STUDIES ON THE GENERALIZED SHWARTZMAN REACTION*

IV. Prevention of the Local and Generalized Shwartzman Reactions with Heparin

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It has been shown that thrombosis of small blood vessels is a constant pathological feature of both the generalized and local Shwartzman phenomena (1–3). Studying the dermal Shwartzman reaction, Stetson found that leukocyte-platelet thrombi occur in the small blood vessels of prepared skin shortly after the provoking intravenous injection of toxin (3). He attributed this to the development of adhesiveness of the white blood cells and platelets, and associated it with the occurrence of leukopenia following the intravenous injection of meningococcal toxin. On the basis of his observation that occlusion of small blood vessels regularly occurred prior to the development of hemorrhagic necrosis of the skin, Stetson suggested that thrombosis of capillaries and venules plays an essential role in the pathogenesis of the local Shwartzman reaction.

The initial histological event in the development of bilateral cortical necrosis of the kidneys, in the generalized Shwartzman reaction, is occlusion of the glomerular capillaries by acellular, eosinophilic material having tinctorial properties characteristic of "fibrinoid" (4, 5). Although usually homogenous, this material occasionally possesses a fibrillary structure resembling that of fibrin. Neither platelets nor leukocytes are identifiable as a basic constituent of this material (6).

In studies of the pathology of the developing renal lesion (7), it was observed that fibrinoid plugs appeared in the glomerular capillaries between 2 and 4 hours after the second, or provoking intravenous injection of toxin, their formation thus antedating by several hours the appearance of hemorrhagic necrosis of the renal cortex. This sequence of events suggested that bilateral cortical

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necrosis of the kidneys might be due to ischemia resulting from interruption of the glomerular circulation.

In order to obtain further information concerning the role of thrombosis in the pathogenesis of the local and generalized Shwartzman reactions, the effect of anticoagulant doses of heparin on the occurrence of the reactions was investigated. The present paper is concerned with the results of this study.

Materials and Methods

The rabbits used in these experiments were 1 to 2 kilo albino, hybrid rabbits of the stock employed in previous studies (5, 8).

Meningococcal toxin was prepared from agar washings of cultures of a strain of meningococci (44-B) obtained from Dr. Gregory Shwartzman of the Mount Sinai Hospital, New York. The method for preparing toxin was the same as described in a previous paper (5). Various dilutions of the toxin were made in sterile physiological saline solution.

The dermal Shwartzman reaction was produced by giving an injection of 0.25 cc. of a 1–2 dilution of meningococcal toxin intradermally, followed 24 hours later by an intravenous injection of 2 cc. of a 1–20 or 1–40 dilution. With the lots of toxin used, the majority of rabbits treated in this way developed large areas of hemorrhagic necrosis of the skin at the site of the intradermal injection within 3 hours after the intravenous injection.

The generalized Shwartzman reaction was produced by giving two intravenous injections of 2 cc. of a 1–80 dilution of toxin, spaced 24 hours apart. Bilateral cortical necrosis of the kidneys occurred in 80 to 90 per cent of the animals, within 24 hours after the second intravenous injection.

In the text to follow, the initial injection of toxin, whether intradermal or intravenous, will be referred to as the "preparing" injection, and the second injection of toxin will be designated the "provoking" injection.

Cortisone acetate, in a suspension containing 25 mg. per cc., produced under the trade name cortone, was obtained from Merck and Co., Inc., Rahway, New Jersey.

Thorotrast, a 24 to 26 per cent suspension of thorium dioxide, was obtained from Heyden Chemical Corporation, New York. Only materials designated by the manufacturers as lots 204 and 205 were used in these experiments; in previous studies these lots were found to possess greater activity in altering the reactivity of rabbits to meningococcal toxin than several batches designated by higher lot numbers (9).

Heparin solution was obtained from The Upjohn Company, Kalamazoo, Michigan, and from the Eli Lilly and Company, Indianapolis, Indiana. The preparations used contained heparin sodium U.S.P., 1000 units or 10 mg. per cc.

"Depot" heparin, for subcutaneous administration, was obtained from The Upjohn Company. Each cc. of this material contained heparin sodium 200 mg., gelatin 180 mg., and dextrose 80 mg., with sodium ethyl mercurothiosalicylate in a dilution of 1–10,000 as preservative.

White blood cell counts and differential counts were performed on blood obtained from the marginal ear vein, by standard techniques. Platelet counts were performed on ear vein blood by the direct method, using Rees-Ecker diluting solution. Clotting times were measured by the capillary tube technique.

EXPERIMENTAL

The Effect of Heparin on the Clotting Time of Normal Rabbits

In order to determine the dosage of heparin necessary to prevent blood coagulation in rabbits, clotting times were measured by the capillary tube method

prior to and at 30 minute intervals following an intravenous injection of various amounts of heparin. Illustrative results are recorded in Table I. It will be seen that a dose of 30 mg. per kilo regularly produced incoagulability of the blood for a period of 2 hours or longer after injection, and a similar but less prolonged anticoagulant effect was produced by 15 mg. per kilo. The effect of 5 mg. per kilo was of much shorter duration, and prolongation of coagulation time was

TABLE I

The Effect of Heparin Given Intravenously on the Clotting Time of Normal Rabbits

Dosage of heparin	Rabbit No.	Clotting time*						
		Before injection of heparin	Hrs. after injection					
			½	1	1 ½	2	2 ½	3
mg./kg.								
1	1	1′45″	45″	1′30″	50″	50″	45″	45″
	2	1′	1′30″	2′	45″	1′	1′30″	1′30″
	3	1′30″	30″	1′	1′15″	1′	45″	1′
5	4	1′30″	1 hr.	1 hr.	20′	1′45″	45″	1′
	5	45″	1 hr.	15′	5′	45″	45″	1′
	6	40″	1 hr.	3′	3′35″	5′	50″	45″
15	7	1′40″	1 hr.	1 hr.	1 hr.	1 hr.	20′	4′
	8	40″	1 hr.	1 hr.	1 hr.	30′	6′	2′
	9	25″	1 hr.	1 hr.	1 hr.	7′	2′	3′
30	10	45″	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	2′
	11	45″	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	20′
	12	1′30″	1 hr.	1 hr.	1 hr.	1 hr.	40′	5′

* Clotting times were determined by the capillary tube method; the tubes were broken at frequent intervals during the first 10 minutes and at intervals of 5 to 10 minutes thereafter for 1 hour. Clotting times longer than 1 hour were not recorded. The times are indicated as follows: 1 hr., 1 hour; 1′30″, 1 minute and 30 seconds.

‡ Aqueous heparin, in the dosage indicated, was injected intravenously in the marginal ear vein.

not consistently produced for longer than 1 hour after heparin. In a dose of 1 mg. per kilo no anticoagulant effect of heparin was demonstrable.

Additional studies revealed that when heparin was injected intravenously in a dose of 15 or 30 mg. per kilo every 2 hours, incoagulability of the blood could be maintained for periods of 12 hours or longer. Rabbits treated in this fashion did not become ill, and no effects of heparin other than prolongation of coagulation time were demonstrable.

Similar experiments to determine the effect of various doses of depot heparin, administered subcutaneously, are recorded in Table II. It is apparent from these data that incoagulability of the blood was maintained for 8 hours or longer

by a single subcutaneous injection of 40 mg. or more of depot heparin. With doses of 20 mg. or less, the anticoagulant effect was less marked and of shorter duration.

Prevention of the Generalized Shwartzman Reaction with Heparin

In the fully developed generalized Shwartzman reaction, hemorrhagic necrosis occurs in the kidneys, lungs, spleen, and gastro-intestinal tract. The characteristic and identifying lesion is bilateral cortical necrosis of the kidneys. When

TABLE II

The Effect of Subcutaneous Depot Heparin on the Clotting Time of Normal Rabbits

Dosage of depot heparin given subcutaneously*	Rabbit No.	Clotting time‡											
		Before injection of heparin	Hrs. after injection										
			½	1	1½	2	2½	3	4	5	6	7	8
mg./kg.													
10	68	2′30″	2′	1′30″	2′	2′	2′	3′15″	5′	2′	1′	1′30″	1′
	69	1′30″	2′	2′	10′	10′	5′	5′	4′	5′	2′	5′	1′
	70	1′50″	4′	10′	12′	6′30″	50′	10′	15′	10′	5′	5′	2′
20	71	3′	1′30″	2′	30′	20′	1 hr.	1 hr.	1 hr.	20′	10′	10′	2′
	72	1′30″	20′	40′	20′	20′	10′	20′	20′	3′30″	20′	5′	3′30″
	73	2′30″	2′	20″	1 hr.	1 hr.	30′	15′	15′	5′	5′	2′	2′
40	74	1′	10′	1 hr.	5′	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.
	75	1′20″	2′30″	1 hr.	1 hr.	1 hr.	30′	1 hr.	1 hr.	5′	1 hr.	10′	10′
	76	2′	2′20″	3′10″	3′	1 hr.	1 hr.	40′	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.
65	77	2′15″	2′20″	3′	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.
	78	2′40″	1 hr.	3′40″	4′	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.
	79	1′40″	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	40′
100	80	30″	2′20″	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.
	81	1′20″	2′25″	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.
	82	45″	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.	50′	1 hr.	1 hr.	1 hr.	1 hr.	1 hr.

* Depot heparin (Upjohn) was injected subcutaneously into the thigh.

‡ Clotting times were determined by the capillary tube method as in experiments recorded in Table I.

rabbits were treated with doses of heparin sufficient to render the blood incoagulable for a period of 4 to 5 hours following the provoking injection of toxin, the generalized Shwartzman reaction was completely prevented in the majority of instances. This effect of heparin is illustrated in the experiments which follow:—

Fourteen rabbits were injected intravenously with 2 cc. of 1–80 dilution of meningococcal toxin followed 24 hours later by a similar injection. Heparin, in a dose of 30 mg. per kilo, was injected intravenously in seven of the rabbits, immediately following the second injection of toxin and again 2 and 4 hours later. As is shown in Table III, none of the treated rabbits died, and none showed hemorrhagic necrosis in the kidneys or other organs when sacrificed 24 hours after the provoking injection. In contrast, three of the seven control

TABLE III

Prevention of the Generalized Shwartzman Reaction by Heparin Given Intravenously

Experimental procedure*	No. of rabbits	No. dead‡	No. with hemorrhagic necrosis§			
			Kidneys	Spleen	Lungs	Intestines
Intravenous heparin 30 mg./kg. immediately after provoking injection of toxin, and 2 and 4 hrs. later	7	0	0	0	0	0
No heparin	7	3	7	7	7	4

* All rabbits received two intravenous injections of meningococcal toxin (2 cc. of 1–80 dilution) spaced 24 hours apart. Heparin was given at time of second injection.

‡ Figures indicate number of rabbits dying within 24 hours after provoking injection.

§ The kidney lesions indicated by figures consisted of bilateral renal cortical necrosis. The lesions in other organs consisted of multiple areas of hemorrhage and necrosis.

TABLE IV

The Effect of Various Intravenous Amounts of Heparin at the Time of Provocation of the Generalized Shwartzman Reaction Compared with Similar Amounts at the Time of Preparation

Group	Experimental procedure*	Dose of heparin‡	No. of rabbits	Bilateral renal cortical necrosis	
				No. positive	Per cent positive
		mg./kg.			
1	3 injections of heparin: at time of *provocation* and 2 and 4 hrs. later	30	20	1	5
2	Same as in No. 1	15	6	0	0
3	" " " " "	5	8	4	50
4	" " " " "	1	5	5	100
5	3 injections of heparin; at time of *preparation* and 2 and 4 hrs. later	30	8	8	100
6	Same as in No. 5	15	6	5	83
7	No heparin	0	27	24	88

* All rabbits received two intravenous injections of meningococcal toxin (2 cc. of 1–80 dilution) spaced 24 hours apart.

‡ Dose refers to amount of heparin in each injection.

rabbits died, and at autopsy all seven showed bilateral cortical necrosis of the kidneys and hemorrhagic necrosis of other visceral organs.

In the heparin-treated rabbits, the glomerular capillaries did not contain any of the fibrinoid material which characterizes the renal lesion of the generalized Shwartzman reaction.

Effect of Dosage and Time of Administration of Heparin on Prevention of the Generalized Shwartzman Reaction.—The effect of various doses of heparin administered at the time of preparation or provocation of the generalized Shwartzman phenomenon are summarized in Table IV. When heparin was given in doses of 15 or 30 mg. per kilo at the time of the provoking injection of toxin and again 2 and 4 hours later, the development of the generalized Shwartzman reaction was prevented in almost all instances. Little or no protective effect was provided by doses of 5 mg. or less; the incidence of the reaction in these animals was comparable to that in the control group.

TABLE V

The Effect of Intravenous Injection of Heparin at Various Times before and after Provocation of the Generalized Shwartzman Reaction

Time of administration of heparin,* ‡	Bilateral cortical necrosis of kidneys§
At time of provocation	3/4
1 hr. after "	2/4
4 hrs. " "	4/4
At time of provocation and 2 hrs. later	0/8
2 hrs. before and at the time of provocation	3/4
2 and 4 hrs. after provocation	2/4
4 " 6 " " "	4/4
No heparin	4/4

* All rabbits received two intravenous injections of meningococcal toxin (2 cc. of 1–80 dilution) spaced 24 hours apart.

‡ Heparin given intravenously in a dose of 15 mg./kilo in each injection.

§ Numerator refers to number of rabbits with renal cortical necrosis of kidneys. Denominator refers to number of rabbits in the group.

When three injections of 30 mg. per kilo were given at the time of the *preparing* injection of toxin, the generalized Shwartzman reaction was not prevented in any of eight rabbits. This observation indicates that the protective action of heparin is directed against events associated with provocation rather than preparation.

Previous studies of the histological development of renal cortical necrosis indicated that occlusion of the glomerular capillaries by fibrinoid material occurred within a few hours after the provoking injection of toxin. Since this material was not present in the glomeruli of animals in which renal necrosis was prevented by heparin, it seemed of importance to determine whether the effect of heparin was exerted during the period when glomerular occlusion ordinarily occurs. Accordingly, groups of rabbits were given heparin at varying times before and after provocation of the generalized Shwartzman reaction.

As is shown in Table V, a single injection of 15 mg. heparin did not prevent

the reaction when given simultaneously with toxin, nor when given 1, 2, or 4 hours after toxin.

When two doses of 15 mg. each were given at the time of the provoking injection of toxin and again 2 hours later, the reaction was prevented in all of a group of eight rabbits (Table V). In contrast, the same doses were ineffective when given 2 hours before and at the time of provocation, or when given 2 and 4, or 4 and 6 hours after provocation. These observations indicated that a

TABLE VI

Prevention of Generalized Shwartzman Reaction with Subcutaneous "Depot" Heparin

Group	Experimental procedure*	Dose of heparin‡	No. of rabbits	Bilateral renal cortical necrosis	
				No. positive	Per cent positive
		mg./kg.			
1	Depot heparin subcutaneously 1 hr. prior to *provoking* injection of toxin	100	5	0	0
2	Same as in No. 1	65	8	0	0
3	" " " " "	40	8	3	37.5
4	" " " " "	20	8	6	75.0
5	" " " " "	10	8	7	87.5
6	Depot heparin subcutaneously 1 hr. prior to *preparing* injection of toxin	100	8	7	87.5
7	Same as in No. 6	65	6	5	83.5
8	No heparin	0	10	9	90

* All rabbits received two intravenous injections of meningococcal toxin (2 cc. of 1–80 dilution) spaced 24 hours apart.

‡ Heparin given subcutaneously into thigh.

sustained effect of heparin throughout the first 4 hours after provocation was necessary for prevention of the reaction.

Prevention of the Generalized Shwartzman Reaction with Heparin Administered Subcutaneously.—In Table VI are summarized results obtained when depot heparin was employed in various doses to prevent the generalized Shwartzman reaction. When administered 1 hour prior to provocation, in doses of 65 or 100 mg. per kilo, depot heparin was uniformly protective. A dose of 40 mg. per kilo provided only partial protection, and 20 and 10 mg. per kilo failed to protect. It is of interest that the latter doses were also incapable of producing sustained incoagulability with regularity for periods of 2 hours or longer in normal rabbits (Table II).

When depot heparin was given in various dosages at the time of the first, or preparing injection of toxin, there was no inhibition of the generalized Shwartzman reaction.

Prevention of the Dermal Shwartzman Reaction with Heparin

The local Shwartzman reaction was regularly produced when an intradermal injection of meningococcal toxin was followed in 18 or 24 hours by an intravenous injection of toxin. The administration of heparin, in doses capable of inhibiting coagulation for 4 to 5 hours after the provoking injection of toxin, resulted in prevention of the dermal Shwartzman reaction, as is shown in the following experiments:—

Eight groups of rabbits were injected intradermally with 0.25 cc. of a 1–2 dilution of meningococcal toxin, followed 24 hours later by an intravenous injection of 2 cc. of a 1–20 dilution. Seven of the groups were given various forms of treatment with heparin, and one group of controls received no heparin. The results are summarized in Table VII.

It will be seen that both aqueous heparin and depot heparin, when given in sufficiently large dosages at the time of the provoking injection of toxin, prevented the local Shwartzman reaction. Of 18 rabbits which received three injections of 30 mg. intravenously at the time of provocation, and 1 and 3 hours later, only two developed Shwartzman reactions. Of 26 animals which received 100 mg. per kilo of depot heparin, 1 hour before provocation, five developed reactions.

With doses of aqueous or depot heparin which had previously been found incapable of causing prolonged incoagulability, no protection against the Shwartzman reaction was observed. For example, as is shown in Table VII, three intravenous injections of aqueous heparin in a dose of 5 mg. each, or a single subcutaneous injection of 20 mg. of depot heparin, had no effect on the reaction.

As in the generalized Shwartzman reaction, heparin had no effect when injected at the time of preparation, as is shown in Table VII. Amounts of aqueous or depot heparin which provided complete protection when given at the time of the provoking injection were without effect when given at the time of preparation of the skin.

Prevention by Heparin of Provocation of the Dermal Shwartzman Reaction with Glycogen, Tissue Extracts, and Human Serum.—That the dermal Shwartzman reaction may be provoked by the intravenous injection of materials other than Gram-negative endotoxins has long been known (1, 10). Various non-bacterial substances, including glycogen, agar, tissue extracts, kaolin, antigen antibody complexes, and normal human serum, may be injected intravenously as the provoking stimulus in rabbits whose skin has been suitably prepared by an intradermal injection of endotoxin. Hemorrhagic necrosis of the skin occurs

more quickly following provocation with non-bacterial agents than when produced by an intravenous injection (3, 11, 12). The earlier appearance of hemorrhagic necrosis after the injection of non-bacterial provoking agents suggests that these substances may act differently from bacterial toxin in provoking the Shwartzman reaction. In light of this possibility, it seemed important to

TABLE VII

Prevention of Dermal Shwartzman Reaction with Heparin

Group	Experimental procedure*	Dose of heparin‡	No. of rabbits	Dermal Shwartzman reaction	
				No. postive	Per cent positive
		mg./kg.			
1	3 intravenous injections of heparin: at time of provocation, 2 and 4 hrs. later	30	18	2	11
2	Same as in No. 1	5	6	6	100
3	Depot heparin subcutaneously 1 hr. prior to provocation	100	26	5	18
4	Same as in No. 3	20	6	5	83
5	3 intravenous injections of heparin: at time of *preparation*, 2 and 4 hrs. later	30	6	6	100
6	Depot heparin subcutaneously, 1 hr. prior to preparation	100	12	12	100
7	Untreated controls	0	30	29	96

* All rabbits received 0.25 cc. of a 1–2 dilution of meningococcal toxin intradermally, followed 24 hours later by an intravenous injection of 2 cc. of a 1–20 dilution.

‡ Dose of heparin refers to amount given in each injection.

determine whether treatment with heparin would inhibit provocation of the Shwartzman reaction by such materials.

In the experiment described below it is shown that heparin given either subcutaneously or intravenously prevents provocation of the Shwartzman reaction with glycogen, liver suspension, and human serum.

Thirty-three rabbits were injected intradermally with 0.25 cc. of a 1–2 dilution of meningococcal toxin. 23 hours later 15 were injected subcutaneously with depot heparin in a dose of 100 mg. per kilo. 1 hour later, all 33 rabbits were injected intravenously with 200 mg. of rabbit liver glycogen prepared as described in an earlier report (10). The results are summarized in Table VIII. Of the 18 control rabbits given no heparin, 14 developed severe Shwartzman reactions at the prepared skin site. In contrast, only 3 of 15 heparin-treated

animals showed hemorrhagic necrosis of the skin. A similar protective effect of heparin was observed in rabbits in which the Shwartzman reaction was provoked by a 10 per cent saline suspension of rabbit liver tissue, or by 5 cc. of normal human serum.

TABLE VIII

Prevention by Heparin of Provocation of the Dermal Shwartzman Reaction with Glycogen, Human Serum, and Liver Suspension

Group	Provoking agent*	Heparin treatment	No. of rabbits	Dermal hemorrhagic necrosis	
				No. positive	Per cent positive
1	Rabbit liver glycogen‡	Depot heparin, 100 mg./kg.§	15	3	20
		None	18	14	77
2	Rabbit liver suspension‖	Depot heparin, 100 mg./kg.	4	1	25
		None	4	4	100
3	Human serum¶	Depot heparin, 65 mg./kg., 1 hr. prior to provocation and 2 hrs. later	8	3	37.5
		None	8	8	100
4	Human serum	30 mg./kg. heparin intravenously at time of provocation and 2 hrs. later	5	0	0
		None	5	5	100

* All rabbits received 0.25 cc. meningococcal toxin intradermally followed 24 hours later by an intravenous injection of rabbit liver glycogen, liver suspension, or normal human serum.

‡ 200 mg. rabbit liver glycogen suspended in 5 cc. physiological saline solution.

§ Depot heparin was given subcutaneously 1 hour prior to provocation.

‖ 10 cc. of 10 per cent suspension of homogenized normal rabbit liver in physiological saline solution.

¶ 5 cc. normal human serum.

Prevention by Heparin of Bilateral Cortical Necrosis of the Kidneys in Cortisone and Thorotrast-Treated Rabbits

Previous studies showed that rabbits treated with either cortisone or thorotrast develop bilateral cortical necrosis of the kidneys following a single intravenous injection of meningococcal toxin. In this respect, the animals react as though they are prepared for the generalized Shwartzman reaction. Evidence has been presented indicating that the development of bilateral cortical necrosis of the kidneys following a single intravenous injection of toxin in these rabbits

may be due to interference by cortisone or thorotrast with protective functions of the reticuloendothelial system (5, 8, 9). The effect of heparin on the incidence of lesions in animals treated with these materials was investigated in the following manner.

Sixteen rabbits were injected intramuscularly with 25 mg. of cortisone for 4 days. On the 3rd day of cortisone treatment, each rabbit was given 2 cc. of a 1–40 dilution of meningococcal toxin. Immediately thereafter eight of the rabbits were given heparin intravenously

TABLE IX

Prevention by Heparin of Bilateral Cortical Necrosis of the Kidneys in Cortisone- and Thorotrast-Treated Rabbits

Group	Experimental procedure	Heparin treatment	No. of rabbits	Bilateral cortical necrosis of the kidneys	
				No. positive	Per cent positive
1	Cortisone 25 mg. daily for 4 days. Meningococcal toxin intravenously on 3rd day*	30 mg./kg. at a time of injection of toxin and 2 and 4 hrs. later	8	0	0
2	Same as in No. 1	None	8	6	75
3	Thorotrast,‡ 3 cc. per kilo intravenously, 6 hrs. prior to injection of toxin§	Same as group 1	13	0	0
4	Same as in No. 3	100 mg./kg. depot heparin subcutaneously 1 hr. prior to injection of toxin	6	0	0
5	" " " "	None	19	18	91

* Meningococcal toxin given intravenously in a dose of 2 cc. of a 1–40 dilution.

‡ The thorotrast used was from a batch designated as lot 204.

§ Meningococcal toxin given intravenously in a dose of 2 cc. of a 1–1280 dilution.

in a dosage of 30 mg. per kilo. This dose of heparin was repeated 2 and 4 hours later. The results are recorded in Table IX.

It will be seen that untreated rabbits regularly developed bilateral cortical necrosis of the kidneys, while those treated with heparin showed no renal lesions.

Similarly, 18 of 19 thorotrast-treated rabbits injected intravenously with 2 cc. of a 1-1280 dilution of meningococcal toxin, 6 hours after thorotrast, developed bilateral cortical necrosis of the kidneys, while none of 19 heparin-treated rabbits given identical injections of thorotrast and toxin developed renal necrosis. As is shown in Table IX, a single subcutaneous injection of depot

heparin provided complete protection against renal cortical necrosis in thorotrast-treated rabbits.

Prevention by Heparin of Hemorrhagic Skin Reactions in Thorotrast-Treated Rabbits

In previous studies (8, 9, 13, 14) it was found that rabbits treated with either cortisone or thorotrast developed hemorrhagic necrosis at the site of an intradermal injection of meningococcal toxin, instead of the usual reaction of edema and erythema. The hemorrhagic skin lesions were in the gross and histologically similar to dermal Shwartzman reactions, and occurred between 12 and 24 hours following the intradermal injection of toxin. Similar reactions were noted

TABLE X

Prevention with Heparin of Hemorrhagic Necrosis of Skin and Kidneys Following Intradermal Injection of Meningococcal Toxin in Thorotrast-Treated Rabbits

Experimental procedure*	Heparin treatment	No. of rabbits	No. with skin hemorrhage	No. with bilateral cortical necrosis of kidneys
Thorotrast followed by intradermal toxin	30 mg./kg. intravenously every 3 hrs. for 24 hrs. following intradermal injection of toxin	8	0	0
Same as in experiment above	None	8	6	7

* Thorotrast, administered intravenously in a dosage of 3 cc. per kilo, was followed 6 hours later by an intradermal injection of 0.5 cc. of a 1–2 dilution of meningococcal toxin.

by Bennett (15) following intradermal injections of *Serratia marcescens* toxin in thorotrast-treated rabbits.

In addition to these skin lesions, it was also observed that renal cortical necrosis frequently occurred after an intradermal injection of meningococcal toxin in rabbits treated with cortisone or thorotrast, implying an enhanced degree of absorption of toxin from the skin (8, 9). In order to determine whether such skin and kidney lesions could be prevented by heparin, the following experiment was performed:—

Sixteen rabbits were given thorotrast intravenously in a dosage of 2 cc. per kilo, followed 6 hours later by an intradermal injection of 0.5 cc. of a 1–2 dilution of meningococcal toxin. Eight were given aqueous heparin in a dose of 30 mg. per kilo by vein, every 3 hours for 24 hours after the injection of toxin, and eight control rabbits were given no heparin. The results are summarized in Table X.

It will be seen that none of the heparin-treated rabbits developed primary hemorrhagic necrosis of the skin or bilateral cortical necrosis of the kidneys.

In contrast, six of eight untreated controls had hemorrhagic skin lesions, and seven developed bilateral cortical necrosis of the kidneys.

Failure of Heparin to Interfere with the Lethal Action of Toxin

Severe prostration and death occur when large doses of meningococcal toxin are injected intravenously in rabbits (1, 5). The intravenous injection of thorotrast several hours prior to the injection of toxin results in marked enhancement of the lethal action of endotoxin (16, 9).

TABLE XI

The Failure of Heparin to Protect Rabbits against the Lethal Action of Meningococcal Toxin

Group	Experimental procedures	Heparin treatment	No. of rabbits	No. dead*
1	Meningococcal toxin intravenously‡	Heparin 30 mg./kg. intravenously at time of injection of toxin and 2 and 4 hrs. later	8	4
2	Same as in No. 1	None	8	3
3	Thorotrast intravenously followed 6 hrs. later by an intravenous injection of meningococcal toxin§	Same as 1	6	6
4	Same as in No. 3	Depot heparin 100 mg./kg. intravenously 1 hr. prior to injection of toxin	6	5
5	" " " " "	None	6	6

* No. dead refers to the number of rabbits dying within 12 hours after the intravenous injection of meningococcal toxin. None of these rabbits developed the generalized Shwartzman reaction.

‡ Meningococcal toxin from a batch known to be capable of causing severe prostration and death in approximately 50 per cent of rabbits was given intravenously in a dose of 2 cc. of a 1–20 dilution.

§ Thorotrast was injected intravenously in a dose of 3 cc. per kilo and was followed 6 hours later with 2 cc. of a 1–320 dilution of meningococcal toxin.

In order to determine whether treatment with heparin would inhibit the lethal action of Meningococcal toxin, the following experiments were carried out:—

Sixteen rabbits were injected intravenously with 2 cc. of a 1–20 dilution of a batch of meningococcal toxin known to be capable of causing severe prostration and death in 30 to 40 per cent of rabbits. Eight of the animals were given heparin in a dose of 30 mg. per kilo by vein, immediately after the administration of toxin, and again 2 and 4 hours later. Eight controls were not treated. The results are shown in Table XI. Four of the heparin-treated rabbits, and three of the control group died within 12 hours after the injection of toxin.

The failure of heparin to protect against the lethal effect of toxin was also observed in animals receiving meningococcal toxin following an injection of thorotrast, as indicated by the following experiment:—

Eighteen rabbits were injected by vein with thorotrast in a dosage of 3 cc. per kilo. Six hours later each rabbit received an intravenous injection of 2 cc. of a 1–320 dilution of meningococcal toxin. Six rabbits were given heparin intravenously at the time of injection of toxin, and 2 and 4 hours later. Six others were given depot heparin subcutaneously 1 hour prior to injection of toxin. Six control rabbits received no heparin. As is shown in Table XI, heparin provided no protection against the lethal effect of toxin in this circumstance.

Hematological Observations in Rabbits Injected with Heparin and Toxin

The intravenous injection of meningococcal toxin causes a marked polymorphonuclear leukopenia, which appears after approximately 30 minutes and

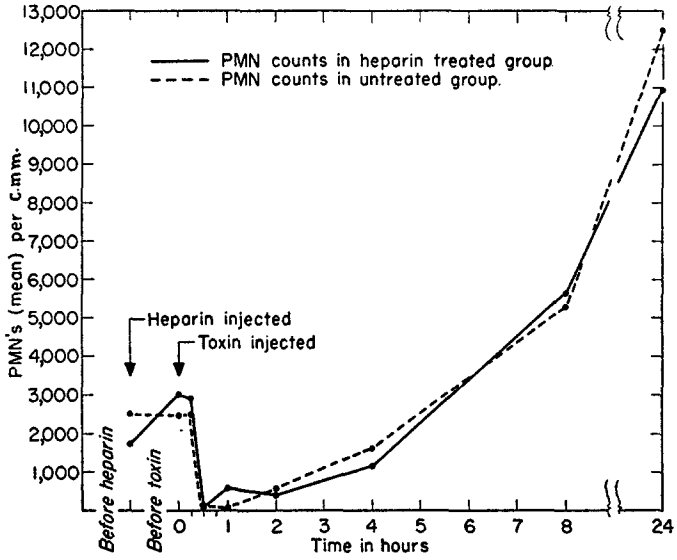

FIG. 1. The effect of an intravenous injection of meningococcal toxin on the polymorphonuclear leukocyte count in heparin-treated and untreated rabbits. The mean cell count in 3 rabbits is represented by each point on the graph.

persists for 3 or 4 hours. It has been suggested by Stetson (3) that an aggregation of leukocytes, associated with this event, may play an important role in the development of the vascular occlusion leading to the dermal Shwartzman reaction. The demonstration that the local and generalized Shwartzman reactions are inhibited by nitrogen mustard, and the correlation between the protective action and the neutropenia produced by this substance, have been interpreted as indicating an essential role of polymorphonuclear leukocytes in both the local and generalized Shwartzman reactions (17, 18, 5). In view of these findings, it seemed of importance to determine whether the prevention of the Shwartzman reaction by heparin was also associated with an effect on leukocytes. In the experiment which follows, it was shown that treatment with

heparin, in doses that completely prevented the Shwartzman reaction, did not cause leukopenia, nor did it interfere with the sudden fall in numbers of circulating neutrophils which is produced by an intravenous injection of meningococcal toxin.

Three rabbits were injected subcutaneously with 100 mg. of depot heparin 1 hour prior to the intravenous injection of 2 cc. of a 1–160 dilution of meningococcal toxin. Three rabbits given toxin without heparin served as controls. Total white blood cell and differential counts were made prior to injection of heparin, prior to the injection of toxin, and 15 minutes, 30 minutes, 1, 2, 4, 8, and 24 hours after the intravenous injection of toxin. The results are summarized in Fig. 1. In this figure the mean numbers of circulating neutrophils plotted against time are compared for the two groups of rabbits.

No significant differences between the heparin-treated and untreated animals were noted in the numbers of neutrophils in the circulating blood at any time before or after the intravenous injection of toxin.

Effect of Heparin on the Numbers of Circulating Platelets.—The intravenous injection of heparin has been found to cause a transitory depression of the number of platelets in the circulating blood (19–21). In order to determine whether thrombocytopenia was involved in the protective action of heparin, platelet counts were performed at frequent intervals after a subcutaneous injection of depot heparin, in a dose of 65 mg. per kilo. No significant alteration in the level of circulating platelets was demonstrable, indicating that the protective effect of heparin is not due to a reduction in the number of available platelets.

DISCUSSION

Treatment of rabbits with heparin provided complete protection against the local and generalized Shwartzman reactions. Prevention occurred when heparin was given by either the intravenous or subcutaneous route at the time of the second, or provoking injection of toxin, but not when heparin was administered with the initial, preparing injection of toxin. In preventing the generalized reaction, heparin also prevented the appearance of fibrinoid material within the glomerular capillaries, which is considered to be an initiating step in development of bilateral cortical necrosis of the kidneys. The occurrence of bilateral cortical necrosis of the kidneys following a single injection of toxin in cortisone- or thorotrast-treated animals was also prevented by heparin, as well as the hemorrhagic skin reactions which follow an intradermal injection of toxin in such rabbits. Provocation of the dermal Shwartzman reaction with non-bacterial agents such as glycogen, liver suspensions, or human serum was also prevented by treatment with heparin.

The prevention of these manifestations of the Shwartzman reaction involved certain critical time relationships. It was necessary that heparin be given in a manner which provided a maximal anticoagulant effect during a period of

several hours immediately following the provoking injection of toxin. When the dosage was less than the amount needed to maintain incoagulability for 4 hours, or when heparin treatment was not begun until 2 hours after the provoking injection of toxin, no protection was demonstrable. The dosage and timing requirements are of interest in the light of Stetson's demonstration that capillary and venous thrombosis takes place during the 1st hour following provocation of the local Shwartzman reaction (3), and the finding in this laboratory that occlusion of the glomerular capillaries by fibrinoid material occurs within the first 4 hours in the development of the generalized Shwartzman reaction (5, 7, 8).

It is probable that the effect of heparin does not involve a direct action on toxin itself, since no protection occurred when heparin was given with the preparing injection of toxin. Moreover, heparin did not protect animals against the lethal effect of a single, overwhelming dose of toxin, nor did it interfere with the capacity of toxin to produce profound polymorphonuclear leukopenia.

Since very large amounts of heparin, in terms of the effective dosages in human beings, were necessary for prevention of the Shwartzman reaction in the rabbit, it is possible that properties other than the anticoagulant action of this material may be responsible for its effect on the Shwartzman reaction. The capacity of heparin to combine with other substances in the blood, and its effects on the colloidal stability of proteins and lipid constituents, are well recognized (22, 23). On the other hand, the amounts necessary to inhibit the Shwartzman reaction were the same as the amounts required to produce *in vivo* a sustained incoagulability of the blood of rabbits. This fact, together with the observation that the protective effect of heparin is exerted during the period of time when thrombosis occurs in untreated animals, suggests strongly that the inhibiting action of heparin is mediated through its anticoagulant effect. The nature of the disturbance in the coagulation mechanism which leads to vascular occlusion in the local and generalized Shwartzman reactions is not known, and heparin should provide a useful tool for further study of this problem.

It is of incidental interest that the prevention by heparin of bilateral cortical necrosis of the kidneys, in rabbits receiving a single injection of meningococcal toxin following treatment with cortisone or thorotrast, provides additional evidence for a basic similarity between these lesions and the generalized Shwartzman reaction as produced in the conventional manner. In earlier reports, it was shown that the lesions were morphologically indistinguishable, and were prevented by prior administration of nitrogen mustard (8, 9). The present observations indicate that the effect of the provoking injection of toxin is the same in animals prepared by a previous intravenous injection of toxin as in rabbits treated with cortisone or thorotrast. Whether this effect is exerted directly on components of the blood coagulation mechanism, or indirectly by a

damaging action on vascular endothelium, remains to be determined by further investigation.

SUMMARY

In order to explore the hypothesis that the occurrence of thrombosis of small blood vessels is an essential stage in the development of the local and generalized Shwartzman reactions, the effect of heparin was studied.

Aqueous heparin, administered intravenously, and "depot" heparin, injected subcutaneously, prevented completely the occurrence of the local and generalized Shwartzman phenomena. The amounts of heparin required for protection were similar to the amounts required to produce sustained incoagulability of the blood of rabbits for a period of at least 4 hours.

The local and generalized Shwartzman reactions were prevented when heparin was given at the time of *provocation*, but not when heparin was administered during the period of *preparation*.

Heparin prevented the development of bilateral cortical necrosis of the kidneys following a single intravenous injection of meningococcal toxin in rabbits previously treated with cortisone or thorotrast.

Hemorrhagic necrosis of the skin which follows an intradermal injection of toxin in thorotrast-treated rabbits was also prevented by heparin.

Provocation of the dermal Shwartzman reaction with glycogen, saline suspension of rabbit liver, and human serum was prevented by treatment with heparin.

Heparin itself, in the preparations and dosages used, had no consistent effect on either white blood cell or platelet counts. Heparin had no effect on the occurrence of polymorphonuclear leukopenia which follows an intravenous injection of meningococcal toxin.

Treatment with heparin did not interfere with the lethal effect of single, large doses of meningococcus toxin.

In animals in which bilateral cortical necrosis of the kidneys was prevented by heparin, occlusion of the glomerular capillaries by "fibrinoid" material did not occur.

These observations support the concept that vascular occlusion plays an essential role in the development of the local and generalized Shwartzman reactions.

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