EFFECT OF THE ANTIBACTERIAL SERUM FACTOR ON STAPHYLOCOCCAL INFECTIONS

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ABSTRACT

YOTIS, W. W. (Loyola University Medical School, Chicago, Ill.). Effect of the antibacterial serum factor on staphylococcal infections. J. Bacteriol. **83**:137-143. 1962—Intracerebral injections of mice with 1 to 5 × 10^6 washed viable cells previously exposed for 1 hr at 4 C to 2 mg/ml of the serum factor resulted in 0 to 30% mortality when three recent isolates of yellow, hemolytic, coagulase-positive strains of *Staphylococcus aureus* were used. Mice inoculated in the same manner with the above strains, but exposed to an inactive preparation of the serum factor, showed a 60 to 90% mortality.

Addition of partially purified coagulase to the serum factor neutralized the protective action of the serum factor.

The serum factor was found primarily in the supernatant obtained following 62% $(NH_4)_2SO_4$ saturation of the water-soluble globulin portion and precipitated by one-half volume of undiluted 95% ethanol. Plate counts, manometric techniques, and animal protection studies were employed to follow purification of the serum factor. If dry weight is taken as the criterion of purification, the active substance showed a 40-fold purification over a previous preparation of this substance.

The protection afforded by human gamma globulin on experimental staphylococcal infections in mice has been described by Sonea, Frappier, and Borduas (1956) and other investigators. More recently, in vitro, a direct demonstration of lytic and lethal action of a serum factor has been made on both coagulase-positive and coagulase-negative strains of staphylococci (Yotis and Ekstedt, 1960).

The serum factor was found primarily in the water-soluble globulin portion precipitated at 25% ammonium sulfate saturation. It was thermostable, free of any lysozyme activity, had a weak anticoagulase activity, and it was contaminated with an agglutinating antibody, apparently similar to that described by Jensen (1958), Neter et al. (1959), and Weld and Rogers (1960).

Results presented here show that protection of mice and rabbits with the serum factor is possible and that addition of partially purified coagulase, prior to exposure to the serum factor, neutralized the protective power of this factor. Further studies on the purification of the antibacterial serum factor will be described.

MATERIALS AND METHODS

Cultures. Five cultures of staphylococci were obtained from the Hektoen Institute for Medical Research (Cook County Hospital, Chicago, Ill.) and represented recent isolates during routine bacteriological examination of clinical specimens. The cultures were checked for purity, hemolysis, and coagulase production by standard bacteriological techniques. Then they were transferred to blood agar slants and stored at 4 C, or grown in brain heart infusion broth (Difco) and stored at −20 C. Strains 92 and 903 are examples of avirulent coagulase-negative staphylococci of the *albus* type and were isolated from the nasopharynx of two healthy individuals. Strains 113, 417, and 500 represent yellow, hemolytic coagulase-positive staphylococci of the *aureus* type. Strain 113 was isolated from an infant with an infected umbilicus, strain 417 from a breast abscess, and strain 500 from a case of furunculosis.

Inocula for protection studies were grown in 100 ml of brain heart infusion broth for 16 hr at 37 C. The cells were harvested by centrifugation, washed once with distilled water, resuspended in 10 ml sterile distilled water, and a smooth suspension was made by manual shaking for 3 to 5 min in a 125-ml Erlenmeyer flask containing 20 glass beads, each having a diameter of 5 mm. The suspension was adjusted with sterile distilled

water to an optical density of 0.134 to 0.140 (67 to 70 Klett units) using the Klett-Summerson photoelectric colorimeter (420-mμ filter).

Protection studies. Groups of 10 normal male Swiss mice, weighing 18 to 21 g, were injected intracerebrally with 0.03 ml of standardized cell suspensions previously exposed for 1 hr at 4 C to active or inactive preparations of the serum factor, using a 0.25-ml syringe and a 27-gauge needle. The inactive preparations of serum factor used in these experiments were the products of unfulfilled experimental conditions stated in the preparation of this factor.

Coagulase preparation. Coagulase was purified by the method described by Tager (1948) and titrated as previously described (Yotis and Ekstedt, 1959). A solution of 1 mg per ml of this partially purified preparation had a titer of 1:10,000.

Serum fractionation. The human serum used in these studies was obtained from professional donors. Blood was drawn into 250-ml Baxter Plasma-Vac bottles without anticoagulant and allowed to clot at room temperature for 1 to 2 hr. The clot was broken up and the serum separated by centrifugation at 0 C. The fresh human serum from four donors was pooled, 400 ml diluted with an equal volume of distilled water, chilled to 2 C, and 140 g of solid, finely ground, cold ammonium sulfate was added, with constant stirring by a magnetic stirrer, over a period of 15 to 20 min to raise the concentration of ammonium sulfate to 25% saturation. The amount of ammonium sulfate added was calculated with the assumption that, at 0 C, 70 g per 100 ml represented 100% saturation. The precipitate was allowed to form for 2 hr at 0 C (pH 7.2 ± 0.2), separated by centrifugation (0 C) at 5,000 rev/min for 30 min (as with all fractions), dissolved in 70 to 80 ml of ice-cold distilled water, and dialyzed against cold running tap water (6 to 10 C) for 20 hr. Upon dialysis the 25% ammonium sulfate fraction separated into water-soluble and water-insoluble globulin portions. The water-insoluble globulin portion was removed by centrifugation and discarded.

Of the water-soluble globulin portion, 13 to 20 ml were kept as control and lyophilized and the remaining portion subjected to further purification.

To 80 ml of the ice-cold water-soluble globulin portion (pH 6.8 ± 0.2), 18.48 g ammonium sulfate were added over a period of 5 to 10 min at 0 C, making the solution 33% saturated. The bulky (600 to 800 mg of lyophilized material), snow-white precipitate, formed at a pH of 6.5 ± 0.2, was removed by centrifugation and placed in the ice-box.

To the supernatant of the 33% fraction, 9.52 g ammonium sulfate were added slowly with constant stirring, giving a final concentration of 50% saturation (pH 6.3 ± 0.2). After 90 min of standing, a very light precipitate (40 to 60 mg of lyophilized protein) was formed, removed by centrifugation, and placed in the ice-box.

Addition of 6.72 g ammonium sulfate to the supernatant (50% fraction) raised the saturation of ammonium sulfate 62%. Standing for 90 min resulted in the formation of a light (50 to 70 mg of lyophilized material), yellow-pink precipitate and lowered the pH to 5.9 ± 0.2. The precipitate was removed by centrifugation and placed in the ice-box.

The three precipitates formed at 33, 50, and 62% saturation with ammonium sulfate were dissolved in 20 ml of ice-cold distilled water and with the supernatant of the 62% fraction, which was designated R_{62}, were dialyzed for 20 hr against cold (6 to 10 C) running tap water and lyophilized.

Warburg studies. Cells for manometric experiments were prepared by inoculating 200 ml of brain heart infusion broth in a 500-ml Erlenmeyer flask with 2 ml of frozen stock culture and incubating at 37 C for 16 hr. The cells were harvested, washed twice with distilled water, resuspended in 10 ml of distilled water, and a smooth suspension made by manual shaking for 3 to 5 min in a 125-ml flask containing 20 glass beads. The suspension was adjusted with distilled water to an optical density of 0.9 to 1.2 (450 to 600 Klett units; 660-mμ filter). This suspension contained 0.1 to 0.14 mg bacterial N_2 per ml, or about a billion viable organisms per ml.

To a series of test tubes, containing 1 to 60 mg of the various active preparations of the antibacterial serum factor, 1.5 ml of a standardized suspension were added. These tubes and the controls (standardized cell suspensions added to inactive preparations of the antibacterial factor) were placed in the ice-box for 1 hr. All tubes were shaken manually every 12 min. At the conclusion of this treatment, 0.5 ml of 5.5×10^{-3} M glucose containing 3 μM adenosine triphosphate (California Foundation for Biochemical Research) was added in the side arm of the Warburg

vessel. In the main flask, 1.0 ml of 0.1 M phosphate buffer (pH 7.0) and 0.5 ml test bacterial suspension were added. The center well contained 0.2 ml of 40% KOH. The gaseous phase was air. All manometric experiments were conducted at 37 C. After 10 min equilibration, the substrate was tipped in and readings were taken every 15 min over a 2 hr period $Q_{O_2}^{N_2}$ values were calculated from data obtained during the first 60 min, have been corrected for endogenous respiration, and are expressed as μliters of O_2 consumed per mg bacterial N_2 per hr.

Paper electrophoresis. A solution (10 μliters) of the R_{62} fraction (6 mg per 0.3 ml distilled water) was applied from a micropipette to the filter paper (Whatman no. 1, 1-in. width) as a streak across the paper ribbon. The horizontal open-strip method, employing the Precision Ionograph, was used in making the electrophoretic experiments. The other experimental conditions were: Veronal buffer, pH 8.6; ionic strength, 0.05; potential, 300 v; current, 15 to 20 ma; and atmosphere, water-saturated air at room temperature. An adequate separation was obtained in 4 hr.

The ionographs were stained with bromophenol blue in Veronal buffer (0.05 M, pH 8.6). Except for minor modifications this procedure was adopted from McDonald et al. (1955).

RESULTS

Fractionation studies. The precipitates resulting from the water-soluble globulin portion, 33, 50, and 62% saturation with ammonium sulfate, as well as the remaining supernatant, R_{62}, were tested separately for their ability to inhibit the respiration of coagulase-positive and coagulase-negative staphylococcal strains. The results shown in Table 1 are those obtained with the R_{62} fraction. This fraction at a concentration of 1 to 2 mg per ml produced a 60 to 90% inhibition in the oxidation of substrate by staphylococci. Those fractions prepared at 50 and 62% saturation with ammonium sulfate, although inactive at the 2 mg per ml level, were found to be five times more active than the whole unfractionated water-soluble globulin portion. When standardized cell suspensions of staphylococci were exposed to these two fractions and tested in the Warburg vessel, a 20 to 30% inhibition in O_2 consumption could be obtained upon exposure of cells to 8 mg per ml of either fraction. The 33% fraction was found to be as active as the whole, unfractionated, water-soluble, globulin portion.

TABLE 1. *Effect of R_{62} fraction on oxygen consumption and viability of staphylococci*

Preparation no.	Strain no.	$Q_{O_2}^{N_2}$		Number of viable organisms	
		Exposed to R_{62}	Controls	Exposed to R_{62}	Controls
1	113	67	598	2.05×10^8	1.24×10^9
2	113	250	763	8.30×10^7	1.90×10^9
3	92	109	675	3.16×10^8	1.39×10^9
4	903	130	651	9.90×10^7	9.50×10^8
5	417	161	683	1.70×10^8	7.40×10^9

It has been shown in previous studies (Yotis and Ekstedt, 1960) that staphylococci were affected in their respiration by 30 to 40 mg per ml of the water-soluble globulin portion. If dry weight is taken as the criterion of purification, it is clear that the R_{62} fraction showed a 40-fold purification over our previous preparations of the serum factor.

Attempts were made without success to precipitate the active substance in the supernatant of the 62% fraction by increasing the concentration of ammonium sulfate to 100% saturation. However, the active substance may be precipitated from the supernatant of the 62% fraction by one-half volume of undiluted 95% ethanol. In the use of ethanol the chief precautions of slow addition of alcohol and the maintenance of low temperatures (−10 to −15 C) should be followed. Furthermore, precipitation of the active material may be accomplished by careful lowering of pH to hydrogen ion activities varying between 4.2 to 5.3.

Under the conditions of electrophoretic separation, the edge of the R_{62} band migrated 30 to 40 mm from the origin and constituted one-fifth to one-sixth (185 to 230 mg of lyophilized protein) of the total water-soluble globulin portion. Furthermore, it appeared to be homogeneous by this method.

When the whole water-soluble globulin portion was subjected to paper electrophoresis, usually three bands were formed. The bulk of the material remained at the origin, a faint band migrated 5 to 10 mm from the origin, and the leading edge of the R_{62} band migrated 30 to 40 mm from the origin.

Protection studies. To study the effect of the R_{62} fraction on staphylococcal infections, strains 113, 417, and 500 were prepared for inoculation

as previously described (Table 2). As can be seen, intracerebral injections of mice with 2.1 × 10^6 washed viable cells, previously exposed for 1 hr to 2 mg per ml of the R_{62} fraction, resulted in 16% mortality when three recent isolates of hemolytic, coagulase-positive strains of *Staphylococcus aureus* were used. Mice inoculated in the same manner with the above strains, but exposed to an inactive preparation of the serum factor, showed a 72% mortality.

Quantitative aspects of animal protection studies. The amount of active substance necessary to show a protective effect was determined. Standardized cell suspensions of two coagulase-positive strains of staphylococci were prepared and exposed to 0, 0.5, 1.0, 1.5, 2.0, and 3 mg per ml of the R_{62} fraction by methods previously described. By observing the virulence of bacterial suspensions exposed to various concentrations of the R_{62} fraction, it was possible to obtain a measure of the optimal concentration of this portion for the experimental system (Table 3). It appears that 2 to 3 mg of the active substance will be sufficient for the experimental system.

Relationship between protection and infective dose. To determine the protective power of the R_{62} fraction against intracerebral infections of mice with successive dilutions of an infective dose, cell suspensions of staphylococci were prepared. The suspensions were adjusted with sterile distilled water to an optical density of 1.0 (500 Klett units; 660-mμ filter). Such suspensions contained 1 to 5 × 10^9 viable organisms per ml, or about 1 × 10^7 cells per dose (0.03 ml). Solutions (1 ml) of the undiluted suspension, a 1:10, and a 1:100 dilution of the standardized suspension were exposed in the ice-box for 1 hr to 2 mg of the R_{62} fraction. At the conclusion of this treatment, groups of 10 mice were injected intracerebrally with 0.03-ml portions. As can be seen (Table 4), mice injected with staphylococci exposed to R_{62} fraction withstood an infective dose 1 to 2 $\log_{10}$ higher than that resisted by controls (mice injected with staphylococci and exposed to an inactive preparation of the serum factor).

TABLE 2. *Effect of the antibacterial serum factor on staphylococcal infections in mice*

Expt. no.	Inoculum	No. of animals	Mean mortality after 14 days	
			Mice injected with cells treated with serum factor	Controls
			%	%
1	5.0 × 10^6	10	20	80
2	2.1 × 10^6	10	10	80
3	4.3 × 10^6	10	0	70
4	1.6 × 10^6	10	30	80
5	1.8 × 10^6	10	20	50

TABLE 3. *Effect of concentration of the serum factor on staphylococcal infections in mice*

Strain no.	No. of animals	Concn.	Mortality		
			1 day	7 days	14 days
		mg	%	%	%
417	10	0.0	60	60	60
	10	0.5	50	70	90
	10	1.0	20	30	50
	10	1.5	0	0	0
	10	2.0	10	20	20
	10	3.0	0	10	10
500	10	0.0	40	50	80
	10	0.5	10	40	60
	10	1.0	20	30	40
	10	1.5	0	10	20
	10	2.0	0	0	10
	10	3.0	10	10	20
R_{62} control	20	3.0	0	0	0

Neutralization of the protective power of R_{62} fraction by coagulase. The standardized cell suspensions (5 ml; 0.134 to 0.140 OD) were mixed with 5 mg coagulase dissolved in 0.2 ml sterile distilled water, incubated at room temperature for 30 min, and a 2.5-ml portion was exposed for 1 hr at 4 C to 5 mg of the R_{62} fraction. At the conclusion of this treatment, groups of 10 mice were injected intracerebrally with 0.03-ml portions. Coagulase was heated at 60 C for 30 min before it was added to cell suspensions to inactivate any alpha toxin present. The effect of the R_{62} fraction and coagulase on the viability of staphylococci was also determined by methods previously described (Yotis and Ekstedt, 1960). It is evident that, in every case in which the organisms were coated with coagulase and then exposed to the R_{62} fraction, there was a marked increase in mortality and plate counts observed (Table 5).

Experiments with rabbits. To determine whether the route of injection or the animal species employed had any effect on the antibacterial action of the R_{62} fraction, groups of four young rab-

TABLE 4. *Relationship between antibacterial action and infective dose*

Strain no.	No. of animals	Dose (viable cells)	Mortality after 14 days	
			Mice injected with cells treated with serum factor	Controls
			%	%
500	10	3.5×10^5	0	50
	10	2.6×10^6	30	90
	10	3.0×10^7	60	100
113	10	1.3×10^5	0	40
	10	1.0×10^6	0	90
	10	1.4×10^7	80	100

bits weighing about 2.0 kg were injected intravenously through the marginal ear vein with 1×10^8 to 1×10^9 viable organisms which had been previously exposed to 2 mg per ml of the R_{62} fraction. Controls were injected similarly with staphylococci that had been previously exposed to an inactive preparation of the R_{62} fraction. It can be seen (Table 6) that intravenous injections of rabbits with 1×10^8 to 1×10^9 washed viable cells, previously exposed to the active substance, showed significantly lower mortality rates than controls following challenge with two coagulase-positive strains of staphylococci.

DISCUSSION

The evidence presented in this report shows that intracerebral injections in mice, with washed viable cells previously exposed to the serum factor, resulted in 0 to 30% mortality when three coagulase-positive strains of *S. aureus* were used. Mice inoculated in the same manner with the above strains but exposed to an inactive preparation of the serum factor showed a 60 to 90% mortality. This effect appears to be related to the ability of this factor to reduce the infective dose to a level which can be readily managed by host defense mechanisms. The results described show also that addition of coagulase, prior to exposure to serum factor, neutralized the antibacterial action of this factor. This finding confirms the in vitro studies of Ekstedt and Nungester (1955), Ekstedt (1956*a*,*b*), and Yotis and Ekstedt (1959).

The function of coagulase in reversing the antibacterial activity of the serum factor remains to be satisfactorily explained. It has been shown in previous studies (Ekstedt and Yotis, 1960) that staphylococci can firmly absorb coagulase from solution. It was postulated that coagulase seals specifically the active sites of labile substrate on the bacterial cell, preventing its disintegration upon later exposure to the serum

TABLE 5. *Neutralization of the protective power of serum factor by coagulase*

Expt. no.	Dose (viable cells)	No. of animals	Mortality after 14 days			
			Cells + R_{62}	Cells + R_{62} + coagulase	Cells + coagulase	Cells
			%	%	%	%
1	1.5×10^6	10	30	70	70	80
2	3.6×10^6	10	10	80	100	100
3	7.5×10^6	10	20	90	100	90
4	6.1×10^6	10	10	80	60	80
Mean plate counts after treatment			2.7×10^5	2.2×10^6	4.7×10^6	

TABLE 6. *Effect of serum factor on staphylococcal infections in rabbits*

	Strain no.	No. of animals	Dose	1 day	7 days	14 days	28 days
Deaths of animals injected with cells treated with active serum factor	113	4	2.1×10^8	0	0	0	0
	417	4	1.4×10^9	0	2	—	—
Deaths of animals injected with cells treated with inactive serum factor	113	4	2.1×10^8	4	—	—	—
	417	4	1.4×10^9	4	—	—	—

factor. The function, then, of coagulase in the experimental system may depend upon this ability to neutralize the activity of the serum factor and thus maintain the infective dose to a lethal level.

The beneficial effect of gamma globulin, given alone or in combination with antibiotics, has been stressed by many investigators (Sonea, Frappier, and Borduas, 1956; Harris and Schick, 1954; Waisbren, 1957; Knouf, 1957; Cameron, 1949; Fisher, 1957). Although their results may find apparent support in the experiments described here, they are admittedly, difficult to assess because of the different conditions under which they were obtained.

According to Lambert (1960), the reported protective effects of gamma globulin in acute lethal infections in mice may be attributed to its content of staphylococcal antitoxin. Although antitoxin plays a role in the evolution of staphylococcal infections (Forssman, 1936; Smith, 1937; Elek, 1959), the data shown here and the experimental conditions under which they were obtained do not support staphylococcal antitoxin contamination as the underlying mechanism of action. The experimental evidence points to a lethal substance distinct from lysozyme, anticoagulase, or agglutinating antibodies and perhaps similar to the lysins reported by Pettersson (1924).

The method of purification of the serum factor has been presented because of the ease with which active yields of the protective substance may be obtained. The protective substance has been used for intravenous and intracerebral injections with no ill-producing side reactions and with apparent therapeutic activity. Plate counts, manometric techniques, and animal protection studies were employed to follow purification of the serum factor. An active preparation could be obtained by fractionation with ammonium sulfate and ethanol precipitation under controlled conditions of temperature, hydrogen ion concentration, ionic strength, and protein concentration. Fraction R_{62} showed a 40-fold purification over a previous preparation of serum factor (Yotis and Ekstedt, 1960).

Although, under the conditions of electrophoretic separation, the R_{62} fraction appeared to be homogeneous, it has not been definitely established whether the factor represents a single entity and is free from other contaminants.

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LITERATURE CITED

CAMERON, H. S. 1949. The role of plasma fractions in passive immunization and treatment. Am. J. Vet. Research **10**:265–267.

EKSTEDT, R. D., AND W. J. NUNGESTER. 1955. Coagulase in reversing antibacterial activity of normal human serum on *Micrococcus pyogenes*. Proc. Soc. Exptl. Biol. Med. **89**:90–94.

EKSTEDT, R. D. 1956*a*. Further studies on the antibacterial activity of human serum on *Micrococcus pyogenes* and its inhibition by coagulase. J. Bacteriol. **72**:157–161.

EKSTEDT, R. D. 1956*b*. The effect of coagulase on the antibacterial activity of normal human serum against selected strains of *Micrococcus pyogenes*. Ann. N. Y. Acad. Sci. **65**:119–131.

EKSTEDT, R. D., AND W. W. YOTIS. 1960. Studies on staphylococci. II. Effect of coagulase on the virulence of coagulase-negative strains. J. Bacteriol. **80**:496–500.

ELEK, S. D. 1959. *Staphylococcus pyogenes* and its relation to disease. The Williams and Wilkins Co., Baltimore.

FISHER, M. W. 1957. Synergism between human gamma globulin and chloramphenicol in the treatment of experimental bacterial infections. Antibiotics & Chemotherapy **7**:315–321.

FORSSMANN, J. 1936. Studies in staphylococci. VII. On active immunity against staphylococcal infections and its relation to known antibodies against staphylococci or products of these bacteria. Acta Pathol. Microbiol. Scand. **13**:459–485.

HARRIS, J. R., AND B. SCHICK. 1954. The use of gamma globulin in infection refractory to antibiotics. J. Mt. Sinai Hosp. N. Y. **21**:148–161.

JENSEN, K. 1958. A normally occurring staphylococcal antibody in human serum. Acta Pathol. Microbiol. Scand. **44**:421–428.

KNOUF, E. G. 1957–1958. The combined use of gamma globulin and chloramphenicol in the

treatment of refractory infections. Antibiotics Ann., p. 585–592.

LAMBERT, H. P. 1960. Gamma globulin in experimental staphylococcal infections. J. Lab. Clin. Med. **56**:701–710.

MCDONALD, H. J., R. J. LAPPE, E. P. MARBACH, R. H. SPITZER, AND M. C. URBIN. 1955. Ionography. Electrophoresis in stabilized media. Year Book Publishers Inc., Chicago.

NETER, E., E. A. GORZYNSKI, A. M. DRISLANE, A. H. HARRIS, AND E. RAJNOVICH. 1959. Detection of staphylococcal antibodies in human gamma globulin and serum by hemagglutination tests. Proc. Soc. Exptl. Biol. Med. **101**:484–487.

PETTERSSON, A. 1924. Über die thermostablen bakteriolytischen Substanzen des Normalserums. Z. Immunitätsforsch. **40**:43.

SMITH, M. L. 1937. Circulating antitoxin and resistance to experimental infection with staphylococci. J. Pathol. Bacteriol. **45**:305–310.

SONEA, S., A. FRAPPIER, AND A. BORDUAS. 1956. L'action protective de la gamma globuline humaine contre une infection staphylococcique experimentale. Union méd. Canada **85**:1028–1032.

TAGER, M. 1948. Concentration, partial purification, properties and nature of staphylocoagulase. Yale J. Biol. Med. **20**:487–501.

WAISBREN, B. A. 1957. The treatment of bacterial infections with the combination of antibiotics and gamma globulin. Antibiotics & Chemotherapy **7**:322–333.

WELD, J. T., AND D. E. ROGERS. 1960. Staphylococcal immunity. Production of staphylococcal hemagglutinins in rabbits receiving staphylococcal vaccine. Proc. Soc. Exptl. Biol. Med. **103**:311–314.

YOTIS, W. W., AND R. D. EKSTEDT. 1959. Studies on staphylococci. I. Effect of serum and coagulase on the metabolism of coagulase-positive and coagulase-negative strains. J. Bacteriol. **78**:567–574.

YOTIS, W. W., AND R. D. EKSTEDT. 1960. Studies on staphyococci. III. Further studies on purification and mechanism of action of an antibacterial human serum factor. J. Bacteriol. **80**:719–725.