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Failure to Provoke the Local Shwartzman Reaction with Thrombin.* (31841)

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In the classical local Shwartzman reaction Gram-negative endotoxin is first injected intradermally (preparatory injection) and then, 18-24 hours later, intravenously (provoking injection). Within hours after the provoking injection an area of hemorrhagic necrosis develops at the site of the preparatory injection.

Many agents besides endotoxin can be used for the *preparatory* injection. Apparently, any material which causes polymorphonuclear leukocytes to accumulate perivascularly and to liberate proteolytic enzymes from their specific granules will prepare the skin for the reaction(1,2,3,4). A variety of substances (*e.g.*, starch, kaolin powder, serum, and glycogen) can also be used for the *provoking* injection, but their common mechanism of action is not yet clear. It has been suggested that *the* essential requirement of a provoking agent is its ability to trigger intravascular coagulation(5). If so, one should be able to prepare the skin by an intradermal injection of endotoxin and, 24 hours later, to provoke the reaction with an intravenous infusion of thrombin. Experiments to test this possibility are reported herein. They establish that an episode of diffuse intravascular coagulation is in itself insufficient to provoke the local Shwartzman reaction at a prepared site.

Materials and methods. Female albino rabbits between 1.5 and 2.4 kg in weight were used. The *endotoxin* was *E. coli* lipopolysaccharide 0111:B4 (lot 463135, Difco Laboratories, Detroit), which was dissolved in isotonic saline just before use. Bovine thrombin (Thrombin, Topical; Parke-Davis) was adsorbed with barium sulfate powder(6) to remove impurities and then stored frozen as a stock solution of approximately 1000 NIH units per ml.

Preparatory injection: The abdomen was shaved the day before, and the desired concentration of endotoxin dissolved in 0.5 ml of isotonic saline was injected into the skin of the right upper quadrant. *Provoking injection:* Twenty-four hours after the intradermal injection, either endotoxin or thrombin was given intravenously. Endotoxin was given as a 150 gamma per ml solution over a period of about 1 minute. Thrombin was added to 150 ml of sterile isotonic saline and infused at a rate of approximately 1 ml per minute. Thrombin concentrations were determined from thrombin times of dilutions of the stock solution, which were converted to NIH units from a reference curve made with standard thrombin (Division of Biologic Standards, National Institutes of Health, Bethesda, Md.). The thrombin solution did not lose activity during infusion.

Assays: Blood was obtained by needle puncture of a marginal ear vein as described earlier(7) and collected into several 0.4 ml plastic micro-test tubes (Beckman Instruments, Fullerton, Calif.), each of which contained 40 lambda of citrate-citric acid anticoagulant(6). The blood was centrifuged in a microcentrifuge (Beckman/Spinco Model 152), and the plasma was pooled and stored frozen in plastic vials. Fibrinogen was measured by the method of Jacobsson(8) as modified by Blombäck and Blombäck(9) and Godal(10). Factor VIII (antihemophilic globulin) was assayed by a slight modification of an activated partial thromboplastin time technique described elsewhere(11) and expressed as per cent of a pooled citrated rabbit plasma standard.

Results and discussion. In preliminary experiments the intradermal injection of 130-260 gamma of endotoxin prepared for the local Shwartzman reaction when the reaction was provoked by the intravenous injection of 150-215 gamma of endotoxin per 1.5 kg of animal. Therefore, 200 gamma of endotoxin was selected as the standard preparatory injection.

Table I summarizes the data obtained when

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TABLE I. Provocation of the Local Shwartzman Reaction with Endotoxin and the Associated Changes in Fibrinogen and Factor VIII.

Animal No.	Local SR	Fibrinogen (mg %) Before	Fibrinogen (mg %) After 1h	Fibrinogen (mg %) After 2h	Fibrinogen (mg %) After 4h	Factor VIII (%) Before	Factor VIII (%) After 1h	Factor VIII (%) After 2h	Factor VIII (%) After 4h
1	4+	533	680			90	115		
2	"	381	560			220	112		
3	"	338	364			148	188		
4	"	405		407		135		80	
5	"	331		331		330		200	
6	Neg.	395		440		100		42	
7	4+	462			324	155			32
8	"	296			299	150			65
9	"	448			369	55			50

TABLE II. Failure to Provoke the Local Shwartzman Reaction with Thrombin Despite Marked Falls in Fibrinogen and Factor VIII.

Animal No.	Thrombin infused (NIH units)	Local SR	Fibrinogen (mg %) Before	Fibrinogen (mg %) After*	Factor VIII (%) Before	Factor VIII (%) After*
1	150	Neg	305	296	148	60
2		"	439	267	200	75
3		"	416	343	140	60
4	300	Neg	186	141	70	15
5		"	582	432	130	41
6	640	Neg	364	199	210	125
7		"	367	145	250	15
8		"	304	42	210	4
9	800	Neg	279	190	140	10
10		"	408	213	140	14
11		"	536	377	125	23
12	1200	Neg	220	92	200	6
13		"	320	58	135	6
14		"	445	103	130	7

* Samples drawn immediately after completing the 2½ hr infusion of thrombin.

9 animals were given this standard intradermal injection followed, 24 hours later, by intravenous injection of 150 gamma of endotoxin per 1.5 kg of animal. Fibrinogen and Factor VIII were assayed in samples taken before and at 1, 2 or 4 hours after the intravenous injection. Eight of the nine animals developed a 4 plus(12) local Shwartzman reaction with large areas of hemorrhagic necrosis. Interestingly, fibrinogen levels fell only slightly and only in the samples drawn at 4 hours, which was after changes in the prepared skin were already visible. The drop in Factor VIII was more definite and was found in both the 2- and 4-hour specimens.

Table II summarizes the different results obtained when 14 animals were given the standard intradermal dose of endotoxin followed, 24 hours later, by an infusion of thrombin of 150-1200 NIH units (150 ml of saline containing 1.0-8.0 NIH units per ml given at the rate of 1 ml per minute). Not one animal developed the local Shwartzman reaction. Yet, much more intravascular coagulation was produced by the larger doses of thrombin than by the endotoxin as reflected by far greater falls in Factor VIII and fibrinogen found in samples taken immediately after the infusion.

Thus, extensive intravascular coagulation induced by thrombin failed to provoke the local Shwartzman reaction in animals prepared with endotoxin, whereas an intravenous injection of endotoxin provoked the reaction with less evidence of associated intravascular coagulation. Nevertheless, intravascular coagulation must play a role in triggering the local Shwartzman reaction for these reasons:

thrombi in capillaries and venules are a key histopathologic feature of the reaction(1); Gram-negative endotoxin promotes coagulation both *in vitro*(13) and *in vivo*(14); anticoagulants, either heparin(15) or Dicumarol® (16), prevent the reaction; and, finally, fibrinolytic agents also prevent the reaction(17). But clearly, the ability to trigger diffuse intravascular coagulation can not be the sole essential requirement—the least common denominator— of all agents capable of provoking the local Shwartzman reaction. Diffuse intravascular coagulation did not cause thrombi to localize at the prepared site, which strongly suggests that all of the factors needed for localization were not provided by the preparatory agent. Apparently, the provoking agent must also contribute to the processes which localize the platelet-white cell-fibrin thrombi at the prepared site with the consequent production of the hemorrhagic necrosis of the local Shwartzman reaction.

Summary. An infusion of thrombin will not provoke the local Shwartzman reaction in rabbits prepared with an intradermal injection of Gram-negative endotoxin. Therefore, the ability to trigger an episode of diffuse intravascular coagulation can not be the sole requirement of a provoking agent for the local Shwartzman reaction.

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