

STAPHYLOCOCCUS AUREUS ANTIGENS IN HYPERSENSITIVITY REACTIONS AND EXPERIMENTAL ARTERIAL SENSITIZATION

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INTRODUCTION

INVESTIGATION into the subject of bacterial sensitivity has been hampered by the physical state of the bacterial cell itself participating in in vivo antigen-antibody reactions. It is always difficult to evaluate the role of an intact cell as a true antigen in studying hypersensitivity phenomena, as its particulate nature and cellular function might suggest a biologic effect apart from a suspected role in immunologic reactions. Then, too, biochemical laboratory procedures designed to fractionate the living cell into protein, carbohydrate, and lipid components entail the risk of creating new compounds that might result from the splitting and/or rearrangement of the labile, complex, molecular aggregates that may have been characteristic of intracellular structure.¹ The report of Dworetzky and co-workers² has demonstrated the physiologic and immunologic action of *Staphylococcus aureus* preparations obtained by grinding live microorganisms in a ball mill, and our studies³ have demonstrated the retained antigenicity of *Staphylococcus aureus* cytoplasm recovered from the disruption of live bacterial cells when exposed to ultrasonic vibrations. This availability of antigenic bacterial cytoplasm has furnished a convenient tool for the further study and consideration of bacterial hypersensitivity for in a soluble state biologically active *Staphylococcus aureus* bacterial preparations may lend themselves to participation in such attempted in vivo antigen-antibody reactions as anaphylactic shock, Arthus cutaneous reactions, and experimentally induced lesions of vascular sensitization in experimental animals.

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Soluble bacterial cell protoplasm employing ultrasonic vibrations was prepared under the direction of Dr. Sam Beiser, Department of Microbiology, Columbia University, College of Physicians and Surgeons, New York.

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EXPERIMENTAL PROCEDURES AND RESULTS

Antigens.—All bacterial antigens used for immunization and challenging procedures were derived from broth cultures of the same strain of *Staphylococcus aureus*, Wood 46.

1. *Vaccine*: Saline suspension of phenol killed whole bacterial cells.

2. *Undenatured cellular protoplasm*: Prepared by subjecting saline suspensions of whole live bacterial cells to the effects of ultrasonic waves at 17,000 cycles per second. Bacterial debris consisting chiefly of cell walls was separated from the soluble cellular contents by centrifugation and Seitz filtration. The resultant sterile filtrate contained 1 mg. nitrogen per milliliter.

3. *Toxoid*: A culture filtrate of *Staphylococcus aureus* treated by pepsin digestion and containing 10,000 dermonecrotizing units per milliliter.

Experimental Animals.—Male rabbits varying between 2.5 and 3.5 Kg. in weight were used throughout in active immunization procedures for the production of antisera and for all passive sensitization and challenge experiments.

Antisera.—

1. *Antibacterial sera*: This was prepared by immunizing rabbits with intravenous and intradermal injections of vaccine for a total dose of 21 billion bacterial organisms administered in divided doses over a twenty-one-day period. Seven days after the last injection the rabbits were bled by cardiac puncture; the sera were pooled and sterilized by Seitz filtration. The antiserum was capable of agglutinating *Staphylococcus aureus* bacterial cells in in vitro macroscopic tests in a serum titer of 1:2,560 and formed interfacial precipitate when overlayed in test tubes with saline dilutions of *Staphylococcus aureus* soluble cytoplasm and toxoid.

2. *Antitoxin*: This was prepared by immunizing rabbits with intramuscular injections of toxoid adsorbed on aluminum hydroxide cream adjuvant at multiple sites for a total dose of 100,000 dermonecrotizing units. Twenty-one days later the animals were bled by cardiac puncture; the sera were pooled and sterilized by Seitz filtration. This antiserum was capable of agglutinating *Staphylococcus aureus* bacterial cells in in vitro macroscopic tests in a serum titer of 1:80 and formed interfacial precipitate when overlayed in test tubes with saline dilutions of *Staphylococcus aureus* soluble cytoplasm and toxoid.

Anaphylaxis.—Anaphylaxis was uniformly produced in normal rabbits following passive sensitization and challenge experiments with *Staphylococcus aureus* antisera-antigen systems. Passive sensitization was accomplished by the intravenous administration of 5 ml. of homologous species antisera capable of agglutinating *Staphylococcus aureus* bacterial cells in a serum titer of 1:2,560.

Thirty minutes later they were challenged with a soluble preparation of *Staphylococcus aureus* cellular protoplasm. Within 2 to 3 minutes after receiving the minimal critical dose of 0.25 mg. N, typical signs and symptoms of fatal anaphylactic shock appeared with the resultant gross pathologic finding of right heart dilatation. Abnormal signs and symptoms were completely absent in the control series of animals receiving either the 5 ml. of antiserum or 0.25 mg. N of soluble bacterial cell protoplasm alone.

Cutaneous Reactions.—The intradermal injection of saline dilutions of *Staphylococcus aureus* soluble cell protoplasm preparations in normal rabbits resulted in the development of cutaneous lesions of erythema, edema, and induration over a twenty-four to forty-eight-hour period at the site of injection and usually progressed to resemble the dermonecrotic lesions observed in rabbits following the intradermal injection of *Staphylococcus aureus* culture filtrate or toxoid. The development of these skin lesions following antigen injections alone prevented the study and evaluation of the ability of *Staphylococcus aureus* antigen-antibody systems to participate in in vivo cutaneous hypersensitivity reactions of the Arthus reaction type in sensitized rabbits prepared for challenge by the intradermal route. Cutaneous reactions failed to develop in rabbits in whom local skin sites were prepared by intracutaneous injections of antibacterial agglutinating antiserum or precipitating antitoxin and subsequently challenged with the intravenous administration of soluble bacterial cell protoplasm and toxoid, respectively.

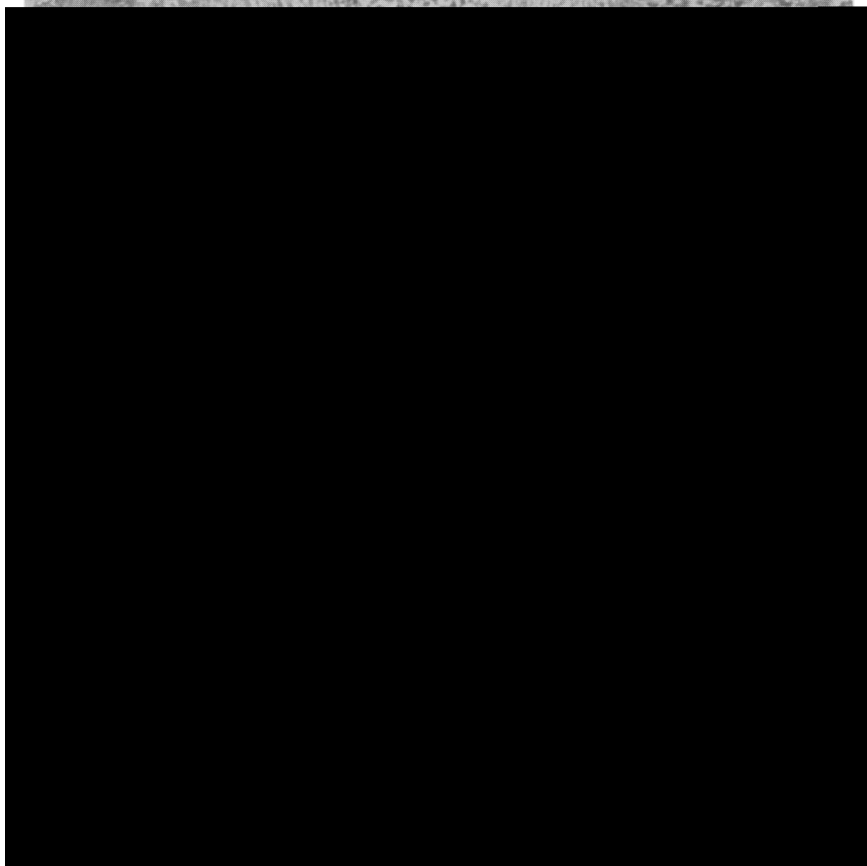
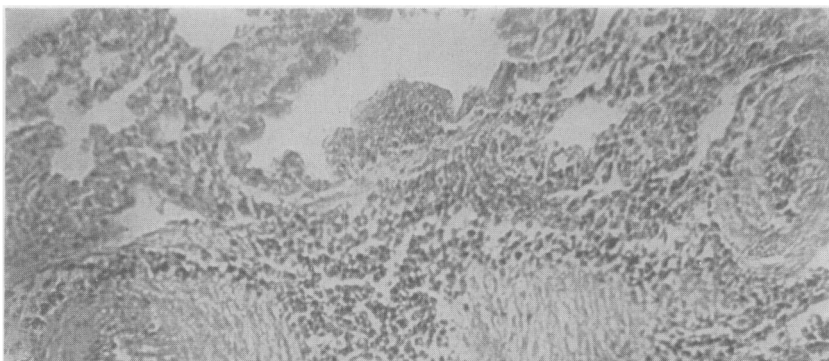
Arteritis.—The techniques previously described, favorable for the production of lesions of pulmonary periarteritis and panarteritis with horse serum-anti-horse serum⁴ and streptococcus toxin-antitoxin⁵ systems, were extended to evaluate potential vascular sensitizing properties of *Staphylococcus aureus* antigen-antibody systems. In this procedure normal animals are passively sensitized with the intravenous administration of a high-titer antiserum. After an incubation period of thirty minutes the subsequent intravenous challenge

TABLE I. THE NUMBER OF RABBITS DEVELOPING LESIONS OF PULMONARY PERIARTERITIS AND PANARTERITIS FOLLOWING PASSIVE SENSITIZATION WITH HOMOLOGOUS SPECIES RABBIT AGGLUTINATING ANTISERA VERUS STAPHYLOCOCCUS AUREUS BACTERIAL CELLS OR ANTITOXIN AND SUBSEQUENTLY CHALLENGED WITH SOLUBLE CELL PROTOPLASM OR TOXOID OF THE SAME BACTERIAL SPECIES

NO. OF ANIMALS	SENSITIZING ANTISERUM VERUS STAPHYLOCOCCUS AUREUS	CHALLENGING ANTIGEN STAPHYLOCOCCUS AUREUS	NO. OF ANIMALS WITH LESIONS
6	5 ml. antiserum for whole cell	Cell protoplasm 0.25 mg. N	5
6	5 ml. antiserum for whole cell	—————	0
6	—————	Cell protoplasm 0.25 mg. N	0
5	5 ml. antiserum for whole cell	Toxoid 5,000 dermonecrotizing units	4
5	5 ml. antitoxin	Cell protoplasm 0.25 mg. N	4
5	5 ml. antitoxin	Toxoid 5,000 dermonecrotizing units	4
6	—————	Toxoid 5,000 dermonecrotizing units	0

The control series of animals received only the antisera or preparations of the bacterial antigens.

A.



B.

Fig. 1.—Sections of lung tissue from rabbits that had been passively sensitized with homologous species *Staphylococcus aureus antisera* and subsequently challenged with the intravenous administration of subanaphylactic doses of soluble *Staphylococcus aureus* antigens. The involved arteries and arterioles show marked perivascular and adventitial infiltration of eosinophils and some polymorphonuclear leukocytes and, to a lesser extent, of a few lymphocytes. Some intimal reaction can be seen in *A* and *C*. Eosinophils can be seen penetrating the media and intima in *E*. Collections of eosinophils are found within the lumen of vessels seen in *B* and *C*, and early thrombus formation has occurred within the lumen of one vessel seen in *B*. High-power magnifications of sections *E* and *F* demonstrate the eosinophilic cellular perivascular and adventitial infiltrates.

(Fig. 1, *C* to *G* on opposite page.)

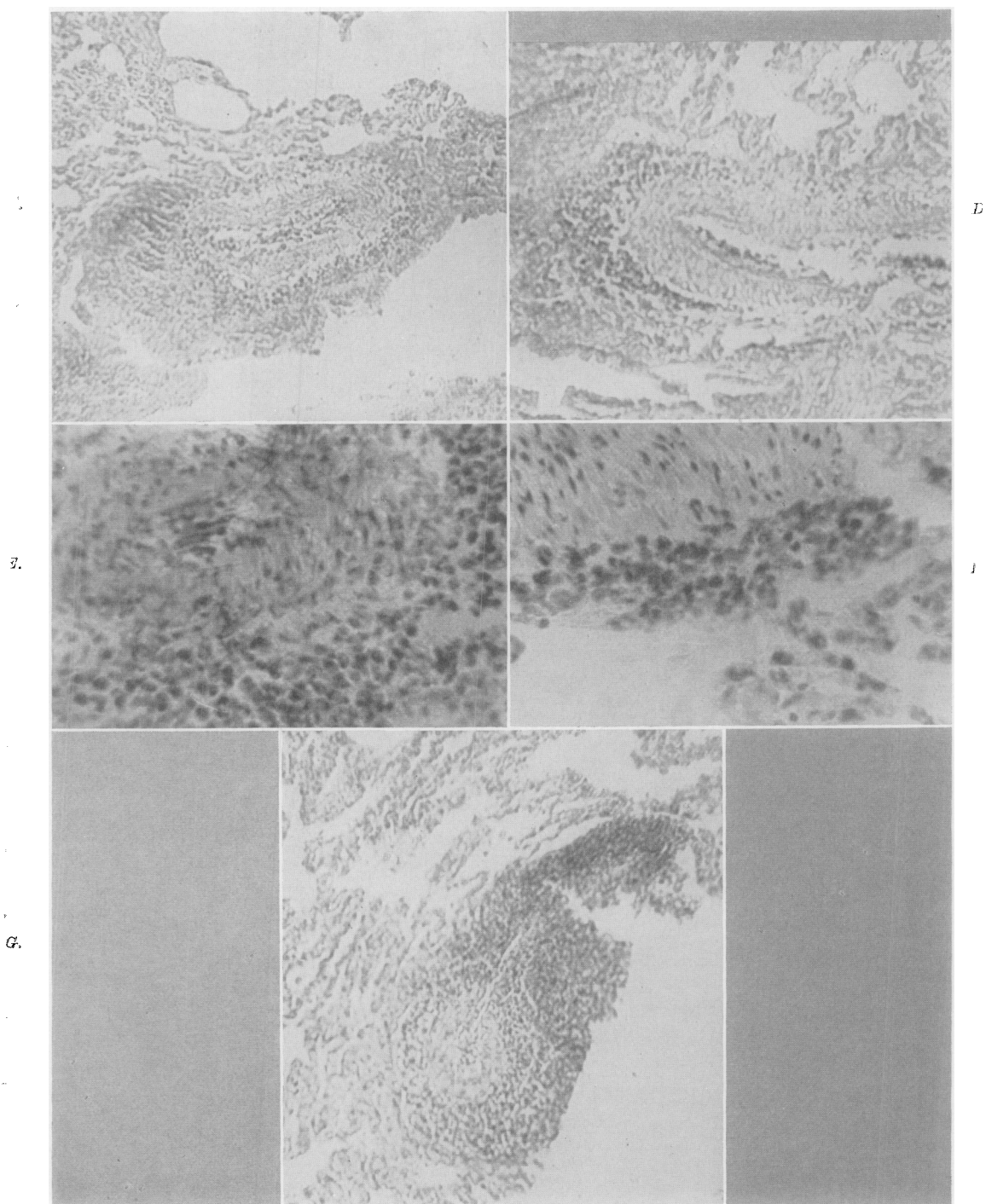


Fig. 1.—C to G. (*For legend, see opposite page.*)

of these animals with the soluble specific antigen is started. A total dosage of antigen previously determined to be the minimal critical dose capable of producing fatal anaphylactic shock is divided into four fractional dosages, each given at fifteen-minute intervals in graded increasing concentrations over the period of one hour in an attempt to effect an asymptomatic subacute anaphylactic reaction. Twenty-four hours later the animals are sacrificed by air embolism; sections are taken from the internal organs, prepared, and stained for histologic study.

The *Staphylococcus aureus* heterologous species rabbit antisera used for passive sensitization consisted of 5 ml. of either an antiserum for the whole cell with a bacterial agglutinating titer of 1:2,560 or a precipitating antitoxin. The *Staphylococcus aureus* antigens used in challenging the passively sensitized rabbits consisted of either: (1) soluble bacterial cell protoplasm in divided doses of 0.025, 0.05, 0.75, and 0.1 mg. N, or (2) toxoid in divided doses of 500, 1,000, 1,500 and 2,000 dermonecrotizing units. The results are summarized in Table I.

Passive sensitization with antisera for the whole bacterial cell: When challenged with soluble cell protoplasm, five out of six rabbits showed pulmonary vascular lesions. When the animals were challenged with toxoid, pulmonary vascular lesions were demonstrated in four out of five rabbits.

Passive sensitization with antitoxin: When challenged with toxoid, four out of five rabbits showed pulmonary vascular lesions. When the challenge was made with soluble cell protoplasm, pulmonary vascular lesions were demonstrated in four out of five animals.

Lesions of the pulmonary vasculature could not be found in six rabbits receiving only antiserum for the whole cell, five rabbits receiving antitoxin, six rabbits receiving only soluble cell protoplasm, and six rabbits receiving toxoid alone.

In those series of animals where lesions were demonstrated following passive sensitization and challenge experiments, the pulmonary vasculature was found to be chiefly involved (Fig. 1). The small pulmonary arteries and pulmonary arterioles showed marked perivascular and adventitial infiltration of eosinophils and, to a lesser extent of polymorphonuclear leukocytes and some lymphocytes. Eosinophils were also found infiltrating the intima and media of the small arteries and arterioles, and in several instances collections of eosinophils and early thrombus formation were found within the lumina of these vessels. In several sections varying degrees of hyperplasia of the endothelial cells of the intima, cloudy swelling of the muscle fibers with hydropic degeneration of the media, and some adventitial edema were found. Scattered and light interstitial infiltration with some eosinophils was seen occasionally within some alveolar walls.

DISCUSSION

Typical symptoms and gross pathologic findings of anaphylactic shock in rabbits were uniformly elicited in animals passively sensitized by the intravenous

administration of homologous species *Staphylococcus aureus* antibacterial agglutinating sera and subsequently challenged with the soluble cellular protoplasmic contents of the same bacterial strain. Bacterial cells have previously been shown capable of causing anaphylactoid reactions in sensitized animals, but the role of the cells as antigen in these instances has been difficult to evaluate because of the toxicity of the bacterial preparations, per se, and because of their particulate nature. Thus, it has not been possible to rule out their role as intact particles in possible thrombus formation and/or embolization.⁶ However, the reduction of cells to undenatured cellular antigen by the gentle but effective disrupting action of ultrasonic waves on the cell wall and the employment of this soluble material as the challenging antigen would tend to avoid such complicating factors and offer evidence to suggest a true sensitizing role for *Staphylococcus aureus* cytoplasm in such in vivo reactions of hypersensitivity as anaphylaxis. Little need be said regarding the value of the established technique of passive sensitization and challenge in allowing for the controlled evaluation of in vivo effects of the action of either antibacterial serum or the *Staphylococcus aureus* antigen. This further lends itself to the establishment of a minimal critical dose of challenging antigen prerequisite for anaphylaxis. In this regard, its value in circumventing the interfering factor of possible anaphylactoid reactions in nonsensitized animals resulting from toxic effects of larger doses of *Staphylococcus aureus* bacterial antigen is evident.

It was impossible to evaluate any potential for localized skin-sensitizing effects of intracutaneously administered soluble bacterial cell protoplasm because of the local cutaneous reactions of erythema, edema, induration, and progression to necrosis that resulted from the local injection of this material in dosages considered prerequisite for interreaction with specific antibody-containing homologous species serum for the production of local Arthus reactions. Kahn⁷ has noted similar inflammatory type "flare-up" reactions in rabbits injected intradermally with killed suspensions of staphylococcus and attributed these to the natural immunity possessed by this animal to these microorganisms. However, the results of agar gel diffusion plates in an attempt to resolve *Staphylococcus aureus* cytoplasm into constituent antigens would suggest that the dermonecrotizing effects observed may be partially due to the actual exotoxin content demonstrated within the bacterial cell itself in the particular strain of this microorganism studied and reported here.³

Previous studies^{4, 5, 8-10} employing the technique of passive sensitization and challenge have demonstrated that pulmonary lesions of periarteritis and panarteritis in the rabbit arise as a result of in vivo antibody-antigen reaction and probably as a tissue manifestation of subacute anaphylaxis. The results of the experimental procedure concerned with *Staphylococcus aureus* antibody-antigen systems reported here would tend to offer further evidence in this regard. These lesions may arise as a result of hypersensitivity reactions involving either the cellular cytoplasm and/or the exotoxin of *Staphylococcus aureus* and do not differ significantly in histologic detail from those resulting from the action of other protein antigen-antibody systems, such as horse serum-antihorse

serum. Their development, therefore, probably depends upon a tissue response to reactions of hypersensitivity rather than to any particular specific characteristic of the nature of the bacterial antigen or antiserum employed.

In vitro cross reactions in macroscopic agglutination and precipitin tests and precipitin band reactions of identity in agar gel diffusion plates have been observed between various *Staphylococcus aureus* cellular protoplasmic and toxoid antigens when tested against their respective antisera.³ These probably arise as a result of each sharing some immunologically similar or identical component antigens. The fact that tissue lesions of vascular sensitization can arise in rabbits after challenge with either staphylococcus cellular protoplasm or toxoid, regardless of whether the animal has been passively sensitized to the bacterial antisera or to the antitoxin, would point out the possibility of similar cross reactions occurring in vivo, as well as in in vitro test procedures. Ehrlich¹¹ has observed the development of primary mesenchymal reactions in rabbits following the intravenous injection of whole bacteria. However, the control studies utilizing only preparations of cell protoplasm, toxoid, antibacterial serum, or antitoxin suggest that lesions of pulmonary periarteritis and panarteritis described here arise as a result of tissue response to *Staphylococcus aureus* antibody-antigen reactions apart from any other tissue effect, toxic or otherwise, that may possibly be exerted by *Staphylococcus aureus* antigens or the presence of their antisera.

SUMMARY

1. The sensitizing properties of *Staphylococcus aureus* (Wood 46 strain) were studied, utilizing soluble cell protoplasm obtained by exposing live bacterial cells to the disrupting effects of ultrasonic vibrations and pepsin-digested culture filtrate (toxoid) as antigens.
2. In rabbits passively sensitized with high-titer bacterial agglutinating antisera, fatal anaphylaxis could be uniformly elicited by subsequent intravenous challenge with a soluble preparation of bacterial cytoplasm.
3. When the previously determined critical anaphylactic dose of soluble bacterial cellular protoplasm was administered intravenously in fractional dosages over a sixty-minute time period to rabbits passively sensitized with the same high-titer bacterial agglutinating antiserum, lesions of pulmonary periarteritis and panarteritis were demonstrated twenty-four hours later.
4. Lesions of pulmonary panarteritis and periarteritis were also observed when similar techniques were employed in rabbits passively sensitized with high-titer bacterial agglutinating antiserum and challenged with toxoid and in rabbits passively sensitized with antitoxin and challenged with either toxoid or soluble bacterial cellular protoplasm.
5. Neither anaphylactoid reactions nor the development of lesions of pulmonary periarteritis or panarteritis were observed in normal rabbits receiving only the same bacterial agglutinating antisera, antitoxin, soluble bacterial protoplasm, or toxoid.

6. The intracutaneous injection of preparations of soluble bacterial cellular protoplasm in normal nonsensitized rabbits resulted in the development of cutaneous lesions of erythema, edema, infiltration, and necrosis. This resembled the dermonecrotizing action of *Staphylococcus aureus* toxin and may have been due to the antigenic exotoxin components previously demonstrated within *Staphylococcus aureus* bacterial cells.

7. The sensitizing properties of the cytoplasm and toxin of *Staphylococcus aureus*, their antigenic relationships, and their ability to participate in such in vivo reactions of hypersensitivity in the rabbit as anaphylaxis and the development of tissue manifestations of vascular sensitization have been demonstrated.

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