

STUDIES OF MATERIALS DERIVED FROM STAPHYLOCOCCUS

III. PROTECTIVE ACTION OF GAMMA GLOBULIN AGAINST THE ANAPHYLACTOID EFFECTS OF A STAPHYLOCOCCAL BACTERIAL FRACTION*

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SALINE extracts prepared in this laboratory from three strains of *Staphylococcus aureus*, washed free from culture filtrate, have been reported to cause anaphylactoid reactions in the isolated guinea pig ileum.^{1, 2} This toxic effect was limited to a fraction precipitated at pH 3.5 (fraction A), which is probably a nucleoprotein.¹ In contrast to the anaphylactoid effect of bacterial extract and fraction A from pathogenic strains of bacteria, extract and fraction A from nonpathogenic staphylococci had no toxic properties.¹ Furthermore, the toxic activity of the extract was quite different from the toxic effect of culture filtrates of the same strains of *Staphylococcus aureus*.²

In the process of extending this work, the protective effect of horse anti-staphylococcal serum upon the anaphylactoid reaction of toxic fraction A was studied. However, control studies revealed that *normal* horse serum had the same protective effect as the antiserum. The present article reports the results of investigations of this protective effect.

MATERIALS AND METHODS

One- to two-inch segments of fresh guinea pig ileum, suspended in a 5 ml. bath containing Tyrode's solution, maintained at 37° C. were used. The upper end of the segment was tied to a simple lever which recorded longitudinal movements of the segment on a smoked drum rotating at a speed of 2.5 cm. per minute.

In each experiment 0.2 ml. of serum, or equivalent doses of serum fractions, were added to the bath. Thirty seconds to one minute later, toxic fraction A was added and the record was continued for one and one-half minutes, to allow a contraction, if any, to occur. In order to assure complete removal of the serum or serum fractions, the bath was then rinsed three times, followed by five additions of 0.1 mcg. of histamine standard with three washes

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between each addition. Toxic fraction A was then added, without serum or serum fractions, to demonstrate the anaphylactoid reaction described in previous reports.^{1, 2}

In a few of the earlier experiments, rather than adding the materials separately, serum was first mixed with fraction A and incubated at 37 degrees for thirty minutes prior to addition to the bath. This prolonged incubation was found to be unnecessary.

A total of 110 segments of ileum from seventy-three guinea pigs were used (Table I). Sixty segments were used to study the protective effects of serum or gamma globulin against the toxic staphylococcal extract or fraction A, and the remaining fifty segments were used in control studies. These included investigation of the effect of substances other than whole serum or gamma globulin upon the toxic activity of bacterial extract or fraction A, as well as determination of the effect of serum or gamma globulin on the action of myotonic substances other than bacterial extract or fraction A.

TABLE I. DISTRIBUTION OF 110 EXPERIMENTS IN STUDY (73 ANIMALS)

SUBSTANCES STUDIED FOR PROTECTIVE EFFECT	MYOTONIC AGENTS						
	STAPHYLOCOCCUS AUREUS			HISTAMINE	COBRA VENOM	ANAPHYLAXIS	TOTAL
	WHOLE EXTRACT	FRACTION A	CULTURE FILTRATE				
Serum,* whole	24	7	1	8		1	41
Serum gamma globulin	6	12	5	3	2	8	36
Serum beta globulin	5						5
Serum alpha globulin	2	1					3
Serum albumin	3						3
Serum fibrinogen		1					1
Serum, whole, rat	6	5					11
Egg white	3						3
Egg yolk	2	2					4
Cobra venom	1						1
Glycerin	2						2
Totals	54	28	6	11	2	9	110

*Human, equine, bovine, rabbit, and guinea pig.

Bacterial extract, fraction A, and culture filtrate were prepared as described previously.¹⁻³ Culture filtrate was dialyzed against distilled water to remove histamine which is regularly present.

Naja nivea (cobra) venom was obtained in crystalline form* and was used in aqueous solution.

Human, horse, rabbit, guinea pig, and rat sera were used in various experiments.

*Kindly supplied by Dr. Charles N. Bogert, American Museum of Natural History, New York, N. Y.

The following serum fractions were used: bovine serum fibrinogen; albumin; alpha, beta, and gamma globulin,* pooled human gamma globulin,† and one individual human alpha and beta globulin sample.‡

In addition, 20 per cent aqueous solutions of egg white and egg yolk and a 25 per cent aqueous solution of glycerin were used for some of the control studies. Histamine standard contained 1.0 mcg. of histamine base per milliliter.

RESULTS

To demonstrate protection, two criteria must be fulfilled: (1) absence of contraction of the ileum following addition of bacterial extract or fraction A (F-10) to the bath containing serum, serum fractions, or controls, and (2) typical anaphylactoid response to bacterial extract or fraction A, after removal of serum, fractions, or controls, followed by tachyphylaxis (that is, loss of responsiveness to bacterial extract or fraction A after the initial contraction).

Experiments in which the reaction to extract or fraction A was unaffected by the presence of serum, fractions, or control materials were considered "no protection." Those in which only one of the two criteria was fulfilled were considered "undetermined." This classification was necessary because, unless a reaction to extract or fraction A could be demonstrated after removal of the protective substance, one could not be certain that the ileum segment was capable of response originally, a prerequisite for the demonstration of protection.

Whole serum or gamma globulin protected the isolated guinea pig ileum against the anaphylactoid effects of pathogenic staphylococcus bacterial extract or fraction A (F-10) (Fig. 1). This was demonstrated by the absence of initial contraction in the presence of serum or gamma globulin (criterion 1), and after washing out the serum or gamma globulin, by the typical anaphylactoid reaction, followed by tachyphylaxis, when a second and a third dose of extract or fraction A were added to the bath (criterion 2). Of forty-nine experiments with whole serum or gamma globulin from human, horse, rabbit, guinea pig, and bovine sources, protection was demonstrated in forty-one. Human serum was employed in ten instances, human gamma globulin in fifteen, horse serum in seven, rabbit serum in eleven, guinea pig serum in three, and bovine gamma globulin in three. Protection was demonstrated with all of these.

The heat stability of the protective effect was tested on seven ileum segments from seven guinea pigs by challenge with whole bacterial extract in three instances and with toxic fraction A (F-10) in four, following the initial addition of pooled human gamma globulin, previously heated at 56 degrees for thirty minutes. In all seven cases protection was unaffected, being present to the same degree as in the unheated gamma globulin.

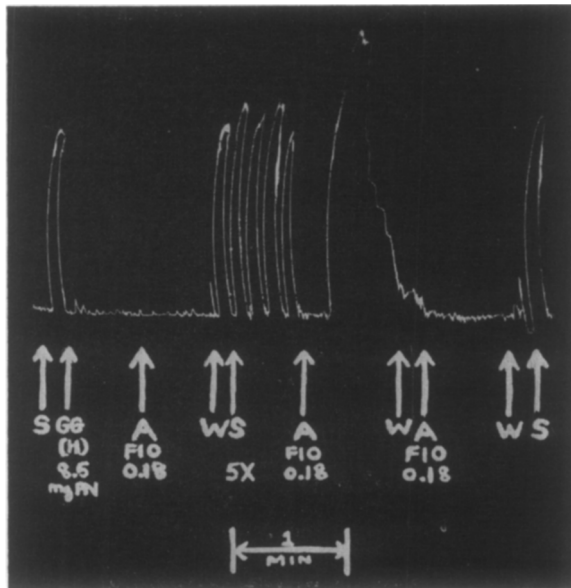
In only one of the forty-nine experiments did serum or gamma globulin from the species mentioned fail to protect against the anaphylactoid reaction

*These fractions were kindly supplied by the Armour Laboratories.

†Kindly supplied by Pitman-Moore Company.

‡Kindly supplied by Dr. Leonhard Korngold, Sloan Kettering Institute for Cancer Research.

In seven of the forty-nine experiments, the degree of protection was regarded as "undetermined," because the required criteria for protection were not fulfilled. Five of the seven showed slight or no reaction to the toxic bacterial extract or fraction A, when given with or immediately after serum, fulfilling the first criterion for protection. After washing out the perfusion bath, however, addition of extract or fraction A again resulted in slight or no reaction, therefore failing to fulfill the second criterion for protection. This may



After five additions of histamine standard, with three washes between each, two additions of fraction A (F-10), separated by three washes, demonstrate the customary contraction with the first addition and lack of response (tachyphylaxis) with the second (second criterion for designation of "protection").

have resulted from the production of tachyphylaxis without a preceding contraction, or it may have been due to a segment of ileum which was unresponsive to toxic fraction A. Such a reaction could also result from incomplete rinsing of the bath, allowing a sufficient amount of serum to remain behind to protect against the second dose of fraction A.

Of the latter five cases of "undetermined" protection, two involved normal rabbit serum, one involved horse serum, and two involved human serum. However, five other tests with normal rabbit serum, three with Coombs' rabbit anti-gamma globulin, six with normal horse serum, and twenty-three with human serum or pooled human gamma globulin all showed protection against the toxic fraction A.

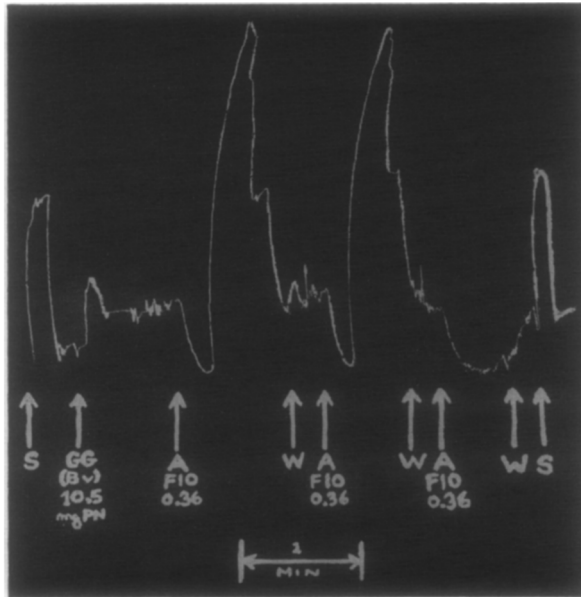


Fig. 2.—"Indeterminate protection" by gamma globulin against anaphylactoid effect of *Staphylococcus aureus* (F-10) fraction A upon isolated suspended segment of guinea pig ileum. S and W as in Fig. 1.

At G.G. (B.V.), 10.5 mg. protein nitrogen of pooled bovine gamma globulin added to bath, followed in one minute by addition of 0.36 mg. protein nitrogen of fraction A. Maximal contraction in fifteen seconds, followed by relaxation in thirty seconds.

After three washes at A (F-10), 0.36 mg. protein nitrogen of fraction A (F-10) again added. Maximum contraction, as before (that is, no tachyphylaxis).

After three washes, addition of same dose of fraction A as above results in no reaction (that is, tachyphylaxis).

Finally, two experiments with bovine gamma globulin gave undetermined protection against toxic fraction A (Fig. 2), which differed in type from the above five examples of "undetermined" protection. These utilized adjacent segments of ileum from a guinea pig which had been sensitized by four daily intradermal injections of 0.5 ml. of a 20 per cent solution of egg white four to five weeks prior to the experiment. In both instances there was a marked contraction to the initial dose of toxic fraction A, added while bovine gamma globulin was in the perfusion bath, indicating that there had been no protection. However, after thorough washing, maximal contraction followed a second dose of fraction A, free of bovine gamma globulin. A third addition of fraction A in both experiments resulted in no contraction, demonstrating

tachyphylaxis. It is of interest that protection by gamma globulin was demonstrated with a third segment of ileum from the same guinea pig and with five segments from other guinea pigs sensitized to egg white, not included in the present data.

With the exception of bovine beta globulin, no other materials studied protect the ileum against the anaphylactoid effects of fraction A (F-10). Serum albumin (bovine), fibrinogen (bovine), and alpha (human and bovine) and beta (human) globulin all failed to confer protection against the toxic fraction A, indicating that the protective effect is more or less limited to gamma globulin.

In three experiments, bovine beta globulin protected against bacterial extract (F-10) in one and showed indeterminate protection in two. No protection was demonstrated in two experiments with human beta globulin.

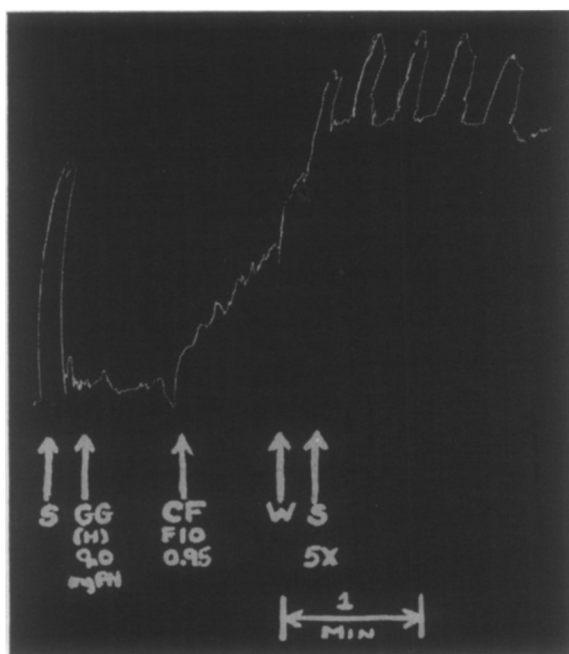


Fig. 3.—Absence of protection by pooled human gamma globulin (G.G. [H] 9.0 mg. of protein nitrogen) against myotonic effect of *Staphylococcus aureus* (F-10) culture filtrate (CF F-10 0.95 mg. protein nitrogen) upon isolated suspended segment of normal guinea pig ileum. S and W as in Fig. 1.

Studies of the protective effect of rat serum were carried out on eleven segments of ileum from six guinea pigs. In seven there was no protection, while four were indeterminate. Absence of protection was found with doses of rat serum as high as 0.5 ml., more than twice the amount sufficient for protection when sera from the other species were used.

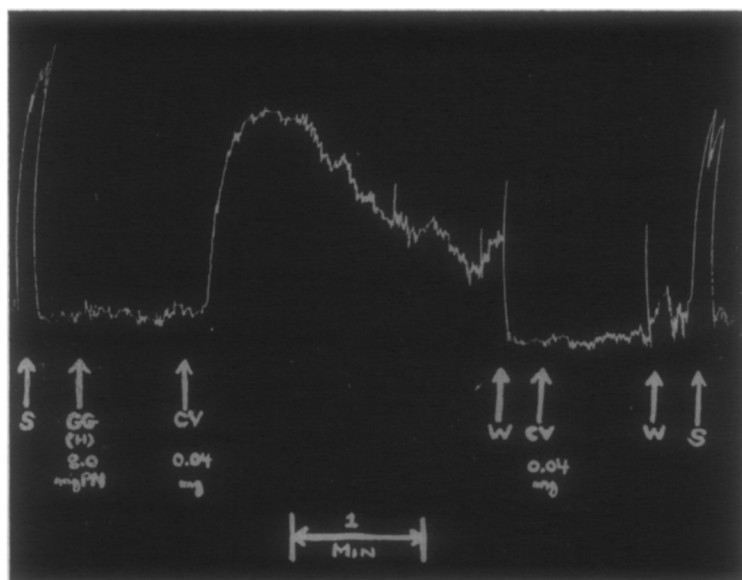


Fig. 4.—Absence of protection by pooled human gamma globulin (G.G. [H] 8.0 mg. protein nitrogen) against the anaphylactoid effect of cobra venom (CV 0.04 mg. of dried venom) upon isolated suspended segment of normal guinea pig ileum.

Note absence of response to second addition of venom (tachyphylaxis).

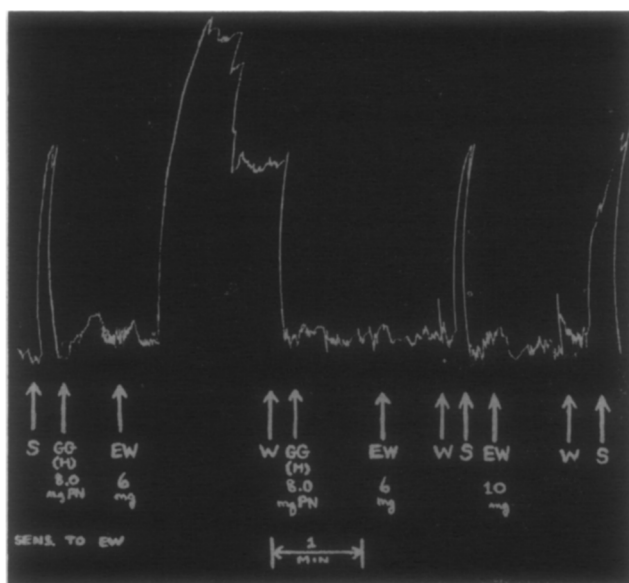


Fig. 5.—Absence of protection by pooled human gamma globulin (G.G. [H] 8.0 mg. protein nitrogen) against anaphylaxis in isolated suspended segment of ileum from guinea pig previously sensitized to egg white.

Note initial maximum anaphylactic response to 6.0 mg. of egg white (EW) and lack of response (desensitization) to second addition of egg white in presence of gamma globulin. Desensitization is complete in the presence of gamma globulin, since there is no response to 10.0 mg. of egg white after the gamma globulin is removed.

Twenty-five per cent solutions of glycerin, egg white, and egg yolk had no protective effect. Cobra venom was used in sufficient dosage to cause an anaphylactoid contraction and, following the demonstration of tachyphylaxis to venom, the ileum still reacted typically to staphylococcal bacterial extract or fraction A (F-10).

Neither serum nor gamma globulin conferred protection against the myotonic effects on the guinea pig ileum of staphylococcal culture filtrate (Fig. 3), cobra venom (Fig. 4), or histamine.

It was also found that neither serum nor gamma globulin had any noticeable effect upon anaphylaxis in the isolated guinea pig ileum from animals sensitized to egg white (Fig. 5), streptococcal proteinase, or two protein fractions derived from a nonpathogenic strain (F-7) of staphylococcus isolated in our laboratory and prepared as previously reported.^{1, 3}

DISCUSSION

We have found that serum of several animal species protects the isolated guinea pig ileum against the anaphylactoid reaction regularly produced by a nucleoprotein fraction derived from washed, ground *Staphylococcus aureus* organisms. The protective effect is confined to the gamma globulin fraction. It is present in sera from all species thus far tested except the rat. The protective substance is stable at 56 degrees for thirty minutes. Its absence in rat serum apparently eliminates the properdin system from consideration as a factor in this phenomenon.⁴

The protection is specifically directed against the anaphylactoid effects of fraction A. Serum or gamma globulin has no effect upon other myotonic agents similarly studied, such as hemolytic staphylococcal culture filtrate, cobra venom, or histamine.

Of particular interest are the two instances of "indeterminate protection" (Fig. 2), in which gamma globulin failed to protect the segment of guinea pig ileum against the toxic fraction A, but in which, after washing, a second and third addition of fraction A without gamma globulin resulted in a typical anaphylactoid reaction with tachyphylaxis. If there had been no protection, one would have expected the ileum to be refractory to subsequent additions of fraction A. This could represent a situation in which only partial protection was conferred by the gamma globulin with incomplete tachyphylaxis, and therefore subsequent addition of fraction A caused a contraction. Another possible explanation is that the ileum was from an animal which had become hypersensitive, through natural means, to fraction A or a closely related protein. If this were so, the contraction of the ileum when fraction A was added in the presence of gamma globulin would represent an anaphylactic reaction, the gamma globulin protecting against the toxic effect of the fraction A, but allowing the anaphylactic reaction to occur. Subsequent addition of fraction A in the absence of gamma globulin would represent the toxic effect of fraction A. This explanation correlates with our findings that gamma globulin did not protect against anaphylaxis in the isolated ileum. It also fits in with the fact that the two segments of ileum that reacted in this way were adjacent

segments from the same animal. The third adjacent segment did not so react, but we have found, in confirmation of Kempf and Feinberg,⁵ that if adjacent segments of ileum from sensitized animals are tested, some nonreactive segments occasionally can be found. The fact that the guinea pig from which these two segments of ileum demonstrated this unusual type of "indeterminate" protection had been made hypersensitive to egg white by prior injections is probably unrelated to the phenomenon, since segments of ileum from other guinea pigs, similarly sensitized to egg white, reacted no differently from segments from normal animals.

If these two instances of "indeterminate" protection are due to hypersensitivity to fraction A, as postulated, it should be possible to induce and demonstrate anaphylaxis to this fraction, despite its primary toxicity, by prior addition to the bath of gamma globulin, which would protect the ileum segment against the toxic effect of fraction A while permitting the anaphylactic reaction to occur. Such studies are in progress.

CONCLUSION

A heat-stable factor in human, horse, rabbit, bovine, and guinea pig serum protects the isolated guinea pig ileum against the anaphylactoid effects of a toxic nucleoprotein fraction derived from washed, ground *Staphylococcus aureus* organisms. Rat serum was the only serum studied that failed to protect. The protective effect is found almost exclusively in the gamma globulin fraction, and possibly to some extent in the beta globulin fraction.

The protective effect is specific, since it is not directed against certain other myotonic agents, such as hemolytic staphylococcal culture filtrate, cobra venom, or histamine. Neither whole serum nor gamma globulin protects against anaphylaxis in the isolated guinea pig ileum.

It is suggested that gamma globulin, by protecting the ileum against the anaphylactoid effect of the nucleoprotein fraction and allowing anaphylaxis to occur, may make it possible to demonstrate anaphylaxis in animals rendered hypersensitive to this primarily toxic fraction.

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