little part in affecting native resistance when there were such obvious, large variations among bacterial strains in their sensitivity to the serum.

Except for the three cases described, all sera, whether from normal donors or from patients with diseases in which a serum defect might be suspected to be present, killed any given sensitive bacterial strain at a remarkably uniform rate. All sera were consistently ineffective against resistant strains. Whether the anticomplementary effect of some hyperglobulinemic sera such as that described in Case 1 has any relationship to decreased resistance to infection is unknown. This anticomplementary quality certainly has a profound effect on the bactericidal system *in vitro,* allowing even the most sensitive strains to survive. That the anticomplementary activity of the sera of patients with multiple myeloma interferes with the bactericidal effect *in vitro* has been noted before (6).

In both Cases 2 and 3, sera from patients with prolonged bacteremia lacked bactericidal activity against their own infecting strains but possessed full potency against other test strains. If this finding were caused by a lack of a specific antibody, it would seem that such a deficiency would have appeared occasionally among the sera from the 76 other individuals tested. It seems more likely that this phenomenon is caused by antibody interfering with the bactericidal system (18) and is related to the Neisser-Wechsberg effect (19). The latter describes the inability of a serum containing high concentrations of antibody against a gram-negative bacillus to kill that strain in the presence of complement although the bactericidal effect can be demonstrated if the antiserum is diluted. In the cases described, the sera from both patients were tested at times when antibody in large concentrations should have appeared. The agglutinin titers of their sera were not tested because the possibility of antibody interference had not occurred to us. A situation like that described has been reproduced in the rabbit. As this animal is immunized with a strain of *E. coli,* the undiluted serum loses its normal bactericidal effect for the immunizing strain but retains its activity

for other test organisms (20). What role, if any, this *in vitro* finding plays in native immunity is uncertain.

With the exception of the three cases described above, the sera from individual patients and normal donors were very much alike in their ability to kill any given sensitive test strain. When a strain was resistant to one serum, it was resistant to all the sera tested. In all, antibiotic-free sera from six cases of bacteremia other than Cases 2 and 3 were available for study. All were effective against sensitive and moderately sensitive test strains. In five cases, a lack of effect against the infecting strains, even if present, could not be demonstrated because these strains were resistant to serum. In the other case, the patient's serum was fully active against the moderately sensitive strain of Klebsiella causing the infection as well as against other test strains. Such an occurrence might have several explanations. Perhaps the sensitive organisms were being constantly fed into the blood stream from a protected infected focus. They may have been sheltered by an intracellular location within phagocytes. The bactericidal system may not be nearly as effective *in vivo* as *in vitro*.

On the basis of the evidence presented, it would appear that changes in the bactericidal potency of undiluted serum as measured *in vitro* rarely take place even in the serum of patients with bacteremia or with diseases often associated with infection.

On the other hand, the finding that the great majority of the enteric bacillary strains causing bacteremia were resistant to killing by serum indicates that the system may be of importance as a factor limiting the number of strains capable of invading or persisting in the blood stream. Since the enteric strains causing bacteremia ordinarily gain entrance from either the intestine or urinary tract, the data showing that the majority of strains isolated from these sources are moderately or highly sensitive suggest that there is a limitation of the number of strains capable of causing bacteremia. The finding of one instance in which bacteremia of at least two days' duration was caused by a strain moderately sensitive to the patient's own serum indicates that the limitation is not absolute.

The percentages of resistant and of sensitive strains isolated from the urine were not significantly different from those isolated from the stool. This finding supports the belief that there is actually a difference between strains isolated from these two sources and those from the blood, and is compatible with the belief that the kidney is not ordinarily infected via the blood stream.

From the results of this study, one might hypothesize that one characteristic of a systemically pathogenic enteric bacillary strain would be its resistance to killing by serum. There are few studies to confirm this theory, but those which have been reported are in agreement with it. Jensen was able to derive a strain of *Salmonella typhimurium* nonpathogenic for the mouse from a pathogenic strain by passing it *in vitro* at gradually increasing temperatures of incubation for 206 passages. This strain had the same antigenic qualities and fermentative characteristics as the parent strain (21). Maaløe showed the parent strain to be resistant to, and the derived strain sensitive to, the complement-dependent serum factor (14). He showed the two strains to be equally toxic and infective for the mouse, but the apathogenic variant appeared unable to multiply and spread. Rowley compared the pathogenicity for mice of strains of *E. coli* sensitive to active guinea pig serum and those resistant. He found that the resistant strains were in each instance the more pathogenic (22). In a recent study it has been found that 18 of 19 strains of gram-negative bacilli isolated from burns were resistant to the bactericidal action of normal, active serum (23).

Obviously, the property of resistance to the bactericidal effect of serum does not alone impart pathogenicity to a bacterial strain. However, once given the opportunity of access to the blood stream, the survival of any particular bacterium and its ability to spread may well depend upon such a quality.

Although, as noted above, a more sensitive strain may be derived from a resistant one by artificial methods and sensitivity can vary with the culture medium used to prepare the inoculum (14, 15), we were impressed by the stability of the quality of sensitivity to serum as a characteristic of a strain if the same medium and cultural conditions were

employed. Our attempts to derive a strain significantly more resistant to killing by serum by serial passage through active human serum have thus far met with no success.

It may be noted from Table V and Figure 2 that a high proportion of the strains of Shigella are sensitive, whereas a high proportion of strains of Salmonella are resistant. If, as our results indicate, resistance to serum is of importance to a strain's ability to cause bacteremia, this distribution may be related to the frequency of cases of bacteremia cause by strains of Salmonella and the rarity of such cases caused by Shigella.

SUMMARY

An investigation of the heat-labile bactericidal properties of undiluted sera from 36 healthy donors and 43 patients with diseases often associated with infection has been conducted. With the exception of the sera from three patients, no major difference in bactericidal ability was found among the various individual sera. Two of these three sera showed a specific inability to kill the organism which was cultured from the patient's own blood. It is hypothesized that the inability of the patient's own serum to kill the infecting bacterial strain is due to interference by antibody with the normal bactericidal effect. The third serum was ineffective against any test strain of bacteria employed because it was anticomplementary.

In studying the sensitivities of strains of gram-negative enteric bacilli to the heat-labile bactericidal quality of blood, it was found that a much higher proportion of bacterial strains isolated from the blood, than of those isolated from the stool or urine, was resistant to killing by serum. The hypothesis is presented that the main role of the heat-labile bactericidal system in native resistance to infection is to prevent many strains from invading and persisting in the blood, and that changes in this bactericidal property are infrequent and of less importance.

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