INHIBITION OF THE SHWARTZMAN PHENOMENON

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In 1928 Shwartzman described a new phenomenon of local skin reactivity to culture filtrates of various microorganisms. The reactivity was induced by the injection of a filtrate into the skin of a rabbit. If an intravenous injection of a potent bacterial filtrate was given to the same rabbit from 20 to 24 hours later, there appeared an extremely severe hemorrhagic necrosis at the site of the previous injection. The factors determining the local skin reactivity were termed skin-preparatory factors and those responsible for the local injury following intravenous injection were called reacting factors. In recent years, an extensive series of studies on the various aspects of this phenomenon has been reported by Shwartzman and other workers.

Gross (1) described briefly an observation concerning inhibition of the Shwartzman phenomenon. The inhibition was obtained when an intravenous injection of a bacterial filtrate was given to a rabbit shortly after or simultaneously with the skin-preparatory injection. No reactions followed the provocative intravenous injection of the same filtrate 24 hours later. The same phenomenon of inhibition was observed independently by the author of this paper in his experiments with *B. coli* culture filtrates (2).

The present paper embodies a series of experiments concerning the inhibition of the Shwartzman phenomenon by means of active bacterial filtrates.

Material and Methods

Typhoid and meningococcus "agar washings" filtrates used were prepared and titrated according to the methods described by Shwartzman.

B. typhosus "agar washings" filtrates (3) were prepared as follows: Kolle flasks

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containing plain veal infusion agar of pH 7.4 were seeded each with 3–4 cc. of 20 hour old plain broth culture of *B. typhosus* (strain T_L) diluted 1:4 with 0.9 per cent NaCl solution. The dilution was made immediately before use. After 20 to 22 hours of incubation the growth of each flask was washed off with 2–4 cc. of 0.9 per cent NaCl solution containing 0.4 per cent phenol. The washings were then pooled, centrifuged within the following 1 to 2 hours and the clear supernatant fluid filtered through Berkefeld V candles shortly after centrifuging.

The method of preparation of *meningococcus "agar washings" filtrates* (4) differed from the above in the following: The inoculum was prepared by inoculation of meningococcus into 1 per cent rabbit blood broth of pH 7.2 – 7.4. The supernatant broth culture, after 20 to 22 hours of incubation, free of red blood cells was used for inoculation of the Kolle flasks. The Kolle flasks contained 0.7 per cent glucose veal infusion agar.

The quantitative measurement of the reacting factors (5) was carried out as follows: The rabbits used for titrations were each injected intradermally with 0.25 cc. of the undiluted filtrate or filtrate diluted 1:2 and divided into groups of three. 24 hours later a single intravenous injection of the filtrate diluted in 0.85 per cent NaCl solution was given to each rabbit. The dose was 1 cc. per kilo of body weight. Each group of rabbits received intravenously a different dilution of the filtrate. The local reactions were read 4 to 5 hours after the intravenous injections. The titrations were carried until the lowest dilution was found which gave no reaction in the four rabbits tested, as well as the highest dilution which gave reactions in one or more rabbits of the group. The minimal dose of reacting factors was then considered as lying between these two figures. If a given filtrate was employed for any length of time, repeated control titrations were done. In these control tests, the dilutions employed were both the highest dilution capable of eliciting reactions and the lowest dilution giving no reactions.

EXPERIMENTAL

1. Titration of Skin-Preparatory Factors against 25 Reacting Units.—Preliminary experiments suggested that it was necessary to employ a minimal skin-preparatory dose for the demonstration of inhibition of the Shwartzman phenomenon. Meningococcus Group III "agar washings" filtrate (T.1968) containing 4,000 reacting units was used. The skin of rabbits was prepared with various dilutions of the filtrate. For the provocative injection 25 reacting units were used in all the groups. The results are recorded in Series 1 of Table I.

As is seen from experiments of Series 1 of Table I, severe reactions were obtained in skin sites of 80 per cent of rabbits prepared with 0.25 cc. of filtrate T. 1968 diluted 1:25 and 24 hours later injected intravenously with 25 reacting units (*i.e.*, dilution 1:100 per kilo of body weight).

2. Inhibition Tests.—Different dilutions of the meningococcus filtrate (T.1968) were prepared. 1 cc. per kilo of body weight of each dilution was injected intravenously into rabbits. Immediately afterwards a preparatory injection of 0.25 cc. of the filtrate diluted 1:25 was made into the skin of the abdominal wall. 20

TABLE I

Series No.	First intravenous injection Dilution	Intradermal injection Dilution	Second intravenous injection Dilution	Total No. of rabbits	No. of deaths	Reactions				
						4+	3+	2+	1+	Negative
1	—	Mg. 44B.* T.1968	Mg. 44B. T.1968							
		1:25	1:160	20	1	16	0	0	0	3
		1:50	"	5	0	0	0	1	1	3
		1:75	"	3	0	0	0	0	0	3
2	—	Mg. 44B. T.1968	Mg. 44B. T.1968							
		1:25	1:400	5	0	1	0	1	0	3
		"	1:1,000	5	0	0	0	0	0	5
		"	1:4,000	5	1	0	0	0	0	4
3	Mg. 44B. T.1968	Mg. 44B. T.1968	Mg. 44B. T.1968							
	1:160	1:25	1:160	18	6	1	0	1	0	10
	1:400	"	"	10	0	1	0	0	0	9
	1:1,000	"	"	5	1	1	1	0	0	2
	1:4,000	"	"	5	0	2	1	0	0	2
4	Mg. 44B. T.1968	Mg. 44B. T.1968	Mg. 44B. T.1968							
	1:160	1:10	1:160	10	3	4	0	0	0	3
	"	1:2	"	10	4	3	0	0	0	3
5	Mg. 44B. T.1968	Mg. 44B. T.1968	Mg. 44B. T.1968							
	1:160	1:25	1:80	6	2	2	0	0	0	2
	"	"	1:40	4	2	1	0	0	0	1

In this and the following tables, all intravenous injections were given in a dose of 1 cc. per kilo of body weight; all intradermal injections were given in a dose of 0.25 cc.

The intensity of hemorrhagic and necrotic lesions noted as 1+, 2+, 3+ and 4+.

* Abbreviation Mg. 44B. designates "agar washings" filtrates of meningococcus, Group III cultures.

to 24 hours after the intradermal injection, 1 cc. per kilo of body weight of the filtrate diluted 1:160 (*i.e.*, 25 reacting units) was injected intravenously. The readings of the skin reactions were made 4 to 5 hours after the last injection. The results are recorded in Series 3 of Table I.

It became obvious from these experiments that the Shwartzman phenomenon to meningococcus filtrate could be almost completely inhibited by an intravenous injection of the filtrate simultaneously with the skin-preparatory injection of the same filtrate in a dose of 0.25 cc. of dilution 1:25. The effective inhibitory doses were dilutions ranging from 1:160 to 1:400. The inhibitory effect of a dilution 1:1,000 was doubtful and higher dilutions remained without effect.

Similar experiments were carried out with increasing concentrations of the skin-preparatory dose and constant doses of the inhibitory and provocative injections. The results are recorded in Series 4 of Table I.

As is seen from these experiments, an intravenous injection of filtrate T. 1968 given simultaneously with the skin-preparatory injection of the same filtrate in dilutions lower than 1:25 failed to inhibit the Shwartzman phenomenon.

In experiments of Series 5 of Table I, constant amounts were used for the inhibitory intravenous injections (*i.e.*, dilution 1:160) and preparatory intradermal injections (*i.e.*, dilution 1:25). Rabbits thus treated were tested with various provocative doses. If the results of Series 5 are compared with those of Series 3, it is obvious that no inhibition of the Shwartzman phenomenon took place if dilutions lower than 1:160 were used for the provocative injections.

From the results thus far recorded, it becomes clear that an intravenous injection accompanying the intradermal preparatory injection may inhibit the Shwartzman phenomenon within the limits of certain quantitative relationships.

The experiments recorded below were planned to determine the optimum time relationships between the inhibitory, preparatory and provocative injections.

In the experiments of Series 1 of Table II, the intervals of time between the inhibitory intravenous injections and the preparatory intradermal injections were varied. The latter injections were given 1, 3 and 4 hours after the inhibitory intravenous injections. The provocative injections were given 20 to 24 hours after the intradermal preparatory injections.

As is seen from Series 1 of Table II, an intravenous injection of meningococcus filtrate T. 1968 diluted 1:160, given 1 hour prior to the preparatory intradermal injection, was capable of inhibiting the Shwartzman phenomenon almost as effectively as an inhibitory injec-

tion given simultaneously with the preparatory injection. An inhibitory intravenous injection given 4 hours before the preparatory intradermal injection remained without effect. A 3 hour interval between the inhibitory and preparatory injections gave only irregular results.

It can be stated, then, that inhibitory effect of an intravenous injection of a bacterial filtrate is of short duration.

In experiments of Series 2 of Table II, the intradermal preparatory injections were given prior to the inhibitory intravenous injections. The number of animals

TABLE II

Series No.	First intravenous injection Dilution	Interval of time	Intradermal injection Dilution	Second intravenous injection Dilution	Total No. of rabbits	No. of deaths	Reactions				
							4+	3+	2+	1+	Negative
		hrs.									
1	Mg. 44B. T.1968		Mg. 44B. T.1968	Mg. 44B. T.1968							
	1:160	1	1:25	1:160	5	0	1	0	0	0	4
	"	3	"	"	5	1	1	0	1	0	2
	"	4	"	"	5	2	3	0	0	0	0
	Intradermally 0.25 cc. one area		First i.v. injection 1 cc. per kilo	Second i.v. injection 1 cc. per kilo							
2	Mg. 44B. T.1968		Mg. 44B. T.1968	Mg. 44B. T.1968							
	1:25	1	1:160	1:160	5	1	1	0	0	0	3
	"	2	"	"	5	2	1*	0	0	0	2
	"	4	"	"	5	2	1*	0	0	0	2

* Already positive before the second intravenous injection.

employed in these experiments was rather small. The facts suggest, however, that an intravenous injection given 1 hour after the preparatory intradermal injection may inhibit the Shwartzman phenomenon. Inhibitory intravenous injections given at longer periods of time, *i.e.*, 2 and 4 hours after the intradermal injection, apparently produced no effect. In some rabbits the state of reactivity was already induced several hours after the preparatory injections. For this reason, the intravenous injections intended to inhibit the Shwartzman phenomenon served as provocative injections and elicited reactions in prepared sites. The fact introduced difficulties in the interpretation of the results of experiments with the 4 hour interval of time between skin-preparatory and intravenous injection.

TABLE III

Series No.	First intravenous injection Dilution	Intradermal injection Dilution	Second intravenous injection Dilution	Total No. of rabbits	No. of deaths	Reactions				
						4+	3+	2+	1+	Negative
1	B.TyT$_L$* T.1976	Mg. 44B. T.1968	Mg. 44B. T.1968							
	1:16	1:25	1:160	10	5	0	2	1	0	2
	B.TyT^L T.1986	"	"							
	1:28	"	"	5	2	1	0	0	0	2
	1:56	"	"	11	4	3	0	0	0	4
	1:100	"	"	15	0	2	0	0	0	13
	1:400	"	"	5	1	2	0	1	0	1
2	—	Mg. 44B. T.1968	B.TyT$_L$ T.1976							
		1:25	1:16	5	0	4	0	0	0	1
	—	"	B.TyT$_L$ T.1986							
			1:28	5	0	2	0	1	0	2
	—	"	1:56	5	0	3	0	0	0	2
	—	"	1:100	5	1	4	0	0	0	0
	—	"	1:800	5	0	1	1	0	0	3
3	*B. coli* filtrate T.1964, 1:3	Mg. 44B. T.1968	Mg. 44B. T.1968							
		1:25	1:160	14	7	2	0	0	0	5
	1:15	"	"	10	3	2	0	0	0	5
	1:30	"	"	5	0	1	0	0	0	4
	1:100	"	"	10	3	4	0	0	0	3
	1:400	"	"	5	0	4	0	0	0	1
4	—	Mg. 44B. T.1968	*B. coli* filtrate							
		1:25	T.1964, 1:3	5	0	3	2	0	0	0
	—	"	1:15	5	0	2	0	1	0	2
	—	"	1:30	5	0	0	0	0	0	5
5	*Streptococcus hemolyticus* filtrate T.1983, undiluted	Mg. 44B. T.1968 1:25	Mg. 44B. T.1968 1:160	5	0	2	1	1	0	1
	Streptococcus hemolyticus filtrate T.1989, undiluted	"	"	3	0	1	1	0	0	1
6	Plain broth 1:2 saline	Mg. 44B. T.1968	Mg. 44B. T.1968							
		1:25	1:160	10	3	4	1	0	0	2
	Horse serum (H.693) undiluted	"	"	5	1	3	0	0	0	1

* Abbreviation B.TyT$_L$ designates "agar washings" filtrates of *B. typhosus*, strain T$_L$ cultures.

As is seen from the results of the above group of experiments, the inhibitory effect of an additional injection may not last longer than 2 hours. It seems to be definitely effective, however, if it is given before the skin preparation takes place.

It is well known that the Shwartzman phenomenon can be elicited by combined injections of heterologous bacterial filtrates. It seemed of interest to determine whether there existed any specificity in the inhibition described.

In the experiments recorded in Series 1, 3, 5 and 6 of Table III, the Shwartzman phenomenon was elicited by means of meningococcus "agar washings" filtrates T.1968 (*i.e.*, intradermally, dilution 1:25 and intravenously, dilution 1:160). The inhibition was attempted by means of additional intravenous injections of *B. typhosus* T_L "agar washings" filtrate (T.1976 and T.1986, Series 1, Table III); *B. coli* "agar washings" filtrates (Series 3, Table III); *Streptococcus hemolyticus* filtrate T.1983 (Series 5, Table III); plain broth, normal horse serum and physiologic saline solution (Series 6, Table III).

As is seen from the experiments of Table III, no specificity of the inhibition reaction described could be observed. *B. typhosus* culture filtrate in dilution 1:100 was able to inhibit almost completely the Shwartzman phenomenon to meningococcus filtrate. Similarly, *B. coli* filtrate was effective in dilutions 1:30 and 1:100. As is also seen from Table III, additional intravenous injections of *Streptococcus hemolyticus* filtrate and of non-bacterial substances (*i.e.*, plain broth, normal horse serum and physiologic saline solution) failed to inhibit the Shwartzman phenomenon. In preliminary experiments not described in this paper, these agents were also shown to be lacking in reacting potency.

The facts suggest that the inhibitory effect of an additional intravenous injection can be obtained only with substances potent in the elicitation of the Shwartzman phenomenon itself.

COMMENT

It appears from the above experiments that the Shwartzman phenomenon can be inhibited if an additional intravenous injection of a potent bacterial filtrate is given within a certain period of time prior to or following the skin-preparatory injection. The inhibitory effect of the additional intravenous injection takes place within the limits of

certain quantitative relationships. Thus, if the skin is prepared with a large amount of filtrate, the inhibition is absent or incomplete. Similarly, if a large amount of filtrate is used for the provocative injection, there occurs no inhibition. It is also obvious that the inhibition described is of a transitory nature. The additional intravenous injection given several hours before or after the skin-preparation has no inhibitory effect. The inhibition can be obtained only with filtrates capable of eliciting the Shwartzman phenomenon. Bacterial filtrates of low reacting potency (*Streptococcus hemolyticus* filtrate employed in these experiments) as well as non-bacterial substances (*i.e.*, 0.85 per cent NaCl solution, plain broth and normal horse serum) produce no inhibition.

The mechanism of the inhibition remains unknown. Several explanations suggest themselves. Duran-Reynals (6) reported on the presence of spreading factors in bacterial filtrates capable of enhancing tissue permeability. It is possible that an intravenous injection of a bacterial filtrate accompanying the skin preparation may enhance the rate of diffusion of the material injected locally through increase in the capillary or lymphatic permeability. The preparatory factors thus diluted might not bring about a state of reactivity. Gross and histological examination of the tissue where reactions had been inhibited did not disclose, however, any spreading phenomenon. Further studies are under way.

Recently, experiments on generalized Shwartzman phenomenon have been described by Gratia and Linz (7), Apitz (8) and Gerber (9). The phenomenon was elicited by means of two intravenous injections, 24 hours apart. While the work reported in this paper was in progress, Apitz suggested the following explanation for inhibition of the local Shwartzman phenomenon:

The inhibitory intravenous injection may elicit a state of reactivity in the internal organs. Following the second intravenous injection reactions would occur in organs thus prepared. If a small dose is used for the provocative injection, the amount injected may be consumed in the production of lesions in the internal organs and thus an insufficient amount would remain for the elicitation of the reaction in the skin.

To determine whether this assumption could be used for the explana-

tion of the inhibition here described, the internal organs of a large series of rabbits were examined in the gross and microscopically. Rabbits showing complete inhibition of the local Shwartzman phenomenon showed only rarely lesions in the internal organs, possibly because the doses employed were too small for the elicitation of the generalized Shwartzman phenomenon.

The inhibition of the Shwartzman phenomenon cannot be interpreted as an anaphylactic desensitization for the following reasons.

1. Inhibition takes place if the additional intravenous injection is given simultaneously or shortly before and after the preparatory injection. Obviously an anaphylactic desensitization cannot be expected to occur before sensitization is induced.
2. There is no specificity of inhibition.

A great deal of experimental evidence in the literature supports the possibility that processes exemplified by the Shwartzman phenomenon take place in induced and spontaneous bacterial and virus infections. The factors responsible for the Shwartzman phenomenon and deriving from infected foci may induce a state of reactivity in tissues and organs removed from the sites of initial infection. When the state of reactivity establishes itself, discharge of the same factors into the blood stream would then elicit severe hemorrhagic lesions in these reactive sites. The mechanism might then be responsible for pathological lesions scattered through the body, and the inhibitory reaction described in this paper might prevent their occurrence.

SUMMARY

The Shwartzman phenomenon can be inhibited by an intravenous injection of a potent bacterial filtrate within a few hours before or after the preparatory intradermal injection.

The inhibitory effect is produced non-specifically by filtrates potent in the elicitation of the Shwartzman phenomenon, and it is of a transitory nature.

The relation of the observation described to anaphylactic desensitization and to its clinical significance is discussed in this paper.

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BIBLIOGRAPHY

1. Gross, H., *Centr. Bakt., 1. Abt., Orig.*, 1931, **122,** 96.
2. Ogata, T., *Tokyo Igakkwai Zasshi*, 1932, **46,** 2390.
3. Shwartzman, G., *Proc. Soc. Exp. Biol. and Med.*, 1929, **26,** 843.
4. Shwartzman, G., *J. Infect. Dis.*, 1929, **45,** 232.
5. Shwartzman, G., *J. Exp. Med.*, 1930, **52,** 781.
6. Duran-Reynals, F., *J. Exp. Med.*, 1933, **58,** 161.
7. Gratia, A., and Linz, R., *Ann. Inst. Pasteur*, 1932, **49,** 131.
8. Apitz, K., *Virchows Arch. path. Anat.*, 1934, **293,** 1; *J. Immunol.*, 1935, **29,** 255.
9. Gerber, I. E., *Am. J. Path.*, 1935, **11,** 843.