

DEMONSTRATION OF AN INHIBITOR OF THE MULLER PHENOMENON IN HUMAN SERA: ITS IDENTIFI- CATION AS ANTISTAPHYLOKINASE *

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(Submitted for publication June 22, 1961; accepted August 31, 1961)

When coagulase-positive staphylococci are grown on agar plates containing serum and a suitable indicator substrate such as hemoglobin or whole red cells, numerous discrete satellite areas of clearing appear around the colonies after incubation for several days. The phenomenon was first described by Muller in 1927 (3, 4) and is commonly referred to by bacteriologists as the Muller phenomenon. The sera from several mammalian species may be used to provide the serum factor essential for production of the phenomenon (4-7). In addition to hemolysis of red cells, the phenomenon is associated with destruction of hemoglobin and can be demonstrated by substituting hemoglobin or a variety of other protein substrates instead of red cells (8-10). Elek has shown that the phenomenon can be obtained in the absence of bacterial cells by incorporating filtered extracts of agar on which staphylococci have been grown, mammalian serum, hemoglobin, and agar into a sterile system (11).

In earlier studies from this laboratory (12), we have obtained from supernates of staphylococcal broth cultures preparations that are active in the sterile system. Evidence for the identity of the staphylococcal Muller factor and staphylokinase has been obtained, and a similar phenomenon has been produced with streptokinase and with urine containing urokinase. That the Muller phenomenon is a manifestation of the plasminogen-plasmin

system was further suggested by finding in these studies that purified plasminogen could be substituted for whole serum in the sterile system, and that inhibitors of the plasminogen-plasmin system also inhibit the Muller phenomenon.

The studies to be reported here resulted from the observation of a curious discrepancy in the behavior of human and rabbit sera, both of which contain the serum factor essential for production of the phenomenon with the growing colony. In contrast to rabbit sera, however, human sera usually failed to produce the Muller phenomenon in the sterile system. An explanation for the difference between the sera from these two species has been found by the identification of an inhibitor of the staphylococcal Muller factor, which is present in the sera of most human adults but is not normally present in rabbit sera. The nature of this inhibitor has been investigated and a method devised for its quantitative measurement in sera. The distribution and levels of inhibitor in sera from normal human populations of different ages and from patients with evidence of staphylococcal disease are reported.

METHODS

Staphylococci. Strains Q202 and Q303 were obtained from patients with severe purulent lesions. These strains were coagulase-positive and produced α -hemolysin and protease as well as the Muller factor. They were not lysed by any of the available phages.¹

Staphylococcal Muller factor. For most of these studies the supernatant fluids from 16- to 18-hour dialysate broth cultures of Muller-producing staphylococci were used. In some experiments preparations of staphylococcal Muller factor, which had been partially purified by starch zone electrophoresis, were used (12). The method for preparing the dialysate medium (13) and the method

* Presented in part at the 13th Annual Meeting of the Society for Pediatric Research, May 4, 1960, Swampscott, Mass. (1); and published in part in a preliminary report (2). This investigation was sponsored by the Commission on Streptococcal and Staphylococcal Diseases, Armed Forces Epidemiological Board, and was supported by the Offices of the Surgeons General, Department of the Army, Washington, D.C.

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¹ The phage typing was generously done by Dr. Elaine L. Updyke, Communicable Disease Center, Atlanta, Ga. Phages used were 29, 52, 52A, 79, 80, 3A, 3B, 3C, 55, 71, 6, 7, 42E, 47, 53, 54, 73, 75, 77, 42D, 187, 83(VAU), 44A, 81.

for obtaining sterile supernatant fluid from broth cultures has been previously described (12). Dilutions of supernates were made in glycine buffer, 0.01 M, pH 9.0. For immunizing rabbits, concentrates containing the staphylococcal Muller factor and other extracellular products were prepared from 10-L broth cultures of strains Q202 and Q303 by methods previously described (12).

Immunization procedures. Five rabbits (2 to 4 kg, New Zealand albino) were immunized with a concentrate of extracellular products from strain Q202 and two with a similar concentrate from strain Q303. Injections were made subcutaneously twice weekly with 1-ml vol of an emulsion of 1 part staphylococcal extracellular protein concentrate, 1 part Aquaphor, and 2 parts mineral oil. Each animal received approximately 10 mg of bacterial protein over a 2-month period. Two additional rabbits were immunized with a commercial preparation containing streptokinase, Varidase (Lederle), lot 7-1089-491 A. Each animal received a total of approximately 150,000 U streptokinase, in 10 weekly subcutaneous inoculations, with an emulsion containing 1 part Varidase solution, 1 part Aquaphor, and 2 parts mineral oil.

Measurement of inhibitor of the staphylococcal Muller phenomenon by the pour-plate technique. Levels of inhibitor in human sera and in immune rabbit sera were measured by determining the dilution of serum that would prevent the appearance of the Muller phenomenon in an active sterile system: An initial standardization of the amount of staphylococcal Muller factor (staphylokinase) to be added to test systems was accomplished by preparing twofold dilutions of culture supernates in glycine buffer, 0.1 M, pH 9.0; 0.5-ml aliquots of these dilutions, 0.5 ml of normal rabbit serum, 1 ml of hemoglobin solution prepared as previously described (12), 15 ml of tryptose-blood-agar base (Difco), and merthiolate (1:10,000 final dilution) were added to sterile petri dishes. The plates were thoroughly swirled, allowed to solidify at room temperature, and incubated at 37° C for 48 hours. The highest dilution of supernate which resulted in 50 or more areas of proteolysis was determined, and a dilution containing twice this amount was used as the standard amount of staphylococcal Muller factor in the test for inhibitor. Reproducibility from day to day and from lot to lot was controlled by adjusting the concentration of staphylococcal Muller factor to give a constant titer with a standard antiserum.

Before assaying for inhibitor, the proteolytic factor in test sera was inactivated by incubation in a 56° C water bath for 30 minutes. Serial twofold dilutions of the heated test sera were made in glycine buffer, 0.1 M, pH 9.0; 0.5 ml of each serum dilution was added to 0.5 ml of a dilution of staphylococcal supernatant fluid containing the standard amount of Muller factor. These mixtures were incubated in a water bath at 37° C for 30 minutes. The incubated staphylococcal supernate-antiserum mixtures were poured into sterile petri dishes to which 0.5 ml of normal rabbit serum, 1 ml of hemoglobin solution, and 15 ml of warm tryptose-blood-agar base (Difco),

containing merthiolate (1:10,000 final dilution) were added. The plates were thoroughly swirled, allowed to solidify at room temperature, and incubated at 37° C for 48 hours. Inhibitor titers were recorded as those dilutions of sera that caused a 50 per cent reduction in the number of areas of clearing, as compared with a control plate containing buffer instead of antiserum. One or more standard antisera were included in each set of determinations in order to control reproducibility.

Measurement of inhibitors by agar diffusion technique. A double-diffusion technique was also utilized for determining levels of both the inhibitor of the staphylococcal Muller phenomenon and the inhibitor of a similar phenomenon produced with streptokinase preparations (12). Hemoglobin-agar plates with one central well and six peripheral wells, prepared as previously described (12), were used.

For determining the amount of staphylococcal Muller factor (staphylokinase) to be used in these tests, 0.1-ml aliquots of twofold dilutions of staphylococcal culture supernatant fluid or of streptokinase in glycine buffer, 0.1 M, pH 9.0, were placed in the peripheral wells of a hemoglobin-agar double-diffusion plate and 0.25 ml of normal rabbit serum was placed in the central well. The highest dilution of the bacterial factor which resulted in 10 or more areas of clearing after 48 hours' incubation at 37° C was determined. For assaying levels of inhibitor, 0.5 ml of a dilution containing twice this amount of bacterial factor was mixed with two-fold dilutions of heat-inactivated test sera and incubated for 30 minutes at 37° C; 0.1-ml aliquots of these incubated mixtures were placed in each of the six peripheral wells and 0.25 ml of normal rabbit serum was placed in the central well of a hemoglobin-agar double-diffusion plate. Plates were incubated for 48 hours at 37° C. The inhibitor titer was the highest dilution of serum that caused a 50 per cent reduction in the number of cleared areas appearing between the central and peripheral wells as compared with a well containing either staphylococcal supernatant fluid or streptokinase, and buffer instead of antiserum. One or more standard antisera were included with each set of determinations.

This double-diffusion method of assaying inhibitor correlated closely with the results obtained by the pour-plate technique. Since multiple dilutions of a test serum could be assayed on a single plate, this method resulted in conservation of materials.

Electrophoresis of serum. Starch zone electrophoresis of serum was performed by the method of Kunkel and Slater (14), using the modified conditions previously described (12). Serum was concentrated fourfold by dialysis *in vacuo* using collodion bags (Schleicher and Schuell). Glycine buffer, pH 8.6, 0.1 M, was used as the buffer for electrophoresis, for elution of block sections, and for dilutions of enzymes. The serum protein in eluates from 1-cm sections of the starch block was determined by the modified Folin-Ciocalteu method (15). Eluates from starch block sections were assayed for serum inhibitor by the double-diffusion technique described above.

The essential proteolytic factor was determined by a pour-plate technique in which 0.5-ml dilutions of eluates from starch block sections and 0.5 ml of a constant amount of the staphylococcal Muller factor were mixed with 10 ml of 3.5 per cent tryptose-blood-agar base, and 1 ml of hemoglobin solution prepared as previously described (12). After solidification of the agar at room temperature, the plates were incubated at 37° C for 48 hours. The presence of the essential serum proteolytic factor was indicated by the appearance of 10 or more typical areas of clearing.

Sera and patient material. Sera were obtained from 19 adult patients with staphylococcal disease at the University of Minnesota Hospitals and the Minneapolis General Hospital. Eleven of these were patients with post-operative wound infections and the remainder were patients with miscellaneous staphylococcal infections.

Adults without staphylococcal disease included laboratory personnel, patients on the obstetrical service, and blood donors. Children without staphylococcal disease included normal newborn infants, infants ranging in age from 2 months to 2 years,² and pediatric patients hospitalized for noninfectious diseases.

RESULTS

Both rabbit and human sera supported the production of the Muller phenomenon around growing colonies of staphylococci (Figure 1, A and B) although, as noted by previous authors (5, 7, 11), there is some variation in the size, number, and appearance of the discrete areas of clearing with sera from different species. A striking and consistent difference was noted, however, in the behavior of rabbit and human sera in the cell-free system. When sterile supernatant fluid from staphylococcal broth cultures was used as the source of the Muller factor, there was abundant particulate proteolysis in the hemoglobin-agar plates containing rabbit serum, but none in those containing human serum (Figure 1, C and D).

Addition of fresh rabbit serum to the inactive human system failed to produce the phenomenon. This suggested that the failure of the human system was not due to a deficiency of proteolytic factor in human serum. Furthermore, if human serum was added to an active rabbit system, inhibition of the rabbit system resulted. Human serum which had been heated at 56° C for 30 minutes also inhibited the active rabbit system. Thus, the difference in the sera from these two species appeared to be due to a heat-stable inhibitor that

² Dr. Konald Prem generously made available the blood from these infants.

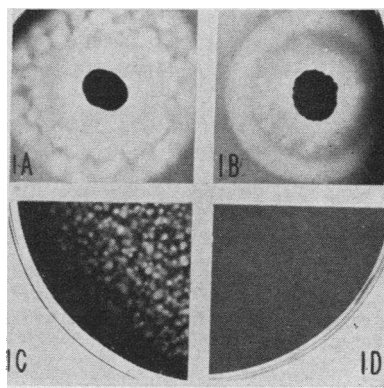


FIG. 1. A COMPARISON OF THE MULLER PHENOMENON USING HUMAN AND RABBIT SERUM. A. The Muller phenomenon around a colony of staphylococci grown on a hemoglobin-agar plate containing rabbit serum. The photograph was taken after incubation for 96 hours at 37° C and is reproduced at approximately actual size. B. The Muller phenomenon around a colony of staphylococci grown on a hemoglobin-agar plate containing human serum. The photograph was taken after incubation for 96 hours at 37° C and is approximately actual size. C. The Muller phenomenon in a sterile system containing rabbit serum in addition to staphylococcal culture supernate, hemoglobin, and agar. The photograph was taken after 48 hours' incubation. D. Failure of the Muller phenomenon to appear in a sterile system containing human serum in addition to staphylococcal culture supernate, hemoglobin, and agar.

was present in human sera and absent from rabbit sera.

Although absent from the sera of normal rabbits, inhibitor was present in the sera of rabbits immunized with preparations containing the staphylococcal Muller factor. Each of seven normal rabbits, whose sera contained the proteolytic factor necessary for the Muller phenomenon, was immunized with approximately 10 mg of staphylococcal extracellular protein containing the staphylococcal Muller factor. Sera obtained from these rabbits after immunization no longer supported the Muller phenomenon; this failure of immune rabbit sera was shown to be due to the appearance of an inhibitor. The inhibitor was demonstrated by incorporating dilutions of immune rabbit serum in hemoglobin-agar pour-plates containing staphylococcal Muller factor and normal rabbit serum. In this system dilutions of pooled immune rabbit serum as high as $\frac{1}{32}$ inhibited the Muller phenomenon (Table I).

The effect of multiple combinations of varying

TABLE I

*Effect of the addition of serum from immunized and nonimmunized rabbits on an active sterile system **

Material added to active sterile system	Dilutions of serum							
	Undil.	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Glycine buffer (control)	4+†							
Nonimmunized rabbit serum	4+	4+	4+	4+	4+	4+	4+	4+
Immunized rabbit serum	0	0	0	0	0	0	2+	4+

* Inhibition was determined by the pour-plate technique.

† Each plus represents approximately 50 particulate areas of hemoglobin clearing in the agar plate.

dilutions of staphylococcal factor with varying dilutions of serum inhibitor was investigated. The results indicate that a given amount of inhibitor neutralized a fixed amount of the staphylococcal Muller factor (Table II). When the Muller factor was present in higher concentration, lower dilutions of immune serum were required for neutralization.

It has been demonstrated that streptokinase preparations produce particulate proteolysis similar to that produced with staphylococcal preparations (12). It was therefore of interest to determine whether the inhibitor induced by injection of the staphylococcal factor would specifically inhibit the staphylococcal phenomenon and not the streptococcal (streptokinase) phenomenon. Inhibitor was assayed by a double-diffusion technique in which normal rabbit serum was placed in the central well of a hemoglobin-agar plate and incubated mixtures of heat-inactivated test serum dilutions and of staphylococcal Muller factor (staphylokinase) or streptokinase were placed in the peripheral wells. The presence of inhibitor was demonstrated by a reduction in the number of areas of clearing which appeared in the zone between the central and each peripheral well (Figure 2).

When assayed by this double-diffusion technique, sera from rabbits immunized with the staphylococcal preparation, which readily inhibited the staphylococcal Muller phenomenon, failed to inhibit the similar phenomenon produced with streptokinase. Conversely, the serum from rabbits immunized with streptokinase (Varidase) failed to inhibit the staphylococcal phenomenon, although these sera did inhibit the phenomenon produced with streptokinase. Thus, no cross reaction was demonstrated between the inhibitors of these two similar bacterial products (Table III).

In order to explore further the nature of the inhibitor of the staphylococcal Muller phenomenon, sera from immunized rabbits were subjected to starch zone electrophoresis. Figure 3 shows the distribution of serum proteins, essential serum proteolytic factor, and Muller factor inhibitor after electrophoresis in glycine buffer, 0.1 M, pH 8.6, for 16 hours at 400 v. The serum inhibitor of the staphylococcal Muller phenomenon was found in the γ -globulin area of the starch block. Electrophoresis resulted in partial separation of the serum proteolytic factor necessary for the Muller phenomenon and the serum inhibitor; the serum proteolytic factor was found in eluates from sections of the starch block containing the β -glob-

TABLE II

*Quantitative relationships between staphylococcal Muller factor and serum inhibitor **

Dilutions, staphyl. supernatant fluid	Buffer	Dilutions of human serum†					Dilutions of immune rabbit serum‡				
		1/2	1/4	1/8	1/16	1/32	1/2	1/4	1/8	1/16	1/32
1/2	4+	3+	3+	3+	3+	3+	0	1+	2+	4+	4+
1/4	4+	0	0	2+	3+	3+	0	0	1+	2+	2+
1/8	2+	0	0	0	0	0	0	0	0	1+	1+
1/16	0	0	0	0	0	0	0	0	0	0	0

* Inhibition was determined by the pour-plate technique.

† Pooled serum from 5 normal healthy adults.

‡ Serum from rabbit immunized with staphylococcal supernatant fluid.

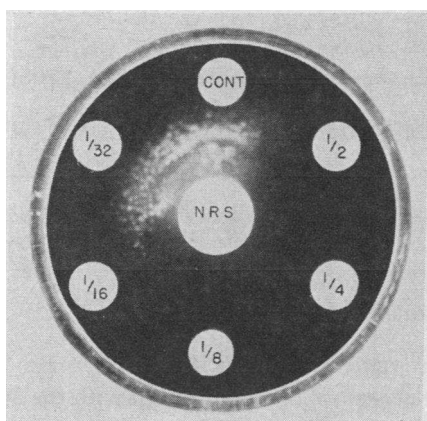


FIG. 2. MULLER FACTOR INHIBITOR TEST (DOUBLE-DIFFUSION TECHNIQUE). Normal rabbit serum (NRS) containing the essential proteolytic factor is placed in the center well of a hemoglobin-agar plate. Mixtures containing a standard amount of the bacterial factor (staphylokinase or streptokinase) and serial dilutions of heat-inactivated antiserum ($\frac{1}{2}$, $\frac{1}{4}$, and so on, as indicated) are placed in the peripheral wells. The control well (CONT) contains the standard amount of bacterial factor plus buffer. In this illustration of the staphylococcal inhibitor system, the titer of inhibitor is $\frac{1}{16}$. The photograph was taken after incubation at 37° C for 48 hours and is reproduced at actual size.

ulins. When normal human serum containing natural inhibitor of the staphylococcal Muller phenomenon was subjected to starch zone electrophoresis, the inhibitor was also found in the γ -glob-

TABLE III
*Specificity of serum inhibitors produced by immunization with staphylococcal and streptococcal preparations **

Material used for immunization	Inhibitor titer for staphylococcal phenomenon	Inhibitor titer for streptococcal phenomenon
Staphylococcal supernatant fluid and adjuvant	1/16	0
Streptokinase preparation and adjuvant	0	1/128
Adjuvant only	0	0

* Inhibitor titers were determined by the double-diffusion technique.

ulin fractions but was less well separated from the serum proteolytic factor (Figure 4).

A quantitative study of the distribution of the inhibitor in human populations was made. Serial twofold dilutions of human sera, which had been heated to inactivate the proteolytic factor, were tested for their ability to inhibit an active rabbit system. The pour-plate technique was used for all of these determinations. Many of the sera were also tested by the double-diffusion technique, and the inhibitor titers obtained were identical. Figure 5 shows typical inhibitor tests on two different human sera as measured by the pour-plate

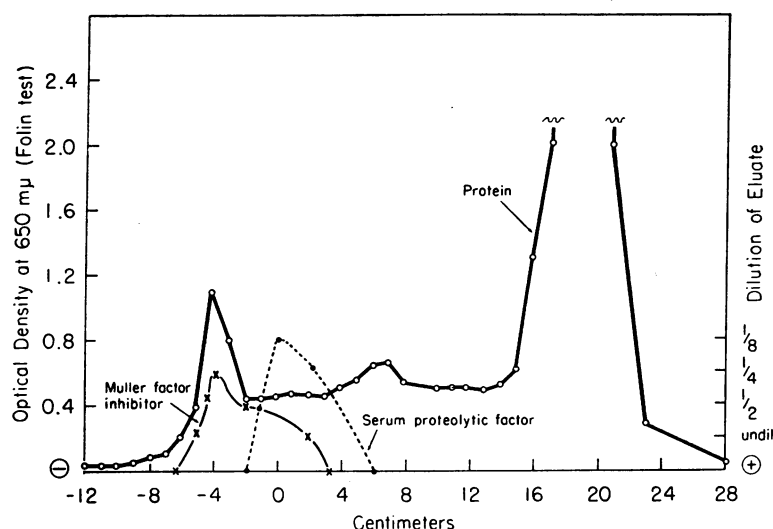


FIG. 3. ELECTROPHORETIC PATTERN, IMMUNE RABBIT SERUM. Inoculum consisted of pooled immune rabbit serum concentrated from 4 ml to 1 ml by vacuum dialysis. The distributions of proteins, of serum proteolytic factor necessary for the Muller phenomenon, and of the staphylococcal Muller factor inhibitor are illustrated.

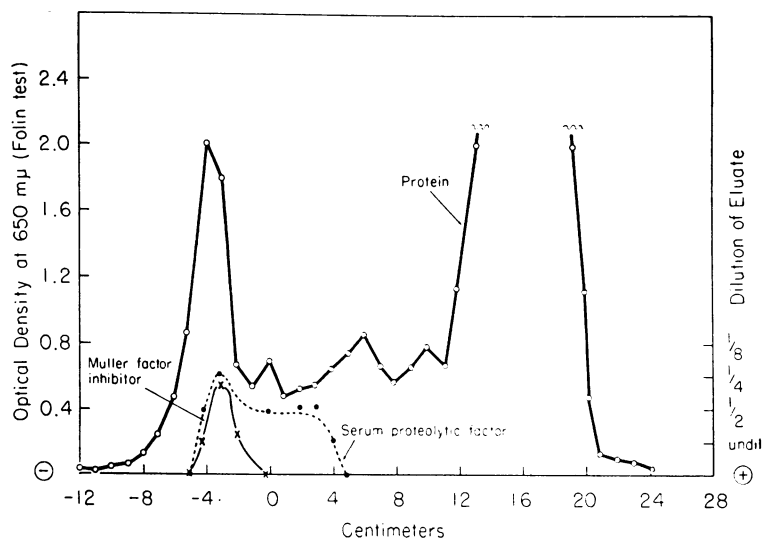


FIG. 4. ELECTROPHORETIC PATTERN, HUMAN SERUM. Inoculum consisted of serum from normal adult human, concentrated from 5 ml to 1 ml by vacuum dialysis. The distributions of protein, of serum proteolytic factor necessary for the Muller phenomenon, and of the staphylococcal Muller factor inhibitor are illustrated.

technique. The inhibitor titer of the serum in the top panel was found to be $\frac{1}{4}$. The other serum contained no measurable inhibitor.

Inhibitor titers were determined on the sera

from 114 normal (i.e., without evidence of staphylococcal disease) individuals of various ages. Although titers varied over a 16-fold or greater range, nearly all the serum samples from adults

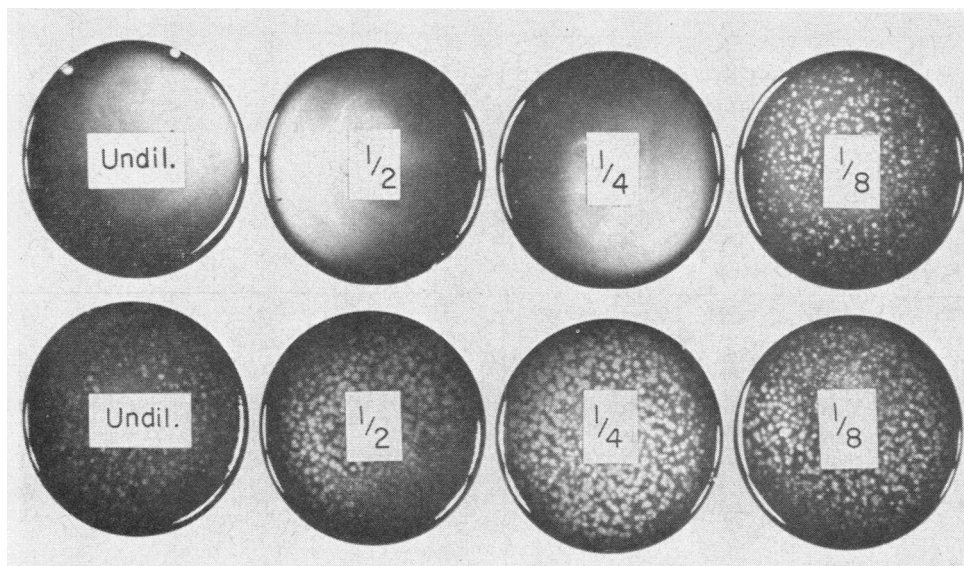


FIG. 5. MULLER FACTOR INHIBITOR TEST (POUR-PLATE TECHNIQUE). Two examples of this quantitative method for determining the Muller factor inhibitor are shown. Serial twofold dilutions of heat-inactivated test sera were made and incubated for 30 minutes with staphylococcal supernatant fluid containing active Muller factor. Pour plates were prepared with this mixture, hemoglobin solution, normal rabbit serum, and agar. The plates were photographed after incubation for 48 hours. The inhibitor titer in one of the serum samples is $\frac{1}{4}$ (top panel). In the second serum sample there is no measurable inhibitor (bottom panel).

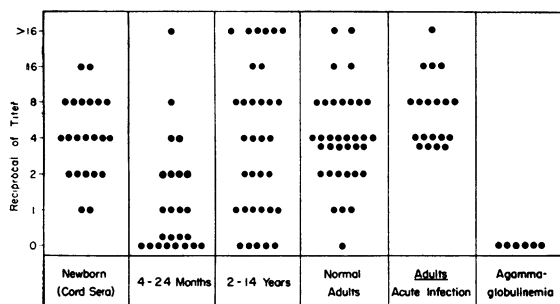


FIG. 6. THE DISTRIBUTION OF STAPHYLOCOCCAL MULLER FACTOR INHIBITOR AMONG SERA FROM DIFFERENT POPULATION GROUPS.

had detectable levels of inhibitor (Figure 6). The distribution of inhibitor in adult sera and in the sera from newborn infants (cord sera) was roughly comparable.

Sera from infants 4 to 24 months of age, however, frequently lacked measurable inhibitor, especially those in the 4- to 7-month age range. Of the 12 infants with no demonstrable inhibitor shown in Figure 6, 10 were in this earlier age group. Beyond 2 years of age, the inhibitor titers were variable, but most were in the same general range as those found in the sera from normal adults.

Although inhibitor titers of less than $\frac{1}{4}$ were not observed among patients with acute staphylococcal infections, the titers in these patients were no greater than those found in the sera of many individuals without evidence of staphylococcal infection. No inhibitor was found in the sera from patients with agammaglobulinemia.

Venous blood from mothers and cord blood

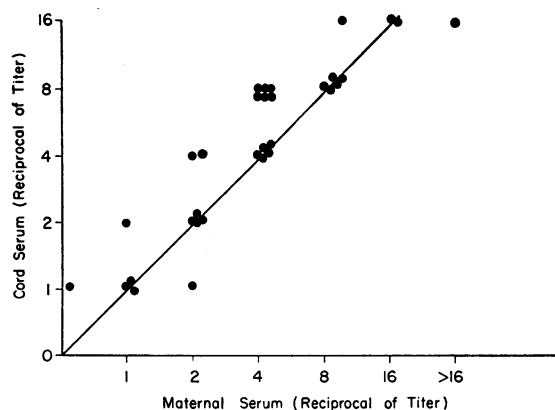


FIG. 7. A COMPARISON OF MULLER FACTOR INHIBITOR TITERS IN CORD SERUM AND MATERNAL SERUM PAIRS.

from corresponding newborn infants were collected, and the inhibitor titers in 32 maternal-cord serum pairs were determined. The titers were found to agree within one-tube dilution in all of the pairs (Figure 7). It was of interest that inhibitor titers were found to be slightly higher in the cord sera from 11 of the pairs.

DISCUSSION

The failure of human sera to produce the Muller phenomenon in the sterile system suggested either a deficiency of proteolytic factor or the presence of an inhibitor in such sera. Deficiency of proteolytic factor seemed unlikely since human sera supported the phenomenon around growing colonies of staphylococci. Moreover, the addition of rabbit serum containing the essential proteolytic factor to an inactive sterile human system failed to activate the system. Evidence for the presence of a heat-stable inhibitor was obtained by demonstrating that an active sterile rabbit system no longer produced the phenomenon upon addition of human serum which had been heated to inactivate the proteolytic factor.

It is curious that the Muller phenomenon often appeared around colonies of staphylococci grown in plates containing human serum, even though these sera contained inhibitor of the staphylococcal Muller factor as demonstrated in the sterile system. In areas adjacent to growing colonies, the bacterial factor was probably present in concentrations which effectively exceeded those of the inhibitor. This interpretation is supported by the finding that the addition of excess staphylococcal supernate (bacterial factor) to a sterile system can overcome the serum inhibitor.

Considerable evidence is presented which indicates that the inhibitor of the staphylococcal Muller phenomenon is an antibody and that the antibody is specifically directed toward the bacterial component of the phenomenon. The inhibitor was stable to heating at 56°C for 30 minutes. It was found in the γ -globulin fraction of electrophoretic patterns and in high titers in commercial preparations of γ -globulin. It was uniformly absent from the sera of patients with agammaglobulinemia.

The development of inhibitor in the sera of rabbits after injection of preparations containing staphylococcal Muller factor is also consistent

with the hypothesis that the inhibitor is an antibody. Like the natural inhibitor found in normal human sera, the inhibitor that developed in these rabbits migrated as a γ -globulin on electrophoresis. Its antibody nature is also suggested by its specificity. Inhibitor of the staphylococcal Muller phenomenon that developed in rabbits injected with staphylococcal preparations failed to inhibit a similar phenomenon produced with streptokinase. Conversely, rabbits injected with streptokinase produced an inhibitor of the streptococcal phenomenon but did not inhibit the staphylococcal phenomenon.

That the inhibitor of the staphylococcal Muller phenomenon is directed toward the bacterial factor rather than toward some other component of the active Muller system is suggested by the fact that the inhibitor effect could be overcome in both normal human sera and in immunized rabbit sera by the addition of excess staphylococcal factor. This conclusion is also supported by the development of inhibitor in the sera of rabbits after injection of the bacterial factor and by the specificity of the inhibitor for the phenomenon as produced with staphylococcal preparations only.

Thus, there seems to be little doubt that the serum inhibitor of the staphylococcal Muller phenomenon is an antibody that is directed toward a specific staphylococcal extracellular product. As reported previously (12), this product is probably identical with staphylokinase.

The absence of the Muller factor inhibitor (anti-staphylokinase) in rabbit sera and the frequency with which it is found in human sera are consistent with the differences in occurrence of other staphylococcal antibodies in the sera of these two species. Jensen (16) has shown that a precipitin which, in his studies, is universally present in human sera is rarely found in rabbit sera. Similarly, Rogers and Melly (17) have shown that most adult human sera contain a thermostable opsonin that is usually absent from normal rabbits but was occasionally found in rabbits maintained in the rabbit colony for several months. The differences between human and rabbit sera demonstrated in both the previously reported and present studies are probably a reflection of the frequency with which exposure to staphylococcal antigens occurs in the two species. Rabbits apparently do not become nasal carriers of staphylococci whereas col-

onization of the anterior nares with coagulase-positive staphylococci is commonly present in humans (18).

The distribution of inhibitor levels in human sera from population groups of different ages is similar to that seen with antibodies to other ubiquitous antigens. Nearly all of the normal human adults tested contained inhibitor of the staphylococcal Muller factor. As with many other antibodies, there was suggestive evidence for placental transfer of the Muller factor inhibitor. The sera from patients, four to seven months of age, when γ -globulin levels are low (19, 20), were frequently lacking in Muller factor inhibitor, whereas titers in newborn infants were equal to or slightly higher than titers found in their mothers. Indeed, the inhibitor titer was one dilution higher in the cord serum in a majority of maternal-cord pairs. A finding of slightly higher antibody titers in cord sera than in maternal sera has been reported for other bacterial antibodies (21, 22). Among adults with staphylococcal lesions (chiefly wound infections), the distribution of inhibitor titers was somewhat higher than in normal human adults. None of the patients with staphylococcal infections showed low titers, but the upper range of titers did not extend beyond that of normal adults. This finding is similar to that reported for other staphylococcal antibodies (23, 24). Further studies will be necessary to demonstrate the pattern of antibody response after acute and chronic staphylococcal infections.

SUMMARY

A heat-stable inhibitor of the staphylococcal Muller phenomenon has been identified in the sera of human adults. It is found in the γ -globulin fraction of serum and is absent in sera from patients with agammaglobulinemia.

Inhibitor is not present in sera from normal rabbits but can be induced by immunizing rabbits with a concentrate of staphylococcal extracellular products. The inhibitor in immune rabbit sera can be partially separated from the essential serum proteolytic factor by starch zone electrophoresis. The inhibitor in immune rabbit sera is specific for the staphylococcal Muller factor; it does not inhibit a similar phenomenon activated by streptokinase. Since previous studies indicate that the staphylococcal factor producing the Muller phenomenon is

probably staphylokinase, it is concluded that the inhibitor in normal adult human serum and in immune rabbit serum is an antibody to this staphylococcal extracellular product.

Inhibitor or antibody levels in human sera from population groups of different ages were measured by a quantitative technique. The distribution of inhibitor levels is similar to that found with antibodies to other ubiquitous antigens. Some inhibitor is demonstrable in the sera from almost all normal adults, and the upper range of titers in sera from adult patients with staphylococcal infections is no higher than that found in many normal adults. Placental transfer of the inhibitor apparently occurs. The sera from infants, aged 4 to 24 months, often lack measurable inhibitor, whereas inhibitor levels in older children approach adult levels.

ACKNOWLEDGMENTS

The authors wish to acknowledge the technical assistance of Miss Aldora Loe.

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