HEMORRHAGES IN SKIN LESIONS OF GUINEA PIGS FOLLOWING INTRAVASCULAR INJECTION OF TOXINS (SHWARTZMAN PHENOMENON)

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In 1924 Sanarelli (1) reported that rabbits infected with cholera vibrios react to *B. coli* or *B. proteus* in a way entirely different from that of normal rabbits. In a study on the virulence of cholera vibrios Sanarelli observed that in every large group of rabbits a few would die from the injection of minute doses, much smaller than the dose lethal for most rabbits. The symptoms resembled anaphylactic shock. In order to explain the mechanism of this phenomenon, Sanarelli studied the fate of cholera vibrios injected into the circulation. He found that the cholera vibrios disappeared from the blood stream within 2 days after their injection. But he also observed that in the rabbits which succumbed following the injection of sublethal doses, *B. coli* or *proteus* bacilli would invade the blood stream. Sanarelli now injected rabbits intravenously with minute doses of cholera vibrios and on the following day gave an intravenous injection of either cholera vibrios or of colon or *proteus* bacilli. Almost all of the rabbits died within from a few hours to 2 days after the second injection. The symptoms and pathological findings were the same for all three kinds of bacteria. Evidently the first injection of cholera vibrios prepared the rabbits, made them susceptible to a second, the injury-producing,[1] injection of bacteria that by themselves were harmless.

In 1927 Shwartzman (2) reported his observations on the local skin reactivity to filtrates of *B. typhosus* cultures. He injected these filtrates into the skin of rabbits and introduced the same material 24 hours later into an ear vein. In the skin at the site of the injection a severe hemorrhagic necrosis appeared from 4 to 5 hours after the second injection. The intensity of the hemorrhagic necrosis did

[1] The "preparatory" factor is the first, the intracutaneous, injection of toxin. The "injury-producing" factor is the second, the intravascular, injection of toxin. The terms "preparatory" and "injury-producing" factors or agents suggest that the preparatory factor does not cause injury. The experiments of Hanger and those reported in the present paper, however, show that the production of injury is essential in the action of the preparatory factor. These terms are employed in this publication because they are generally used in the literature.

not correspond to the severity of inflammation caused by the bacterial filtrate in the skin. Repeated injections into the same area of skin did not result in hemorrhagic necrosis. Turpentine or streptococcus filtrate did not prepare the skin (3).

One month after the publication of Shwartzman's report Hanger (4) published his studies. He observed that when a positive skin test with a filtrate from *Bacterium lepisepticum* is followed by the intravenous injection of the same material, an intensification of the local reaction with bluish discoloration and petechial hemorrhages occurs. Histological examination of the skin lesion showed intense swelling, disruption of the capillary endothelium, and minute thrombi in the blood vessels. The intensity of the hemorrhagic reaction is roughly proportional to the intensity of the inflammation caused by the intracutaneous injection of the filtrate. The hemorrhagic reaction is non-specific since inflammation of the skin induced by a filtrate from streptococci became hemorrhagic after an intravenous injection of a filtrate from *B. lepisepticum.*

The purpose of the present study is to inquire into the nature of the hemorrhagic reaction, particularly to ascertain whether this reaction is limited to the action of bacterial toxins or can also be produced by inflammatory irritants not related to bacterial toxins. In the present paper experiments on guinea pigs are reported.

The Hemorrhagic Reaction in the Guinea Pig

The Shwartzman reaction in its original form has been studied mainly in rabbits. Gratia and Linz (5) reported that rats and mice are refractory and occasional guinea pigs are susceptible. Gross (6) never succeeded in producing the Shwartzman phenomenon in the guinea pig. In view of this contradiction, we injected twelve guinea pigs with filtrate from *B. typhosus*, found highly potent in rabbits. 0.2 cc. of the filtrates was injected into the skin and 1 cc. into the heart of the guinea pigs. None of them reacted with hemorrhage.

The Effect of Intravascular Injection of Bacterial Toxins upon the Specific Inflammation (Arthus Phenomenon) in Guinea Pigs

It was observed recently (7) that the intravascular injection of a filtrate from the culture of typhoid bacilli produces hemorrhagic necrosis in tuberculous guinea pigs at the site of tuberculin reactions. In normal guinea pigs the areas of skin injected with tuberculin were not affected by a subsequent injection of typhoid filtrate. Hence it is

probable that the inflammation produced by tuberculin in the hypersensitive tissue rather than products of the tubercle bacillus *per se* prepared the skin for the action of the typhoid toxin introduced into the blood stream.

In connection with this observation, the question arises whether injury produced by substances other than tuberculin would prepare the skin in guinea pigs. We have studied the action of various antigenic and non-antigenic substances, first of all that of horse serum in the sensitized guinea pig. It is interesting to compare from this point of view the tuberculin reaction with the Arthus phenomenon, for these two specific inflammations are different from each other in many respects.

The tuberculin reaction in guinea pigs is a "delayed" reaction (Zinsser (8)). It appears later than 12 hours and reaches its maximum usually only in 48 hours after the injection. In addition to redness and edema, sometimes purple discoloration is seen and the reaction often becomes necrotic. It cannot be transferred passively. In contrast to the tuberculin reaction, the reaction to horse serum (or to other proteins) is "immediate" (Zinsser). It appears within 2 hours after the injection and reaches its maximum often within 24 hours. It is characterized by redness and edema, purple discoloration and necrosis being conspicuously absent. It can be transferred with immune serum rich in precipitins. Zinsser pointed out that the two types of skin reaction, immediate and delayed, can be distinguished in the guinea pig but not in the rabbit.

We sensitized five guinea pigs to horse serum by injecting 0.1 cc. of horse serum into the subcutaneous tissue three times, 3 days apart. The guinea pigs were tested several times with various dilutions of horse serum. 1 day after the injections of 0.1 and 0.01 cc. of horse serum, redness and swelling was noted at the sites of the skin tests. Hemorrhage was not produced in any of the guinea pigs by the intracardial injection of typhoid filtrate. In guinea pigs sensitized to horse serum, therefore, the specific inflammation in the skin did not act as a preparatory factor.

The Effect of Intravascular Injection of Typhoid Toxins upon the Inflammation Caused by Diphtheria Toxin

The inflammation that is produced by diphtheria toxin injected into the skin and the tuberculin reaction are similar in many aspects.

Both reactions develop slowly, reach their maximum after 2 days, and may be hemorrhagic and necrotic. This similarity suggested that diphtheria toxin might act as a skin preparatory agent.

TABLE I

Diphtheria Toxin as Skin Preparatory Agent in the Guinea Pig

Guinea pig No.	Preparatory agent, 0.1 cc. diphtheria toxin	Injury-producing agent, typhoid toxin	Hemorrhagic reaction
		cc.	
1	1:250 1:500	1	Present
2	1:250 1:500	0.5	"
3	1:400 1:800	1	Absent
4	1:400 1:800	0.5	"
5	1:400 1:800	0	Hemorrhagic before injection of typhoid toxin
6	1:500 1:750	1.5	Hemorrhage increased after the injection of typhoid toxin
7	1:500 1:750	1.5	Present
8	1:1,000 1:5,000 1:25,000	1	Absent
9	1:1,000 1:5,000 1:25,000	1	"

In the experiments with diphtheria toxin, nine guinea pigs were employed. The strength of this diphtheria toxin[2] was 0.0016 cc. 1 M.L.D., 0.15 cc. L+ dose. The dilutions of diphtheria toxin ranged from 1:250 to 1:25,000. Eight of the

[2] Obtained through the courtesy of Dr. J. Reichel of the H. K. Mulford Co.

guinea pigs received intracardial injections of a typhoid filtrate 1 day after the intracutaneous injections of diphtheria toxin; one of the guinea pigs was injected with diphtheria toxin alone.

Table I shows that in two guinea pigs, Nos. 5 and 6, the reactions to the toxin were hemorrhagic, even without the subsequent injection of a bacterial filtrate. One of these two animals, No. 6, reacted with an increase of hemorrhage after the intracardial injection of typhoid filtrate. The other seven guinea pigs showed no purple discoloration 1 day after the injection of diphtheria toxin. When the guinea pigs received intracardial injections of from 0.5 to 1.5 cc. of typhoid filtrate, four of six of those prepared with toxin dilutions varying from 1:250 to 1:750, and none of those injected with toxin dilutions from 1:1,000 to 1:25,000, reacted with hemorrhage. Diphtheria toxin in the guinea pig acts as a skin preparatory factor.

Turpentine, Broth, and Silver Nitrate as Preparatory Factors

Three sterile inflammatory irritants, namely turpentine, concentrated broth, and silver nitrate, non-antigenic in nature, were examined as to their capacity to act as skin preparatory agents.

A 5 per cent solution of turpentine in paraffin oil was injected into the skin of nine guinea pigs. On the day following the injections redness, edema, and necrosis were found in all of them. The site of inflammation did not become hemorrhagic after the intracardial injection of typhoid filtrate.

Negative results were also obtained in experiments with glycerine broth control for tuberculin. This material is prepared by evaporating the glycerine broth used for growing tubercle bacilli to one-tenth of its original volume. Four guinea pigs were injected intracutaneously with various dilutions of glycerine broth control. No hemorrhage was observed after the injection of bacterial filtrate. The observations with turpentine and broth are in harmony with those of Hanger and Shwartzman, in rabbits.

Four control guinea pigs received only intracutaneous injections of silver nitrate solutions. At the site of the injections, gray-green necrosis, surrounded by redness and edema, was observed on the following 2 days. In one of the four animals the area of necrosis was bordered by a sharply defined line of hemorrhage 0.5 mm. wide. The hemorrhagic line appeared 1 day after the injection of silver

TABLE II

Silver Nitrate as Skin Preparatory Agent in Guinea Pigs

Guinea pig No.	Preparatory agent, 0.1 cc. silver nitrate	Injury-producing agent, typhoid toxin	Hemorrhagic reaction	Systemic reaction
		cc.		
1	1:100 1:200* 1:1,000*	2.5	Present	Died in 24 hrs.
2	1:100 1:200 1:1,000	2.5	Absent	
3	1:100* 1:200* 1:1,000*	2	Present Absent "	
4	1:100 1:200 1:1,000	1	"	
5	1:100 1:200 1:1,000	1	Present	
6	1:20 1:200	1	Absent	
7	1:200 1:400 1:1,000	1	Present	" " 24 "
8	1:50 1:200	0.75	Absent	
9	1:500 1:1,000	0.5	Present	
10	1:50 1:200	0.5	"	" " 24 "

* The necrotic area was surrounded by a purple band about 0.5 mm. wide before the injection of typhoid toxin.

nitrate and did not increase in size during the following day. Ten guinea pigs were injected intracutaneously with 0.1 cc. of various dilutions of silver nitrate solution and intracardially with typhoid toxin. The guinea pigs reacted at the site of injection of silver nitrate with gray-green necrosis surrounded by redness and edema. In one guinea pig the necrotic area was surrounded by a purple band 2 mm. wide and a subsequent injection of bacterial filtrate increased the hemorrhage. The area of the hemorrhagic reaction in guinea pigs prepared with silver nitrate was less extensive than in those prepared with tuberculin or diphtheria toxin. In three animals it appeared in the form of a band from 2 to 3 mm. wide, surrounding the area of necrosis, but in two other guinea pigs the hemorrhages extended over areas of 30 x 30 mm. The injection of typhoid filtrate had no effect on the skin in four, and very slight effect in one guinea pig. The hemorrhagic reaction was present in the remaining five guinea pigs. Three of these animals died following the injection of typhoid filtrate and hemorrhages in the spleen and effusion in the peritoneal cavity were found (Table II).

DISCUSSION

The experiments described in the present papers show that the conditions necessary for the hemorrhagic reaction, the Shwartzman phenomenon, are different in the guinea pig and the rabbit. According to our experiments which confirm those of Gross (6), but do not support entirely the conclusions of Gratia and Linz, bacterial filtrates potent in the rabbit are inactive as skin preparatory agents in the guinea pig.

It was found that the intravascular injection of typhoid toxin produced hemorrhage at the site of inflammation caused by diphtheria toxin or silver nitrate. Both of these irritants, namely diphtheria toxin and silver nitrate, elicit, like tuberculin in tuberculous guinea pigs, hemorrhagic necrosis in the skin of some of the guinea pigs even without the subsequent intravascular injection of a bacterial toxin. Arthus phenomenon with horse serum, turpentine, or broth did not act as a skin preparatory agent. It is noteworthy that those irritants that prepared the skin for the hemorrhagic reaction were capable of producing hemorrhages and necrosis in the skin of some of the guinea pigs, whereas horse serum (in guinea pigs sensitized to it), turpentine, and broth lacked both the capacity of causing hemorrhage and that of acting as skin preparatory agents. The capacity to elicit hemorrhage in the skin without subsequent intravascular injection of a toxic substance may be essential for the action of skin preparatory agents. The experiments with diphtheria toxin and silver nitrate show that the skin of guinea pigs can be successfully prepared for the hemorrhagic

reaction by a true exotoxin and by a simple inorganic compound as well.

The experiments described and histological observations (to be published later) seem to support the following explanation of the Shwartzman phenomenon. The substance injected into the tissue at the first injection causes an injury at the site of injection, particularly to the blood vessels of that region. The injury is a transient one. The material introduced into the vascular system by the second injection augments the injury, resulting in rupture of blood vessels, hemorrhage, and thrombosis.

SUMMARY AND CONCLUSIONS

1. Filtrates from *B. coli*, *B. typhosus*, or meningococci injected into the skin of guinea pigs do not produce visible inflammation. When these injections are followed by intravascular injections of the same material, hemorrhages do not occur in the skin.

2. Guinea pigs sensitized to horse serum react with redness and edema to 0.1 or 0.01 cc. of horse serum injected into the skin, and subsequent intravascular injection of typhoid filtrate does not produce hemorrhage at the site of the reaction to horse serum.

3. When guinea pigs are injected into the skin with diphtheria toxin and these injections are followed by intravascular injection of filtrates from *B. typhosus*, hemorrhage occurs in the skin at the site of the reaction to diphtheria toxin.

4. When silver nitrate is injected into the skin of guinea pigs, redness, edema, and necrosis follow, and in a few guinea pigs small areas of hemorrhage can also be noticed. About half of the guinea pigs that have received an intravascular injection of typhoid filtrate react with hemorrhage at the site of the injection of silver nitrate.

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