PRODUCTION OF THE SHWARTZMAN PHENOMENON BY A SINGLE-INJECTION TECHNIC

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Plates 38 and 39

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The basic local Shwartzman reaction (1) is elicited in rabbits by two injections of potent bacterial toxins: the first, preparatory, is administered intracutaneously; the second, provocative, is given intravenously about 24 hours following preparation. Three to 4 hours later, a hemorrhagic necrotizing lesion develops at the site of preparation.

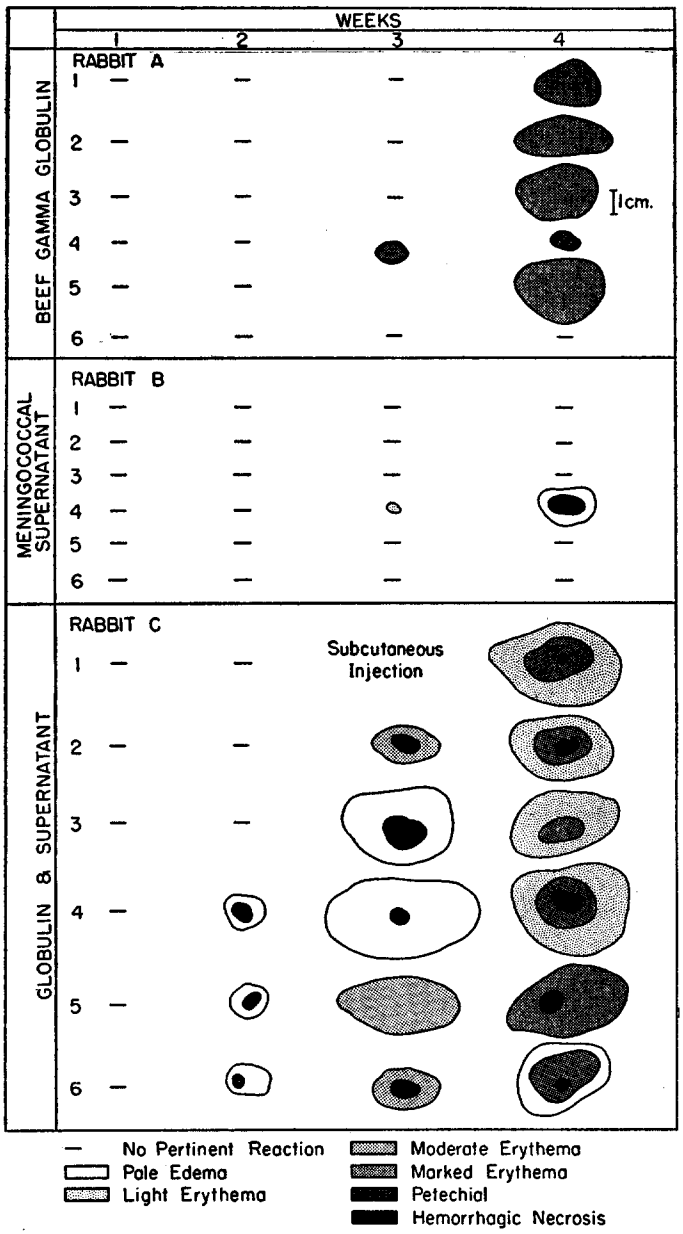
While various materials (1, 2) have been utilized to prepare and provoke the phenomenon, all investigators have used the method outlined above. Recently (2) in a special case, the sequence of injections has been reversed.

The non-specificity of the phenomenon insofar as the preparatory and provocative materials are concerned (as contrasted to the strict specificity of allergic reactions) as well as the wide range of substances capable of preparing and/or provoking the phenomenon suggests that under several circumstances the Shwartzman phenomenon may play an important role in spontaneous disease (1, 3).

Using rabbits rendered hypersensitive to non-bacterial proteins, Shwartzman (1) was able to elicit the reaction by preparing the skin with bacterial toxin, followed, after a suitable interval, by the intravenous inoculation of the homologous protein antigen. The same result could be achieved in a prepared non-sensitized animal if the *in vitro* precipitate of an antigen-antibody reaction was inoculated intravenously. From the numerous experiments reported, it is clear that the product or products of *in vitro* or *in vivo* antigen-antibody reaction are able to provoke the Shwartzman phenomenon. Shwartzman found that a minimum of 6 hours must separate the cutaneous preparation with bacterial toxin and the intravenous injection of homologous non-bacterial protein antigen if a successful result was to be achieved, thus maintaining the pattern of two inoculations, one intradermal, the second intravenous. The purpose of this report is to detail experiments demonstrating the production, in hypersensitive rabbits, of the cutaneous Shwartzman phenomenon by means of a single intracutaneous inoculation.

EXPERIMENTS

The basic experiment was carried out upon 18 rabbits of assorted breeds and sex, weighing between 2000 and 2500 gm. These were divided into three groups of six. Each animal (Text-fig. 1) of control group A received intradermally, at weekly intervals, 0.05 cc. of 3.0 per cent sa-



TEXT-FIG. 1. Experiments A, B, C. All sketches were made 24 hours after inoculation.

Experiment A illustrates the time required to produce the Arthus phenomenon and the extent of reaction achieved by means of small (1.75 mg.) weekly intradermal doses of a purified beef gamma globulin.

Experiment B depicts the relative difficulty with which meningococcal supernatant produces the Arthus reaction by the same method as in Experiment A.

Experiment C shows the reactions elicited by combining the materials of A and B in weekly intradermal inoculations. The lesions are interpreted as Shwartzman phenomena prepared by the meningococcal supernatant and provoked by the interreaction of the globulin (antigen) and its homologous antibody.

line solution of purified beef gamma globulin.[1] Each inoculation was made at a different site in the clipped and depilated abdomen. Control group B was treated in the same way but with inocula of 0.05 cc. of undiluted, sterile, potent *Neisseria meningococcus* supernatant fluid (4).

Group C received a mixture of 0.05 cc. of the 3.0 per cent globulin and 0.05 cc. of the meningococcal supernatant intradermally.

The results of this experiment, insofar as the globulin control is concerned, fulfilled all expectations. Only following the third inoculation did the Arthus reaction begin to manifest itself and then only minimally in one animal: A4 (Fig. 1 A). Twenty-four hours after the fourth injection, 5 of the 6 showed characteristic areas of erythematous induration with petechiae (Fig. 1 B).

Group B, receiving meningococcal supernatant, showed that this preparation is a poor antigen as compared to the globulin, since only one animal developed a clear-cut Arthus reaction and this only after the fourth treatment (Fig. 1 C). The undiluted meningococcal supernatant is highly irritating and when injected in amounts of 0.05 cc. produces, in previously untreated animals, areas of edema measuring up to 4 cm. in greatest dimension with more or less erythema but never hemorrhage or necrosis. Since this response is not one of hypersensitivity, it is indicated in the text-figures with a minus sign.

Group C, in contrast to A and B, shows an early and amplified response to the mixed globulin and meningococcal supernatant. Following the second injection, 3 of the 6 animals showed clear-cut hemorrhagic lesions. After the third inoculation, 4 of 5 rabbits (C1 was given its inoculum subcutaneously) developed large hemorrhagic necrotizing lesions (Fig. 1 D) similar in all respects to a major Arthus reaction or a Shwartzman phenomenon. The response to the fourth injection was hemorrhagic necrosis in all 6 animals.

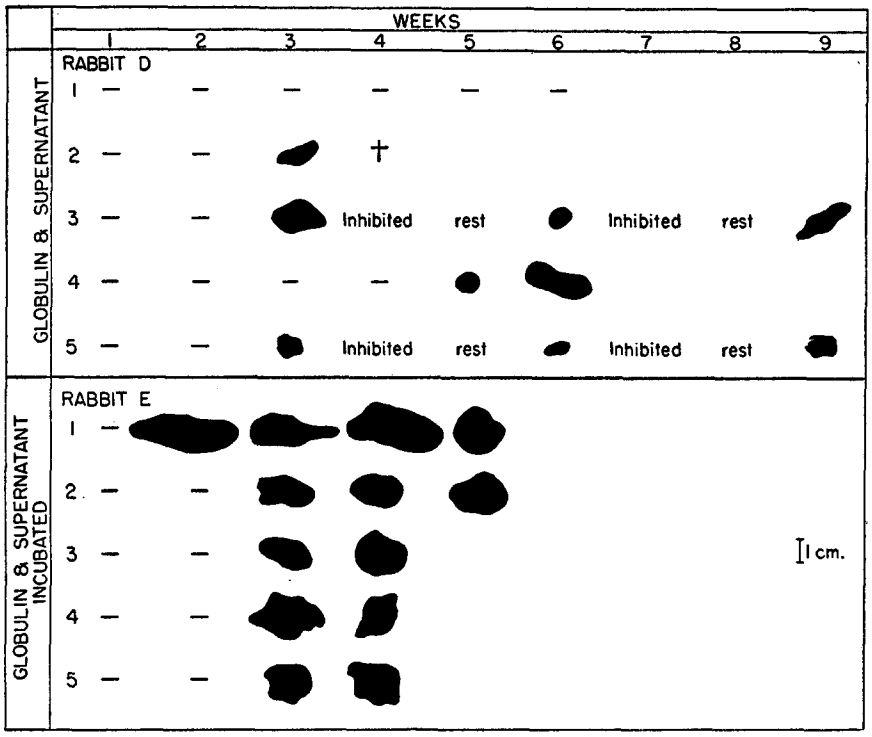
To check these results and investigate the mechanism (Arthus or Shwartzman reaction) the two following experiments were performed and are recorded in Text-fig. 2.

Group D received weekly intradermal inoculations of 0.05 cc. of 3 per cent purified beef gamma globulin plus 0.05 cc. undiluted meningococcal supernatant mixed just prior to inoculation. Rabbit 1 failed to respond even after 6 injections; No. 4 only with the fifth. Animals 2, 3, and 5, however, reacted upon the third inoculation with characteristic hemorrhagic necrosis at the injection sites (Fig. 1 E). The following week these three were given an intravenous inoculum of 5.0 cc. of the 3 per cent beef gamma globulin. About 5 minutes later rabbit 2 died in anaphylactic shock; the other two survived. Four hours later the intradermal injection of mixed globulin and undiluted meningococcal toxin was made. Both animals failed to develop any reaction other than the usual non-specific edema as produced by the toxin in previously untreated animals. After an interval of 2 weeks the rabbits were reinoculated intradermally with the combined materials and yielded hemorrhagic necrotic lesions. As can be seen in Text-fig. 2, this sequence was reproducible.

In group E, inoculated with the mixed material incubated at 37.0°C. for 7 days and tested for sterility, animal 1 reacted promptly upon the second injection with an extensive reaction

[1] Courtesy of Dr. J. B. Lesh, Armour Laboratories, Chicago.

(Fig. 1 F). It must be emphasized that this animal received in its first and only sensitizing injection 1.75 mg. of the beef globulin. All animals responded characteristically thereafter to each weekly injection.



TEXT-FIG. 2. Experiments D, E. These experiments confirm Experiment C. The reactions in D are the result of single weekly intradermal inoculations of combined purified beef gamma globulin and meningococcal supernatant. In E the material was incubated for 7 days prior to use. Only the areas of cutaneous hemorrhage are shown, the surrounding erythema and edema being ignored. All tracings were made 24 hours after injection.

In Experiment D, rabbit 1 was completely refractory, rabbit 4 was relatively refractory. Animals 3 and 5 illustrate the fact that the reaction may be entirely inhibited by intravenous inoculation of homologous antigen (blocking antigen) 4 hours prior to intradermal injection.

Experiment E illustrates in rabbit 1 that a major Shwartzman phenomenon may be elicited 1 week after a single intradermal sensitizing dose of 1.75 mg. of antigen.

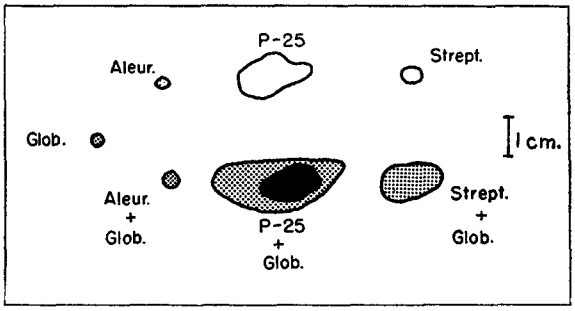
A third group of experiments was carried out to investigate several uncontrolled factors in the preceding experiments.

Experiment F was performed on 4 rabbits, each of which received a sensitizing intravenous injection of 1.0 cc. of the beef gamma globulin solution. Thirteen days later the depilated abdomen was injected intradermally, at different sites, with the following materials: 0.05 cc. of the globulin solution, 0.05 cc. of a 5 per cent suspension of aleuronat, 0.05 cc. of a solution

containing 50 gamma of Shear's polysaccharide P25,[2] and 0.05 cc. of an undiluted *Streptococcus viridans* filtrate. Paired with the last three were injections of the same materials in identical quantities, each mixed with 0.05 cc. of the globulin solution.

It was determined that 50 gamma of P25 could regularly prepare rabbits for the Shwartzman phenomenon provoked by meningococcal supernatant. P25 is also a highly potent and regular provocator of the reaction in doses of 400 gamma.

The streptococcus was a stock organism of the Department of Bacteriology, Duke University. It was grown on brain-heart infusion blood (beef) agar medium for 48 hours, washed off with saline, centrifuged, and the supernatant passed through a Seitz EK filter No. 6 and bottled with 0.05 per cent phenol. It was weakly preparatory in doses of 0.2 cc. (one of 3 animals showed a poor positive response when provoked with meningococcal supernatant). In doses of 2.0 cc. it did not provoke the phenomenon when prepared with a large dose of meningococcal supernatant.



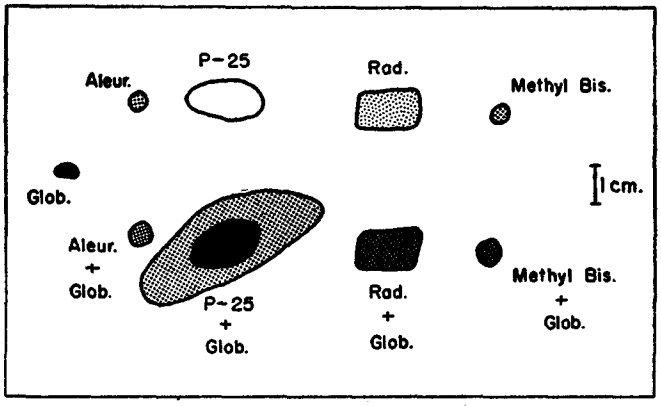
TEXT-FIG. 3. A characteristic reaction pattern in a hypersensitized rabbit is illustrated. Glob. is an Arthus reaction at the site of homologous antigen injection: a purified beef gamma globulin. The upper three sketches indicate the extent and degree of reaction (for key see Text-fig. 1) to aleuronat (aleur.), *Serratia marcescens* filtrate of Shear (P25), and an alpha hemolytic streptococcus filtrate (strept.). The three lower sketches demonstrate the extent and degree of reaction of the skin to the same substances when mixed with the homologous antigen (glob.).

Twenty-four hours after the injections 3 rabbits showed no significant reactions, the fourth (Text-fig. 3) revealed: a small Arthus reaction at the site of globulin injection; an acute erythematous inflammation about the aleuronat particles; surrounding the mixed aleuronat and globulin depot was a very narrow hemorrhagic margin measuring not more than 1 mm. in width. The P25 site showed the usual non-specific pale edema characterizing its inoculation into virgin animals; however, the P25-globulin zone was converted into a characteristic area of hemorrhagic necrosis. The site of the *Streptococcus viridans* filtrate was difficult to find and was finally identified as a small area of edema. The mixed streptococcus filtrate and globulin elicited a moderately severe erythematous reaction without, however, a hemorrhagic component.

As a check upon the above a final experiment, G, was undertaken.

[2] P25, donated by Dr. M. J. Shear, Chief of Chemotherapy Section, National Cancer Institute, Bethesda, is a highly purified polysaccharide obtained from *Serratia marcescens* grown on a synthetic medium.

At weekly intervals 4 rabbits were given 3 sensitizing intradermal inoculations (each 0.1 cc. of 3.0 per cent bovine gamma globulin). About 10 days after the final injection each was treated in the following manner (Text-fig. 4): aleuronat, injected intradermally in the same dose as in Experiment F; radiation, rectangle of skin 1 × 1.5 cm. exposed to ultraviolet rays at 30 inches for 45 seconds; and methyl-bis (β-chloroethyl) amine hydrochloride (nitrogen mustard), 0.001 mg. intradermally in 0.05 cc. of saline. All irritants were administered in their respective dual sites. Twenty-four hours later all areas were inflamed but neither hemorrhagic nor necrotic. At this time the P25 and 0.05 cc. of the gamma globulin was inoculated into the indicated sites. One and one-half hours postinoculation the sites prepared with aleuronat-globulin, radiation-globulin, and nitrogen mustard-globulin were developing a deep erythema. The P25-globulin area was edematous but not erythematous or hemorrhagic. By 3 hours postinjection (Text-fig. 4, Fig. 2 A) the P25-globulin zones in 2 animals showed typical hemorrhagic necrotic lesions. The P25 areas showed characteristic non-specific pale edema.



TEXT-FIG. 4. Experiment G confirms and extends the results of Experiment F. The symbols and titles are the same as in Text-fig. 3. Rad. is the site of an erythema dose of ultraviolet radiation, methyl-bis the locus of a depot of 0.001 mg. of methyl-bis (β-chloroethyl)-amine hydrochloride (nitrogen mustard).

The pure globulin sites had developed into small Arthus reactions. Two of the animals remained negative in all sites, showing only erythema in the aleuronat, radiation, and nitrogen mustard sites and edema in the P25 and P25 plus globulin sites.

A further maneuver was carried out on the 4 animals of this experiment.

After the Arthus and Shwartzman reactions had clearly manifested themselves in their respective sites (4 hours following the globulin and P25 injections) 0.8 cc. of potent meningococcal supernatant was inoculated into each intravenously. Twenty hours later the two positive reactors were found dead, presenting the remarkable appearance illustrated by Fig. 2 B. All sites previously treated with homologous beef globulin antigen along with the P25 control area were converted into characteristic Shwartzman lesions. In marked contrast, the areas in which no homologous antigen or Shwartzman preparatory substance had been introduced: aleuronat, ultraviolet radiation, and methyl-bis (β-chloroethyl) amine hydrochloride, were unchanged. The somewhat more prominent appearance of the petechiae in these three sites in Fig. 2 B as compared to Fig. 2 A is due to three factors: (1) the animal

is dead and as a consequence erythema tends to disappear, heightening the contrast between hemorrhage and the surrounding skin; (2) Fig. 2 B was exposed longer than Fig. 2 A; and (3) the animal in B was photographed closer than the one in A, thus bringing details into greater prominence.

The two non-reactors survived; the only change was the appearance at the P25 sites of typical Shwartzman phenomena.

DISCUSSION

Experiments A through E indicate that mixture of a protein antigen with a Shwartzman potent toxin injected intradermally into an appropriately hypersensitized rabbit regularly results, when fully developed, in an extensive hemorrhagic necrotizing lesion. This is identical in appearance with a Shwartzman reaction or a very severe Arthus reaction. Despite the gross and microscopic morphologic similarity, for the reasons following, it is our studied opinion that the lesions obtained are Shwartzman phenomena.

Experiment A illustrates the minimal time interval necessary to elicit sufficient hypersensitivity to produce a minimal Arthus reaction (Text-fig. 1 A) by means of weekly intradermal inoculations of small amounts of a good antigen: 3 weeks. The fact that a very much more severe lesion (Fig. 1 F) can be elicited by the same dose of homologous antigen when combined with a Shwartzman preparatory substance, as in Experiments C and E, after a preliminary preparation of only one intradermal inoculation of 1.75 mg. of the antigen would appear to adequately exclude from consideration the Arthus phenomenon (5).

Experiments F and G illustrate the dispensability of preliminary treatment with Shwartzman toxin. The animals of Experiment F were prepared with a single intravenous injection of purified beef gamma globulin, and in Experiment G with a series of 3 weekly intradermal inoculations of the same substance. Nevertheless, the reactions followed promptly upon the introduction of the mixed antigen-bacterial toxin complex.

The same two experiments, F and G, indicate that the reaction is not simply the summation of injury by an antigen-antibody reaction and any inflammation. The clear-cut lesions produced by aleuronat, radiation, and nitrogen mustard were accentuated by the antigen-antibody response (Arthus phenomenon as illustrated by the reaction to the pure globulin). The sum total of tissue injury, however, was insignificant when compared to that elicited by the combined antigen and bacterial toxin. When the three toxins,[3] originating from *N. meningococcus*, *S. marcescens* (P25), and alpha hemolytic streptococcus, were tested for potency it was found that the meningococcal toxin and P25 were excellent preparatory and provocatory agents, whereas the streptococcal toxin was weakly preparatory and possessed no provocatory power in intrave-

[3] A filtrate prepared from an *Escherichia coli* culture capable of both preparing and provoking the Shwartzman phenomenon was successfully substituted for both the meningococcal and P25 substances in experiments similar to D and G.

TABLE I

Blood Serum Precipitin Titre

Experimental group and antigen	Rabbit No.	Weeks				
		1	2	3	4	5
A	1	—	1:2	1:4	1:16	1:16
Globulin	2	—	1:16	1:16	1:16	1:16
	3	—	—	—	—	—
	4	—	1:8	1:8	1:32	1:16
B	1	—	—	1:2	—	—
Supernatant	2	—	—	—	—	—
	3	—	—	—	—	—
	4	—	—	—	—	—
A B	1	—	1:4	1:1	1:8	1:16
Globulin	2	—	—	—	1:4	1:8
	3	—	1:8	1:2	1:4	1:16
	4	—	—	1:4	1:4	1:8
A B	1	—	—	—	—	—
Supernatant	2	—	—	—	—	—
	3	—	—	—	—	—
	4	—	—	—	—	—
IV	1	—	1:4	1:8	1:16	1:16
Globulin	2	—	†			
	3	—	1:16	1:8	1:16	1:32
	4	—	1:8	1:8	1:16	1:32
IV	1	—	—	—	—	—
Supernatant	2	—	†			
	3	—	—	—	—	—
	4	—	—	—	—	—

Weekly titres of serial diluted rabbits' serum precipitated by standardized antigen solutions.

Group A, sensitized with weekly injections of 0.05 cc. of a 3 per cent solution of purified beef gamma globulin.

Group B, inoculated weekly with 0.05 cc. of a 1:3 saline dilution of meningococcal supernatant (4).

Group A B, inoculated weekly with a 1:1 mixture of the above globulin and supernatant, incubated together for 7 days.

Group IV, inoculated with 0.05 cc. each of the globulin and supernatant mixed just prior to injection.

—, no reaction.

†, died, cause undetermined.

nous doses as high as 2.0 cc. per rabbit. It is apparent, therefore, that the lesions interpreted as Shwartzman phenomena must be prepared by the bacterial

toxin,—since the strain of streptococcus used possesses little or no provocatory potency,—and must therefore be provoked by the antigen-antibody reaction. These results confirm in full Shwartzman's experience and conclusions (1).

The intravenous injection of the meningococcal supernatant into the 3 animals of Experiment G during the development of the Arthus and the Shwartzman (P) reactions illustrates the ability of the meningococcal material simultaneously to provoke and to prepare the Shwartzman reaction (Text-fig. 4 and Fig. 2). The P25 control area was provoked, and the sites of active antigen-antibody reaction prepared by the same inoculum of meningococcal toxin. The tremendous reactions in these sites stand in marked contrast to the unaltered areas of injury produced in this case by the non-antigenic and non-Shwartzman-phenomenon-preparatory aleuronat, ultraviolet radiation, and nitrogen mustard. Shwartzman (1) has demonstrated that meningococcal toxin selectively concentrates into areas of injury, and if that injury be due to an antigen-antibody reaction, the response may be, as in our experiment, a hemorrhagic necrotizing lesion. From just such experiments, Shwartzman interprets some examples of so called Arthus phenomena in man as representing rather instances of the Shwartzmann phenomenon. These occur in individuals suffering from acute infections who are treated with an antigen to which they have previously been sensitized.

Experiment A demonstrates the indispensability of the antigen-antibody reaction to the production of this form of the Shwartzman phenomenon. Blocking the antibodies by an intravenous dose of the homologous antigen during the 4th and 7th weeks led to complete inhibition of the phenomenon. This also confirms Experiment B anent the poor antigenicity of the meningococcal supernatant since it, being uninhibited, did not produce any significant reaction of hypersensitivity. In the 6th and 9th weeks, respectively, allowing time for the animal to rid itself of blocking antigen, the reactions could again be elicited in the usual manner.

Still another experiment modeled after Experiment G (Text-fig. 1) was carried out with weekly antibody precipitin titrations by means of a constant dose of optimally diluted antigen against serial dilutions of the rabbits' serum. Step dilutions of serum made up to 0.5 cc. with normal saline solution were added to 0.5 cc. of a 1:256 normal saline dilution of 3.0 per cent bovine gamma globulin. The tubes were left at room temperature, approximately 21°C., for 3 hours and the results then read. The results as summarized in Table I indicate that neither the globulin nor the Shwartzman toxin is a potentiated antigen when mixed just prior to inoculation or incubated for 7 days before use.

CONCLUSIONS

In the hypersensitive animal a single inoculation of homologous antigen mixed with Shwartzman preparatory toxin may lead to the production of a

Shwartzman reaction which functions as an amplifier of the antigen-antibody tissue response.

The combination of antigen and bacterial toxin does not alter the antigenicity of purified bovine gamma globulin.

This form of the Shwartzman phenomenon may be useful in the investigation of hyperergic tissue reactions.

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EXPLANATION OF PLATES

PLATE 38

FIG. 1. A, a photograph of the only Arthus phenomenon elicited by a purified beef gamma globulin in the 3rd week of Experiment A (animal 4, Text-fig. 1).

B, another Arthus reaction in Experiment A, animal 5 at the 4th week. This represents the severest reaction of this experiment (Text-fig. 1).

C, the severest Arthus phenomenon elicited by the meningococcal supernatant in Experiment B (animal 4, 4th week, Text-fig. 1).

D, In contrast to A, B, and C above this photograph illustrates the extent and degree of the cutaneous reaction in the 3rd week to the combined materials of Experiment C. It is interpreted as a Shwartzman phenomenon (Experiment C, animal 5, 3rd week, Text-fig. 1).

E, depicts the Shwartzman reaction of rabbit 3 in Experiment D (3rd week, Text-fig. 2, 1:1).

F, the Shwartzman phenomenon of rabbit 1, Experiment E, in the 2nd week (Text-fig. 2).

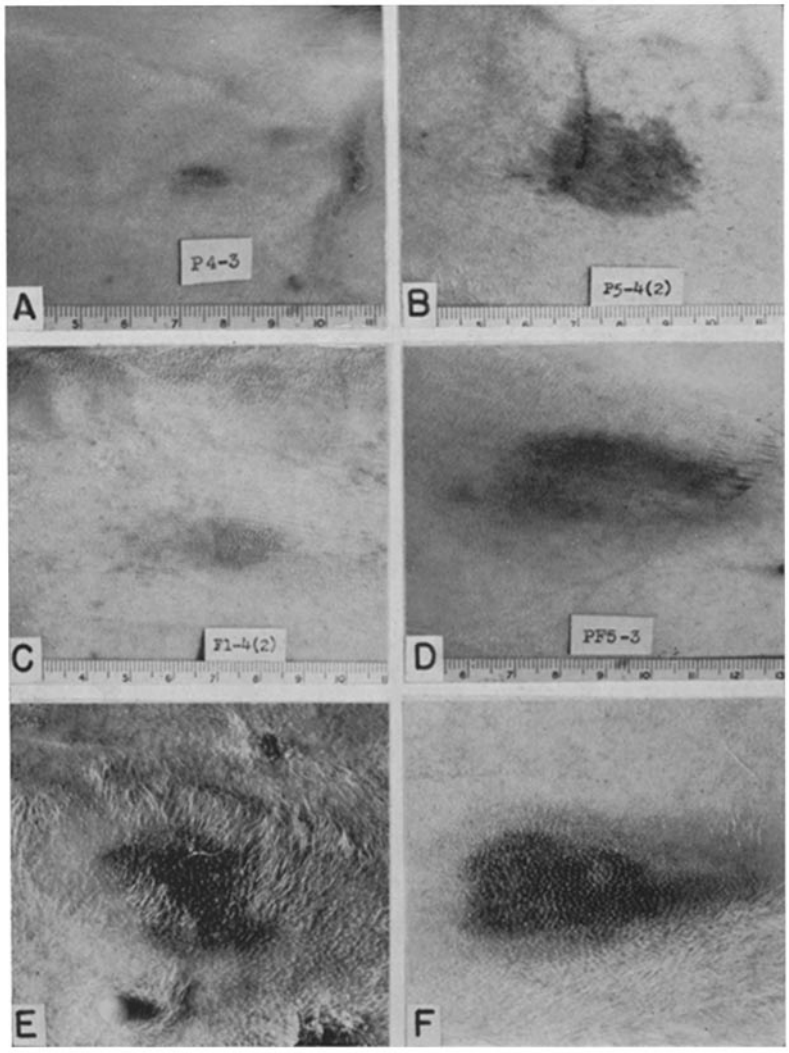

FIG. 1

(Black-Schaffer *et al.*: Production of Shwartzman phenomenon)

PLATE 39

FIG. 2. A, the abdomen of a hypersensitized rabbit, from Experiment G, illustrating an Arthus reaction at *G* (the surrounding intense erythema is an artefact produced by shaving). The lesions above the lettering are the control sites; those beneath the lettering are combined with the homologous antigen, beef gamma globulin *P* = P25 *A* = aleuronate, *R* = ultraviolet radiation, *M* = methyl-bis (β-chloroethyl) amine hydrochloride. The Shwartzman reaction produced by the combined inoculation of P25 and homologous antigen is seen beneath *P*. The relatively mild reactions of all other irritants are clearly seen.

B, the same animal after the intravenous inoculation of 0.8 cc. of potent meningococcal supernatant. All sites of antigen inoculation as well as the P25 control area are converted into Shwartzman lesions. The control non-antigenic and/or Shwartzman preparatory irritants, *A*, *R*, and *M* sites, are unchanged.

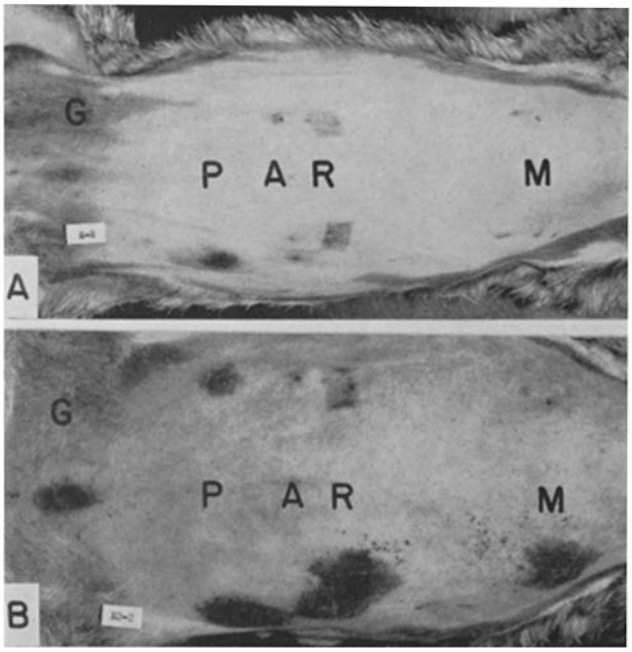

FIG. 2

(Black-Schaffer *et al.*: Production of Shwartzman phenomenon)