HEMORRHAGES IN TUBERCULOUS GUINEA PIGS AT THE SITE OF INJECTION OF IRRITANTS FOLLOWING INTRAVASCULAR INJECTIONS OF INJURIOUS SUBSTANCES (SHWARTZMAN PHENOMENON)

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In the preceding paper it was reported (1) that the intravascular injection of toxins of *B. typhosus*, *B. coli*, or meningococcus produced hemorrhages or hemorrhagic necrosis in the skin of guinea pigs at the site of reaction to diphtheria toxin or silver nitrate. Furthermore, it was described that similar injections of toxins failed to cause hemorrhages in the skin of guinea pigs at the site of injections of other irritants such as horse serum in guinea pigs sensitized to it, or turpentine or broth.

Recently, I have reported very briefly the observation that in a tuberculous guinea pig the skin becomes hemorrhagic at the site of positive tuberculin tests when products of typhoid bacilli are injected intravascularly. The purpose of the present paper is to discuss the occurrence of hemorrhagic reactions in tuberculous guinea pigs at the site of injection of certain irritants following intravascular injections of various injurious substances.

It is known that tuberculous animals respond to various injurious agents with hemorrhage or with hemorrhagic necrosis. Tuberculin injected into the skin causes hemorrhagic necrosis in some guinea pigs, particularly when tuberculosis is advanced and the animals are highly sensitive to tuberculin. Hemorrhages occur in organs containing tubercles when tuberculin is introduced in the subcutaneous tissue or the peritoneal cavity. Tuberculin injected into the skin may produce hemorrhagic necrosis not only in tuberculous guinea pigs, but also in guinea pigs sensitized by large amounts of heat-killed tubercle bacilli (2). In such guinea pigs the tuberculin reaction is characterized by an extensive area of black necrosis and relatively slight edema. Tuberculous guinea pigs respond to various injurious substances not related to the tubercle bacillus, with hemorrhage

or hemorrhagic necrosis more readily than do non-tuberculous guinea pigs. The site of hemorrhage may be the skin, that is free of tubercles, or the organs (lung, spleen, liver) containing tubercles.

Dienes (3) made interesting observations on the occurrence of hemorrhages in skin tests on tuberculous guinea pigs. He studied the systemic reaction to egg white in tuberculous guinea pigs sensitized to egg white. He injected from 10 to 20 mg. of slightly virulent tubercle bacilli into the peritoneal cavities of guinea pigs. From 3 to 8 days later, he injected 0.1 to 1 mg. of egg white (dry weight) into the same site. When from 9 to 10 days after the injection, the guinea pigs were again injected with a larger amount of egg white (10 to 30 mg. dry weight) they died from 4 to 12 hours after the injection, with symptoms resembling tuberculin death. At autopsy, he found hemorrhages in tissues containing tubercles. If the guinea pig lived longer, or survived the shock, he observed bluish or purple discoloration or hemorrhages in the skin at the site of the reaction to egg white and also at the sites of recently healed wounds of operations. In several tuberculous guinea pigs, sensitized to egg white, the site of a tuberculin reaction became hemorrhagic after the injection of egg white, but he could not reproduce this observation in two subsequent experiments.

Paul Bordet (4) studied the effect of *B. coli* upon guinea pigs infected with a non-virulent strain of tubercle bacilli, the Calmette-Guérin bacillus. He injected 5 mg. of B.C.G. into the peritoneal cavities of guinea pigs. 3 weeks later, after the mesenteric lymph nodes had developed tubercles, 10 cc., that is, a large amount of a broth culture of colon bacilli, were introduced into the peritoneal cavities of guinea pigs. Some of the guinea pigs died within 1 day after the injection. Hemorrhages were found in the enlarged mesenteric lymph nodes containing tubercles.

Not only antigens but simple chemical compounds can produce shock symptoms and hemorrhages in tuberculous animals. For instance, an amount of sanocrysin harmless to non-tuberculous animals may kill tuberculous ones, and cause intense congestion and hemorrhages in organs that contain tubercles (5).

Effect of the Injection of Bacterial Toxins upon the Tuberculin Reaction in the Guinea Pig

Groups of guinea pigs were infected with either of the following strains of tubercle bacilli: (1) bovine (Ravenel); (2) human (A_1 D), P_2; (3) B.C.G. The amount of tubercle bacilli was varied: of the strain Ravenel, 0.01 mg.; of A_1 D, 2.5 mg.; of P_2; 0.001 or 0.005 mg.; and of B.C.G., 5 mg. were used for infecting the guinea pigs. The guinea pigs were skin tested with old tuberculin from 6 weeks to 6 months after the infection. The old tuberculin was injected into the skin in dilutions of from 1:15 to 1:500. The bacterial filtrates, potent in the Shwartzman phenomenon, were injected from 18 to 48 hours after the injection of old tuberculin either intracardially or intravenously, or intraperitoneally, or subcutaneously. The intracardial or intravenous injections were made under light ether anesthesia. The bacterial filtrates were prepared by growing the culture on infusion agar in

Kolle flasks. The agar was seeded with 3 to 4 cc. (diluted 1:4 with saline immediately before planting) of a 20 hour plain broth culture. The growth was washed off with 14 to 16 cc. of normal saline solution containing 0.4 per cent phenol. The washings were pooled and centrifuged within 2 hours. The supernatant fluid was filtered through Berkefeld N or a Seitz filter. (See also the description of procedure by Shwartzman (6).) Bouillon cultures of *B. coli*, incubated for 6 to 10 days and filtered through a Seitz filter, were found potent and used in some of the experiments.

The following strains were used for preparing bacterial filtrates: (1) *B. typhosus* T.L., (2) *B. coli*, both obtained from Dr. Shwartzman, and (3) a strain of *B. coli*

TABLE I

The Hemorrhage-Producing Effect of an Injection of Typhoid Toxin upon the Tuberculin Reaction in Guinea Pigs

No. of guinea pigs employed	Amount of typhoid toxin	Route of injection	No. of guinea pigs in which hemorrhage	
			Occurred	Did not occur
	cc.			
2	0.25	Intracardial	0	2
5	0.50	"	3	2
5	1.00	"	4	1
4	1.00	Intraperitoneal	2	2
2	2.00	"	2	0
3	2.00	Subcutaneous	1	2
1	3.00	"	1	0
1	4.00	"	1	0
1	5.00	"	1	0

isolated at New York Hospital. We have also used a filtrate of meningococci received from Dr. Shwartzman. The amount of filtrates injected and the route of injection are stated in Table I.

As shown in Table I, when guinea pigs infected with bovine or human virulent tubercle bacilli were injected into the skin with various dilutions of old tuberculin (from 1:15 to 1:500) and the skin tests followed from 1 to 2 days later by an intravascular, intraperitoneal, or subcutaneous injection of a filtrate from *B. typhosus*, the positive tuberculin tests became hemorrhagic about 4 hours after the injection of the filtrate.

As a rule general shock symptoms were present in guinea pigs injected with 0.5 cc. or more of the filtrate. Almost immediately after the injection of the

filtrate, the guinea pig's hair was ruffled and its breathing became dyspneic. Some guinea pigs lay quietly on their sides, others had spastic movements. Urination and defecation were frequent. Scratching was sometimes noticed. These symptoms simulate non-lethal anaphylactic shock. Some of the guinea pigs died from 3 to 18 hours after the injection of filtrate. At autopsy hemorrhages were found in the organs containing tubercles and exudate in the pleural and peritoneal cavities. The changes found were not distinguishable from those in tuberculin shock.

The extent of the hemorrhagic reaction was proportional to the severity of the tuberculin reaction. This relationship could be best observed by comparing reactions in the same guinea pig, simultaneously injected with different dilutions of tuberculin. This is illustrated by the following protocol.

A guinea pig weighing 450 gm., which had been infected subcutaneously 7 weeks previously, was injected intracutaneously with 0.1 cc. of 1:45, 1:135, and 1:405 dilutions of old tuberculin. 2 days later the reactions at the site of the injections were as follows: Test 1 (1:45), light redness and edema 10 x 10 mm. raised 2 mm.; Test 2 (1:135), pink discoloration and edema 5 x 4 mm. raised 1 mm.; Test 3, doubtful. Immediately after the reading of the skin test 1 cc. of typhoid filtrate was injected intracardially. 1 hour after the injection the discoloration was dull red and 3 hours later the skin reactions were: Test 1 (1:45), mottled dark purple area 7 x 7 mm., edema 10 x 10 mm., raised 2 mm.; Test 2 (1:135), dark purple area 4 x 3 mm., edema 5 x 4 mm., raised 1 mm.; Test 3, doubtful. A few dark purple spots about 1 x 1 mm. were seen around Skin Tests 1 and 2. 1 day later the purple areas described above appeared to be necrotic.

In some guinea pigs not included in Table I, dilutions of old tuberculin from 1:400 to 1:1,200 caused only a slight reaction, *i.e.* a slight redness and edema, which did not change after the injection of bacterial filtrate, although the same animals showed hemorrhagic reactions at the sites of the more intense tuberculin reactions produced by higher concentrations of tuberculin. However, no significant correlation was found between the severity of the tuberculin reaction and the hemorrhagic reaction, when they were compared in different guinea pigs.

We have found some correlation between the appearance of general shock symptoms and the purple discoloration. Guinea pigs injected with amounts of filtrate which did not produce shock symptoms as a rule did not show the hemorrhagic reaction.

In occasional guinea pigs, a purple discoloration appeared at the site of the positive tuberculin reaction without the injection of bacterial filtrates. If such guinea pigs were injected with the filtrate the area of purple discoloration became more extensive.

In experiments not included in Table I, filtrates from *B. coli* and meningococci were used with results almost identical with those in Table I.

The question naturally arises whether tuberculin prepares the skin by itself or by the reaction it produces in the tuberculous guinea pigs.

In four normal guinea pigs, the hemorrhagic reaction failed to occur even when low dilutions of tuberculin (from 1:2 to 1:10) were employed. In the tuberculous guinea pigs, hemorrhages were observed at the sites of injections of tuberculin that showed definite tuberculin reactions but hemorrhages did not appear at the skin sites that were injected with dilutions too high to produce the tuberculin reaction.

These observations show that the inflammation produced in the hypersensitive animal, and not the tuberculin *per se*, is responsible for its preparatory action.

The Effect of the Injection of Bacterial Products upon the Tuberculin Reaction in Rabbits

It is well known that the tuberculous rabbit like the guinea pig is hypersensitive to old tuberculin injected parenterally. Both tuberculous rabbits and guinea pigs die with pleural and peritoneal exudation and hemorrhages in organs containing tubercles after an injection of an amount of old tuberculin apparently harmless to normal animals. However, there is a sharp contrast between tuberculous rabbits and guinea pigs in regard to the skin test with tuberculin. While the skin of tuberculous guinea pigs reacts with inflammation to small amounts of tuberculin, and the skin test is used as a delicate test to detect tuberculosis in guinea pigs, many tuberculous rabbits do not react at all to tuberculin injected into the skin and some of them react slightly. The reaction in the rabbit is very different from that in the guinea pig or other animals. It is characterized by redness and slight edema. Purple discoloration or necrosis does not occur. It was thought that examination of the tuberculin reaction in the rabbit, as a skin preparatory factor, might throw some additional light on the nature of the hemorrhagic reactions.

Rabbits infected intravenously with bovine tubercle bacilli were injected with 0.2 cc. of a fivefold dilution of old tuberculin. On the following day they received an intravenous injection of 0.5 cc. of typhoid filtrate, an amount sufficient to produce the hemorrhagic phenomenon in rabbits when the skin is prepared with filtrates from typhoid or colon bacilli or meningococci. The hemorrhagic reaction did not occur in any of eight rabbits so tested. The failure of the tuberculin reaction to prepare the skin is significant, for the rabbit is very susceptible to the hemorrhagic reaction; and in the rabbit, hemorrhages in the organs containing tubercles occur very regularly after the parenteral injection of old tuberculin.

These experiments indicate that tuberculin does not elicit a necrotic skin reaction in the rabbit and does not act as a preparatory agent for the hemorrhagic reaction.

Effect of an Intravascular Injection of Typhoid Toxins upon the Arthus Phenomenon in Tuberculous Guinea Pigs

Since the specific inflammation caused by tuberculin that prepares the skin of guinea pigs for the hemorrhagic reaction acts only in tuberculous guinea pigs, it seemed desirable to examine the action of horse serum in tuberculous guinea pigs sensitized to horse serum.

Dienes and Schonheit (7) demonstrated that tuberculosis modifies the hypersensitiveness to egg white and horse serum. When guinea pigs are first infected with tubercle bacilli, and then injected repeatedly with egg white, they react to egg white injected into the skin, not only with redness and swelling (like noninfected guinea pigs sensitized to horse serum), but also with a tuberculin type of reaction; *i.e.*, redness and swelling followed by necrosis. Sometimes the necrosis is preceded by a purple discoloration.

In order to study the Arthus phenomenon modified by tuberculosis as a skin preparatory factor in the guinea pig, animals were injected either with the Calmette-Guérin bacillus or with bovine strain Ravenel and sensitized with horse serum.

Tuberculous guinea pigs infected with B.C.G. and sensitized to horse serum, reacted with a necrotic type of inflammation to 0.1 cc. and without necrosis to 0.01 cc., of horse serum. When the guinea pigs so treated were injected with typhoid filtrate, the reactions became hemorrhagic or necrotic in all of the guinea pigs (Table II). The protocol of one typical experiment follows: Guinea Pig 129 was injected with 5 mg. of B.C.G. subcutaneously into the left groin. 1 week later, and twice at intervals of 3 days, 0.1 cc. of horse serum was injected also into the subcutaneous tissue of the left groin. 30 days after the infection, 0.1 cc. of horse serum was injected into the skin. The following day, at the site of the

injection, redness, edema 45 x 40 x 3 mm., and necrosis 8 x 8 mm., were observed. 33 days after the infection, 0.01 cc. of horse serum was injected intracutaneously. The following day, light redness and edema 40 x 40 x 4 mm. were observed. On this day 1 cc. of typhoid filtrate was injected into the heart. 4 hours later purple discoloration appeared in an area of 18 x 12 mm., and 20 hours later purple discoloration in an area of 28 x 25 mm. and necrosis in an area of 6 x 2 mm. was observed.

When we compare the Arthus phenomenon in non-tuberculous[1] and tuberculous (B.C.G.) animals sensitized to horse serum and the

TABLE II

Arthus Phenomenon as Preparatory Agent in Tuberculous Guinea Pigs

Guinea pig No.	Infection before sensitization to horse serum	Necrosis at the site of reaction to 0.1 cc. of horse serum	Hemorrhage at the site of reaction to 0.01 cc. of horse serum after intracardial injection of typhoid toxin	Systemic reaction
1	With B.C.G.	Present	Present	
2	" "	"	"	
3	" "	"	"	
4	" "	"	"	
5	With strain Ravenel	Absent	"	Died in 18 hrs.
6	" " "	"	"	" " 18 "
7	" " "	Present	"	
8	" " "	Absent	"	" " 18 "
9	" " "	Present	"	
10	" " "	"	"	
11	" " "	Present (slight)	Absent	
12	" " "	Present	Present	" " 18 "

effect of bacterial filtrate upon the Arthus phenomenon in tuberculous and non-tuberculous guinea pigs, we find the following differences. In the non-tuberculous animals the Arthus phenomenon elicited by 0.1 cc. of horse serum was characterized by redness and swelling, in tuberculous guinea pigs by redness, swelling, and necrosis; the necrosis was preceded in some instances by purple discoloration. When 0.01 cc. of horse serum was used for testing the guinea pigs no purple discoloration or necrosis occurred in any of the animals. While the

[1] See preceding paper.

intravascular injection of bacterial filtrate had no effect on the Arthus reaction in the non-tuberculous guinea pigs, it caused in the tuberculous guinea pigs hemorrhage or necrosis in the skin at the site of the injection of diluted horse serum.

In nine guinea pigs a virulent bovine strain was employed instead of B.C.G. and the infecting dose (subcutaneous) was reduced from 5 mg. to 0.01 mg. Results are given in Table II.

Only five of the nine guinea pigs reacted with necrosis and one with hemorrhage to 0.1 cc. of horse serum, a smaller proportion than that in the guinea pigs infected with B.C.G. Hemorrhage appeared in the skin at the site of the injection of 0.01 cc. of horse serum, in six of the seven guinea pigs, after the intracardial injection of typhoid filtrate.

There seems to be a relationship between the capacity of guinea pigs to react with necrosis to 0.1 cc. of horse serum and the capacity to react after intravenous injection of bacterial filtrate with hemorrhage at the site of former injection of 0.01 cc. of horse serum. As a rule, the inflammation produced by horse serum acted as a preparatory factor only in those guinea pigs which reacted with necrosis to 0.1 cc. of horse serum.

Effect of an Intravascular Injection of Typhoid Toxin upon Skin Lesions Produced by Silver Nitrate in Tuberculous Guinea Pigs

In contrast to the result in normal animals,[1] hemorrhagic reactions appeared in eight of ten tuberculous guinea pigs at the site of the injection of silver nitrate after the injection of bacterial filtrate (Table III). The area of hemorrhage was, as a rule, considerably more extensive in the tuberculous than in the non-tuberculous guinea pigs described in the preceding paper. The question arises whether the observed difference in normal and tuberculous guinea pigs is due to a difference in the reaction of the skin to silver nitrate itself, or to their reaction to the bacterial filtrate. There was no difference noted in the reaction of the skin to silver nitrate injections in tuberculous and in non-tuberculous guinea pigs. However, tuberculous guinea pigs react differently from normal ones to bacterial filtrates. They die from a relatively small amount of filtrate with hemorrhages in the tissues containing tubercles and effusions in the pleural and peritoneal cavities.

TABLE III

Silver Nitrate as Skin Preparatory Agent in Tuberculous Guinea Pigs

Guinea pig No.	Preparatory agent, 0.1 cc. silver nitrate	Injury-producing agent, typhoid toxin	Hemorrhagic reaction	Systemic reaction
		cc.		
	1:40			
1	1:200	2.5	Present	
	1:1,000			
	1:40			
2	1:200	2.0	"	Died in 24 hrs.
	1:1,000			
	1:100			
3*	1:200	2.0	Absent	
	1:1,000			
	1:100			
4	1:200	1.5	Present	" " 24 "
	1:1,000			
	1:100			
5	1:200	1.5	"	" " 24 "
	1:1,000			
	1:100			
6	1:200	1.5	"	" " 24 "
	1:1,000			
	1:100			
7	1:200	1	"	" " 24 "
	1:1,000			
8	1:20	1	"	" " 24 "
	1:100		"	
9	1:200	1	"	
	1:1,000		Absent	
	1:100			
10	1:200	1	"	
	1:1,000			

* Infected with 0.000,001 mg. human tubercle bacilli 3 weeks before experiment.

This difference between the behavior of the tuberculous and non-tuberculous guinea pigs toward the bacterial filtrate, the injury-producing factor, may explain why the same irritant, silver nitrate, acts as a preparatory factor in the tuberculous and often fails to act in the normal guinea pig.

Tuberculin as Injury-Producing Factor

As described above, the parenteral injections of filtrates from *B. typhosus*, *B. coli*, and meningococci were active in tuberculous guinea pigs as injury-producing agents. Since these filtrates produce anaphylactoid symptoms in the non-tuberculous, and shock not distinguishable from the tuberculin shock in the tuberculous guinea pig, the question arises whether tuberculin would act as an injury-producing factor. Tuberculin is considered non-toxic to normal guinea pigs, since 5 cc. injected intraperitoneally or intravascularly does not produce obvious symptoms. The lethal dose for tuberculous animals is about 0.25 cc.

Seven guinea pigs were infected subcutaneously with from 0.001 to 0.01 mg. of human tubercle bacilli and tested from 6 weeks to 3 months after infection (Table IV). The tuberculin reactions were well defined by redness and edema. There was no purple discoloration or necrosis before the intraperitoneal injection of tuberculin, except in two guinea pigs (Nos. 6 and 7 in Table IV). In these guinea pigs, the extent of hemorrhage was increased after injection of bacterial filtrate.

Table IV shows that tuberculin acted as an injury-producing agent in all guinea pigs that died subsequently from tuberculin shock. Hemorrhage failed to occur in one of two animals that survived the intraperitoneal injections of tuberculin.

Effect of Intravascular Injection of Witte Peptone or Soluble Starch upon the Tuberculin Reaction

Since the hemorrhagic reactions were produced by parenteral injections of the products of various bacteria not related to tuberculosis, and systemic reactions, such as dyspnea, tremor, ruffling of the hair, urination and defecation, spastic movements in some of the guinea pigs, *i.e.* symptoms of stimulation of smooth muscles, were constant, an attempt was made to ascertain whether toxic bacterial products

could be replaced by non-bacterial substances which cause similar anaphylactoid symptoms in guinea pigs. For this purpose we have used Witte peptone (8) and soluble starch (9).

TABLE IV

The Hemorrhage-Producing Effect of Intraperitoneal Injections of Tuberculin upon Skin Reactions to Tuberculin

Guinea pig No.	Preparatory agent, tuberculin injected into the skin	Injury-producing agent, tuberculin injected into the peritoneal cavity	Hemorrhagic reaction	Systemic reaction
		cc.		
1	1:25 1:100 1:400	2	Present	Died with tuberculin shock
2	1:25 1:100 1:400	1.5	" Absent "	" "
3	1:25 1:100 1:400	1.25	Present Absent "	" "
4	1:25 1:100 1:400	1	Present	" "
5	1:25 1:100 1:400	0.6	Absent	Hair ruffled, dyspnea; survived
6	1:25* 1:100 1:400	0.5	Present	Died with tuberculin shock
7	1:25* 1:100 1:400*	0.3	Absent	Hair ruffled, dyspnea; scratching; survived

* Small areas of purple discoloration which increased after the intraperitoneal injection of tuberculin.

In the experiments with Witte peptone twelve guinea pigs injected with virulent tubercle bacilli and five guinea pigs infected with B.C.G. were employed. In the guinea pigs of Table V the reactions to tuberculin, diluted 1:50, 1:200, or

TABLE V

The Effect of Intravascular Injection of Witte-Peptone upon the Tuberculin Reaction

Guinea pig No.	Preparatory agent, 0.1 cc. tuberculin injected into skin	Injury-producing agent, Witte peptone	Hemorrhage after the injection of Witte peptone	Systemic reaction
		gm.		
1	1:50 1:200 1:1,000	0.2	Present " Absent	Died in 6 hrs.
2	1:50 1:200 1:1,000	0.2	Present*	Died within 18 hrs.
3	1:50 1:200 1:1,000	0.2	—	Died in 5 min.
4	1:50 1:200 1:2,000	0.1	Present* " * "	Died in 6 hrs
5	1:50 1:200 1:2,000	0.1	" * " * "	
6	1:50 1:200 1:2,000	0.1	"	
7	1:50 1:200 1:2,000	0.1	" Absent "	
8	1:50 1:200 1:1,000	0.05	Present* Absent "	
9	1:50 1:200	0.05	"	
10	1:50 1:200 1:1,000	0.05	Present* " * "	
11	1:50 1:200 1:1,000	0.05	" * " Absent	
12	1:50 1:200	0.05	Present*	Died within 18 hrs.

* A very small area of hemorrhage was present before the injection of peptone; its size increased definitely after the injection of peptone.

1:1,000, were well defined by redness and edema. In seven guinea pigs there was a purple discoloration at the site of the reactions to a 1:50 or 1:200 dilution of tuberculin. Within 5 hours after the injection of peptone, the hemorrhagic areas increased in size. Hemorrhage occurred after the injection of peptone in all animals except one. The occurrence of hemorrhage was more frequent with dilutions of tuberculin that produced intense inflammation. In some of the guinea pigs scratching, dyspnea, spastic movements, and urination were observed within a few minutes after the injection of peptone. One guinea pig died in 5 minutes

TABLE VI

The Effect of Intravascular Injection of Soluble Starch upon the Tuberculin Reaction

Guinea pig No.	Preparatory agent, 0.1 cc. tuberculin injected into the skin	Injury-producing agent, 10 per cent soluble starch	Hemorrhage after the injection of soluble starch
		cc.	
1	1:50	10	Present*
	1:200		"
	1:1,000		Absent
2	1:50	10	Present
	1:200		Absent
3	1:50	10	Present
	1:200		Absent
4	1:200	10	Present
5	1:50	10	" *
	1:200		

* A very small area of hemorrhage was present before the injection of starch; its size increased definitely after the injection of starch.

and four in from 6 to 18 hours after the injection of peptone. At autopsy no hemorrhages were found in the organs. This is unlike the findings after the injection of typhoid or *B. coli* toxins.

When a similar experiment was made with five guinea pigs infected with B.C.G., only one of them reacted with increase of hemorrhage at the site of tuberculin reaction, after the injection of peptone.

In the experiments with starch five tuberculous guinea pigs were injected with various dilutions of tuberculin and later injected intracardially with 10 cc. of soluble starch. The starch produced no obvious symptoms except dyspnea. Table VI shows that after the injection of starch, hemorrhage either increased or

developed in all of the guinea pigs at the site of the more intense tuberculin reactions.

DISCUSSION

In the previous paper (1) it was suggested that there is a relationship between the capacity of irritants to elicit hemorrhage or hemorrhagic necrosis in the skin of susceptible guinea pigs, and their capacity to act as preparatory agents for the Shwartzman phenomenon. The experiments reported in the present study show that in tuberculous guinea pigs tuberculin, horse serum (in animals sensitized to it), and silver nitrate act regularly as skin preparatory agents. Toxins from *B. coli* or *B. typhosus*, concentrated broth, or turpentine (each of these materials was employed in four to six guinea pigs) failed to prepare the skin for the hemorrhagic reaction. Tuberculin, horse serum, and silver nitrate caused hemorrhages in the skin of some tuberculous guinea pigs, while the latter group of substances did not do so. The observations in tuberculous and non-tuberculous guinea pigs, therefore, are in agreement in relation to the capacity of irritants to cause hemorrhages in susceptible animals and to act as preparatory agents.

It seems significant that, on the one hand, 0.01 cc. of horse serum producing mild inflammation acted as a preparatory factor in tuberculous guinea pigs that reacted with necrosis to 0.1 cc. of horse serum; on the other hand, 0.1 cc. of horse serum was inactive as a preparatory agent in tuberculous and non-tuberculous guinea pigs that reacted to it with redness and edema, but with no necrosis.

Intravascular injections of typhoid toxin caused hemorrhages at the site of injection of silver nitrate more frequently in tuberculous than in non-tuberculous guinea pigs. The area of hemorrhage was usually more extensive in tuberculous animals. The question arises whether the observed difference in normal and tuberculous guinea pigs is due to a difference in the reaction of the skin to silver nitrate itself, or to their reaction to the bacterial filtrate. There was no difference noted in the reaction of the skin to silver nitrate injections in tuberculous and non-tuberculous guinea pigs. However, tuberculous guinea pigs react differently from normal ones to bacterial filtrates. They die from a relatively small amount of filtrate with hemorrhages in the tissues containing tubercles and effusions in the pleural and peritoneal cavities.

This difference between the behavior of the tuberculous and non-tuberculous guinea pigs toward the bacterial filtrate, the injury-producing factor, may explain why the same irritant, silver nitrate, acts as a preparatory factor in the tuberculous, and fails to act in the normal guinea pig.

As injury-producing agents, bacterial toxins from *B. typhosus*, *B. coli*, and meningococci were used with consistent success. Of the three kinds of filtrates, that from meningococci was the most potent. The injury-producing substance can be effectively introduced by the intraperitoneal and subcutaneous routes.

Since the production of hemorrhage by bacterial filtrates and by tuberculin was associated with shock symptoms, peptone and starch, which produce symptoms of anaphylactoid shock in the guinea pig, were examined as injury-producing factors. Both Witte peptone and starch were effective in sublethal doses as injury-producing factors. These observations show that substances of non-bacterial origin that cause anaphylactoid shock symptoms may produce hemorrhages in the skin at the site of the tuberculin reaction.

After the introduction of injury-producing agents, namely *B. coli*, typhoid, or meningococcus toxins, tuberculin or peptone, a considerable number of the guinea pigs died. Large amounts of these substances, as might be expected, caused death more frequently than small amounts. Regardless of the amount of injury-producing substance injected, the animals that died as a rule had hemorrhages in the skin lesions. It appears therefore that the hemorrhage-producing effect of the substances mentioned above is related more closely to the systemic reaction than the amounts injected.

SUMMARY AND CONCLUSIONS

1. When toxic filtrates from cultures of *B. coli*, *B. typhosus*, or meningococci are injected into the blood stream, peritoneal cavity, or subcutaneous tissue of tuberculous guinea pigs, the skin at the site of a tuberculin reaction becomes hemorrhagic. The extent of the hemorrhage is proportional to the severity of the tuberculin reaction demonstrable by tests with various dilutions of tuberculin.

2. Tuberculin does not prepare the skin of non-tuberculous guinea pigs for this hemorrhagic reaction.

3. Tuberculin does not produce an intense or necrotic inflammation in the skin of tuberculous rabbits and fails to prepare the skin for the hemorrhagic reaction.

4. Tuberculin injected into the peritoneal cavities of tuberculous guinea pigs causes a hemorrhage in the skin at the site of a tuberculin reaction.

5. All guinea pigs infected with B.C.G., and most of those infected with a virulent strain of tubercle bacilli, when sensitized to horse serum and injected intracutaneously with 0.1 cc. of horse serum, react with redness, edema, and necrosis; and in some instances the necrosis is preceded by hemorrhage. When horse serum is injected into the skin of these guinea pigs in such dilution that only redness and edema result, the subsequent intravascular injection of typhoid filtrate produces hemorrhage at the site of reaction regularly in those infected with B.C.G. and frequently in those infected with a virulent strain.

6. Filtrates from *B. coli*, *B. typhosus*, or meningococci injected into the skin of tuberculous 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.

7. When concentrated broth or turpentine is introduced into the skin of tuberculous guinea pigs and later typhoid filtrate is injected into the vascular system, hemorrhages do not occur in the skin at the site of inflammation.

8. The majority of guinea pigs that receive an intravascular injection of typhoid filtrate react with hemorrhage at the site of the injection of the silver nitrate. The incidence of hemorrhagic reaction in tuberculous guinea pigs is higher than in non-tuberculous guinea pigs that received similar injections of silver nitrate and typhoid toxin.

9. In tuberculous guinea pigs the skin can be prepared for the hemorrhagic reaction not only by bacterial toxins but also by tuberculin, horse serum, and an inorganic chemical, silver nitrate.

10. In the guinea pig the skin preparatory agents, *i.e.* tuberculin in the tuberculous guinea pig, diphtheria toxin and silver nitrate in both tuberculous and non-tuberculous guinea pigs, tend to produce hemorrhages in the skin even without subsequent injection of a toxic bacterial product. This property of the skin preparatory agents may be essential in their action.

11. Hemorrhages occur in the skin at the site of tuberculin reaction not only after the intravascular injection of bacterial toxins or tuberculin, but also after the injection of substances of non-bacterial origin; namely, peptone or soluble starch.

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